



icmr
INDIAN COUNCIL OF
MEDICAL RESEARCH

NIRRCH
NATIONAL INSTITUTE FOR RESEARCH
IN REPRODUCTIVE AND CHILD HEALTH

वार्षिक प्रतिवेदन
Annual Report
2021-2022

NIRRCH

आई सी एम आर - राष्ट्रीय प्रजनन एवं
बाल स्वास्थ्य अनुसंधान संस्थान

ICMR - National Institute for Research
in Reproductive and Child Health

राष्ट्रीय प्रजनन एवं बाल स्वास्थ्य
अनुसंधान संस्थान
National Institute for Research
in Reproductive and Child Health



icmr
INDIAN COUNCIL OF
MEDICAL RESEARCH

NIRRCH
NATIONAL INSTITUTE FOR RESEARCH
IN REPRODUCTIVE AND CHILD HEALTH

वार्षिक प्रतिवेदन
ANNUAL REPORT

2021-2022

Published by: Dr G M Phadke Memorial Library, NIRRCH, Mumbai

Limited number of copies of the Report available from:

Director

*ICMR-National Institute for Research in Reproductive and Child Health, Parel, Mumbai
400012, India*

Tel.: (022) 24192002, 24192100

Fax: (022) 24139412

E-mail: dir@nirrch.res.in

Website: www.nirrch.res.in

*No part of the report should be reproduced in part or whole without prior permission of the **Director, NIRRCH.***

CONTENTS

From the Desk of the Director

1.	Female Infertility and Associated Reproductive Disorders	1
2	Male Infertility and Associated Reproductive Disorders	24
3.	RTI/STIs/HIV/Microbicides	41
4.	Ageing	59
5.	Maternal Health	64
6.	Child Health	83
7.	Stem Cell Biology	92
8.	Reproductive Cancers	98
9.	Health Technology Assessment and Drug Discovery	115
10.	COVID-19 Research	125
11.	Research Support Facilities	130
12.	Publications	138
13.	Capacity Building	151
14.	Honors and Awards	162
15.	Members of Advisory Committees	164
16.	Extramurally Funded Projects	168
17.	Staff and Students	174
18.	Activities during 2021- 2022	179

Desk of Director...

This year the institute was rechristened as the National Institute for Research in Reproductive and Child Health (NIRRCH). As the addition of a 'Child' completes a 'Mother-Child dyad' or a family, and brings in more responsibilities, for us too, growing from 'NIRRCH' to 'NIRRCH' entails expansion of our responsibilities this necessitates that we address various issues affecting Indian neonates and children such as malnutrition, infections, low body weight, genetic disorders, etc. through research. We now have new targets, but we are confident that through sustained efforts, the institute would be able to contribute towards better health for our children.

During the year, we continued our studies on women's health. One of our studies demonstrated that women with superficial peritoneal endometriosis (SUP) and only one type of endometriotic lesion were more likely to conceive post-surgery. Studies on ovarian cancer showed significantly elevated level of soluble MICA, a ligand of NKG2D, serum cytokines IL-2, IL-5, IL-6, IL-10 and TNF- α in patients. Differential levels of circulatory miRNAs such as miR-182, miR-433 and miR-145 in ovarian cancer patients suggest their potential as prognostic markers. Further, Health Technology Assessment showed that adding Tranexemic Acid to management of PPH saves more lives, prevents surgical interventions or ICU admissions and is cost effective. These results were communicated to Maternal Health Division for incorporation into the guidelines and Dakshata program. We also initiated a study on acceptability and cost-effectiveness of using IV Ferric Carboxy Maltose (FCM) in management of iron deficiency anemia among pregnant women through subdistrict health system in Maharashtra. In addition, we developed online tool Multimorbidity Risk Prediction for Schizophrenia (MRPS). Our technology to detect Y chromosome microdeletions in infertile males was handed over to industry partner APS Lifetech. This will enable availability of this test at affordable rates to genetic and IVF labs in India.

We continued providing COVID-19 diagnostic services to MCGM and peripheral regions like Palghar, Ulhasnagar and Navi Mumbai Municipal Corporations using both manual testing as well as Cobas 6800 high throughput testing. During the reporting year, 1,04,782 samples were tested. Clinical characteristics, outcomes and mortality in pregnant women in the first and second waves of COVID-19 in Maharashtra were also documented. COVID-19 testing diagnostic facility at MRHRU, Dahanu tested 31,311 samples for the presence of SARS-CoV-2 virus during the reporting year. In addition, Leptospirosis diagnostic services were also initiated at MRHRU.

This year also various capacity building workshops and seminars were organized through virtual as well as in-person modes. We conducted two DHR sponsored training courses, each of 4-week duration, on Applications of Technologies in Genomics for Clinical Research and Pediatric Medical Genetics to train medical and para-medical staff

from different medical colleges in India. Around 118 research articles were published in peer-reviewed journals during the year.

We were pleased to receive eminent personalities Hon'ble Union Minister, Dr Mansukh Mandaviya, Ministry of Health & Family Welfare and Ministry of Chemicals & Fertilizers, Government of India and Dr Bharati P Pawar, Hon'ble Minister of State, Ministry of Health & Family Welfare, Government of India at the Institute on February 19, 2022. For all of us, it was a privilege to meet them and get inspired from their words of wisdom and encouragement.

We sincerely thank Prof Balram Bhargava, Secretary, Department of Health Research, Government of India and Director General, ICMR, for his exemplary leadership, continued support and encouragement to our efforts. We are grateful to Sr Deputy Director General (Administration), ICMR; Senior Financial Advisor, ICMR, Head and Staff of the Division of Reproductive Biology and Maternal and Child Health, ICMR for their valuable support and guidance. I would also like to express my gratitude to Dr Neerja Bhatla, chairperson and other members of our Scientific Advisory Committee and experts of Scientific Review Committee for providing valuable suggestions to strengthen our research programs.

I take this opportunity to thank scientific, technical and administrative staff of our institute for their diligence, dedication and enthusiasm. Before I conclude, I extend a cordial invitation to you to join us at the International Conference on Kaleidoscopic Insights into Reproductive and Child Health and Dr Shanta S Rao Centenary Celebrations, to be held on January 23-25, 2023. This conference would mark centenary birth anniversary celebrations of our founder Director, Dr Shanta S Rao, the guiding force behind establishing the institute. We hope that the institute is treading on the path envisioned by her.



Dr Geetanjali Sachdeva

Director

SUMMARY OF RESEARCH ACTIVITIES

1. FEMALE INFERTILITY AND ASSOCIATED REPRODUCTIVE DISORDERS

1.1 Deciphering the Putative Epigenetic Mechanisms Pertaining to Polycystic Ovary Syndrome

Peripheral and tissue-specific alterations in the global DNA methylation (5mC) and hydroxymethylation (5hmC) profiles have been charted as biomarkers for disease prediction. Global and gene-specific epigenetic alterations in the 5mC profiles have shown widespread implications in the etio-pathogenesis of polycystic ovary syndrome (PCOS). However, no attempts have been made to explore correlation between 5mC and 5hmC signatures and the levels of DNA methylating and demethylating enzymes in PCOS. Therefore, we assessed the global 5hmC levels in peripheral blood leukocytes and granulosa cells (GCs) in women with and without PCOS and found that 5hmC content is increased in PCOS women. The transcript and protein levels of DNA demethylating enzymes i.e. TET1, TET2, TET3 and DNA methyltransferases (DNMT1, DNMT3A and DNMT3B) were assessed in GCs. Relative transcript and protein levels of all three TETs were higher in PCOS and were positively correlated with 5hmC levels in GCs. Further, all three DNMTs at transcript level showed altered expression in PCOS although only the downregulated DNMT3A positively correlated with decreasing 5mC levels. These results indicate that DNA demethylation observed in the GCs of PCOS women may arise due to intrinsic alterations in transcriptional regulation of TETs and DNMT3A. DNA methylation and demethylation in an animal model of PCOS is being investigated.

1.2 Follicular Microenvironment and its Relationship with Oocyte and Embryo Quality in Women with Polycystic Ovary Syndrome

Oocyte quality is suboptimal in women with PCOS. This can be attributed to altered microenvironment (follicular fluid-FF and granulosa cells-GCs). Oocyte depends on GCs for supply of metabolites for its nourishing, as it cannot metabolize glucose. It is known that around 50-60% women with PCOS have insulin resistance, which can influence glucose metabolism in GCs, lead to increased glycation and cell damage. We measured the levels of advanced glycation end products (AGE) and its decoy receptor (soluble RAGE-protective in nature) in FF; and found higher AGE and lower RAGE levels in PCOS than controls. AGE levels negatively correlated with pregnancy rate and outcome in both groups. The transcript levels of SORD1 and AKR1B1 (rate limiting enzymes of polyol pathway) in GCs were upregulated in PCOS. Polyol pathway usually accounts for low activity but gets hyper-activated under metabolic stress. Our data indicate altered glucose metabolism and increase in metabolic stress in the follicles of PCOS women.

1.3 Understanding Follicular Angiogenesis in Women with Polycystic Ovary Syndrome

Angiogenesis is crucial for ovarian folliculogenesis. We reported altered expression of several angiogenesis-associated genes/proteins in the GCs and FF of women with PCOS, which may contribute to follicular growth arrest and corpus

luteum (CL) insufficiency in these women. We reanalyzed the publically available microarray datasets and enlisted additional angiogenesis-related genes, which are dysregulated in PCOS. To predict miRNAs regulating dysregulated angiogenesis-related genes in the ovary of women with PCOS, we used *in-silico* approach by querying miRNA databases. We also identified miRNA-mRNA interactions and signaling pathways involved in impaired follicular angiogenesis in PCOS. Further, analysis showed that PI3K/ Akt signaling was the most enriched pathway in the ovary of PCOS. Upon validation, we found expression of some of the miRNAs associated with the PI3K/ Akt pathway up-regulated in the GCs of women with PCOS. This work provides a novel insight into the mechanism of aberrant ovarian angiogenesis contributing to PCOS pathophysiology.

1.4 Unraveling Pathogenetic Mechanisms of Polycystic Ovary Syndrome by Genetic Approach

The genetic underpinning of PCOS has been studied by a variety of approaches including case-control and genome-wide association studies, however a conclusive genetic marker for PCOS is yet to be determined. Genetic loci identified in one ethnic population do not necessarily reflect similar association in other ethnically and geographically different populations. For this, we carried out replication studies for polymorphisms previously identified in GWAS in Han Chinese women, of which rs2479106 polymorphism of DENND1A and rs1894116 of YAP1 showed significant association with increased PCOS susceptibility. We also completed genotyping for polymorphisms in LHCGR, c9orf3 and RAB5B genes, which failed to show association with PCOS risk in our population. Additionally, we initiated genotyping for rs853854 of MAPRE and rs1784692 of ZBTB16 genes. Consequently, our findings show that delineating the genetic predisposition of Indian women with PCOS is essential. For that reason, we have initiated a study on exome sequencing in well-characterized Indian women with PCOS. Initial analysis shows association of known and novel genetic loci with PCOS susceptibility.

1.5 Analysis of Mitochondrial DNA Sequence Variants in Polycystic Ovarian Syndrome Women with Insulin Resistance

Polycystic ovary syndrome is a common cause of infertility among women of reproductive age. Earlier we reported altered mitochondrial DNA copy number and variants in mitochondrial DNA (mtDNA) in women with PCOS. mtDNA copy number was found to be negatively correlated with insulin resistance. In the reporting year, we studied correlation of 60 PCOS women and 45 healthy control women and identified that mtDNA copy number is significantly negatively correlated with AMH, a novel diagnostic marker in PCOS and a marker of ovarian reserve ($p=0.049$). Investigation of mitochondrial ROS in PCOS women ($n=16$) and healthy control women ($n=6$) using a fluorescent probe MitoSOX, a red mitochondrial superoxide indicator, suggest that mitochondrial ROS production is increased significantly in women with PCOS compared to healthy control women ($p=0.022$). This implies the mitochondrial dysfunction in PCOS women plays a crucial role in ROS production thereby causing oxidative stress.

1.6 ICMR Task Force Study on Estimating Prevalence, Phenotypes, Comorbidities and Risk Factors of PCOS - A Multicentric Study

This is an ongoing task force study by ICMR to estimate community based prevalence in India. NIRRH study site recruited a total of 1086 eligible women. Out of which 145 were probable, 18 known cases and 923 controls. Clinical and lab Investigations were completed among 91 probable, 13 known and 144 controls. USG was completed among 90 probable, 12 known and 136 controls. COVID-19 pandemic resulted in large dropouts. Repeated follow-ups were done and many enrolled participants (59 probable/known) were unwilling to come forth for further investigations or became ineligible to continue in the study or migrated and no longer in contact. The study is ongoing.

1.7 Development and Validation of Multi-Scale Dynamic and Steady State Metabolic Network Models for PCOS using Computational and Wet-Lab Methods

To accomplish the aim of developing a dynamic and steady state model for PCOS, a mathematical model of human menstrual cycle was adopted from literature and simulated to study development of PCOS phenotype. The model consisted of 71 parameters of which 28 parameters were found to be associated with PCOS phenotype. These parameters were related to hormone secretion, release, and clearance along with various stages of oocyte development. In addition, using a combination of system biology and qRT-PCR approach, it was demonstrated that FSHR antagonism might lead to PCOS-like state. Further, meta-analysis of microarray datasets associated with PCOS provided novel mechanistic insights into pathophysiology of lean and obese PCOS.

1.8 Studies to Evaluate the Effect of Metformin on Endometrial Functions

Metformin, an antidiabetic drug, has been repositioned in the treatment of several nondiabetic disorders including reproductive disorders such as polycystic ovarian syndrome. It is known to have beneficial effects on reproductive outcomes in PCOS women. However, studies addressing direct effects of metformin on endometrium are still limited and hence, this study was proposed to evaluate the direct effect of metformin on endometrium. We previously conducted studies to profile metformin treated HEC-1A (endometrial cells) at molecular level. In the reporting year, validation experiments were carried out for certain differentially expressed genes. Of the 8 genes chosen for validation, 5 genes (MRPS34, EGR1, GDF15, ODC1 and DDIT4) showed a similar pattern of up or downregulation as revealed by microarray. While 3 genes (CYCS, EDF1 and TP53) showed a similar trend as that of the microarray analysis, but were not statistically significant. Further higher SDHD expression in metformin treated cells corroborated with higher mitochondria number in lower concentration of metformin treated endometrial cells. *In-vivo* studies have been initiated in rat model of thin endometrium to investigate the effects of metformin on endometrial functions.

1.9 Pathways to Oncogenesis in the Pathophysiology of Endometriosis

Endometriosis is a benign condition characterized by the presence of endometrium-like tissue at extra-uterine sites. Several theories of endometriosis

pathogenesis have been proposed but the exact mechanism remains elusive. Few studies demonstrate differential abundance of miRNAs in plasma and/or sera from women with endometriosis. However, there exists a lack of consensus on the identity of a non-invasive marker for endometriosis diagnosis. An integration of transcriptome and miRNome may serve as a novel approach to address this issue. In the reporting year, we searched the existing literature for microarray and high throughput sequencing studies on mRNA and/or miRNA expression profiles of either eutopic endometrial tissues or plasma/sera of women with and without endometriosis. Since a majority of the studies focused on the secretory phase samples, these studies were selected for the reanalysis. Data submitted to GEO database were reanalyzed to identify mRNAs and miRNAs significantly ($p < 0.05$) dysregulated in different studies. Cumulative scores were calculated depending upon the consistency in the expression pattern of various mRNA/miRNA across the selected studies. miRNAs known to target the differentially expressed genes in the eutopic endometrium were compared with the differentially abundant miRNAs in plasma/sera of women with endometriosis. A total of 26 miRNAs (one upregulated and 25 downregulated) emerged as a miRNA panel displaying dysregulation in women with endometriosis. Validation experiments are warranted to explore the potential of these miRNAs in endometriosis diagnosis.

1.10 Investigating the Contribution of DNA Damage, Repair and Demethylation in the Pathogenesis of Endometriosis

Endometriosis is a condition in which endometrium like cells are found outside the uterus. Our previous studies demonstrated that the eutopic endometrium from women with endometriosis encounters higher DNA damage than its control counterpart. The present study was initiated to explore if higher DNA damage observed in the eutopic endometrium of women with endometriosis is associated with alterations in the DNA methylation machinery. DNA repair protein GADD45 (Growth Arrest and DNA Damage Inducible), also involved in DNA demethylation, was found to be upregulated in the eutopic endometrium of women with endometriosis. During the reporting year, studies were conducted to investigate if the expression of MBD4 (Methyl-CpG Binding Domain 4), a DNA repair protein known for its glycosylase activity, is altered in eutopic endometrium of women with endometriosis. Initial data revealed that MBD4 is significantly increased in proliferative phase eutopic endometrium from women with endometriosis. Further, our in-vitro studies revealed increased expression of GADD45a and MBD4 in response to H_2O_2 induced oxidative DNA damage in tHESC cell line. These investigations collectively revealed that DNA damage is associated with higher expression of MBD4 in endometrial cells. Studies are being conducted to assess whether higher DNA damage is associated with alterations in the DNA methylation status in endometrial cells. The study is anticipated to provide insights into mechanisms by which the endometrium acquires differential gene expression signature in women with endometriosis.

1.11 Role of Homeobox Gene HOXA10 in the Pathogenesis of Endometriosis

The study was aimed to understand the role of the transcription factor HOXA10 in the pathogenesis of endometriosis. Endometriosis was induced in mice that were hypomorphic for HOXA10. We observed that loss of HOXA10 leads to epithelial to mesenchymal cell transition by activating the transcription factors like Twist2. These cells underwent fibrosis only in the ectopic but not the eutopic endometrium of HOXA10 hypomorphs. The project is completed.

1.12 Pregnancy Outcomes in Indian Women with Endometriosis and Biomarker Correlation: A Cohort Study

In a cohort of 204 women with endometriosis-associated infertility, we observed deep infiltrating endometriosis (DIE) as the most common lesion (40%), followed by ovarian endometrioma (OMA) [31%] and superficial peritoneal endometriosis (SUP) [29%]. Women recruited in eastern India had the highest proportion of SUP (41%). OMA was most commonly seen in the southern Indian population (40%), followed by the north Indian population (33%). The highest proportion of DIE was observed in the northern region of India (83%). The study demonstrated that women with SUP and only one type of endometriotic lesion were more likely to conceive post-surgery. Therefore, we recommend that infertile women with endometriosis, younger than 35 years of age with SUP or with only one lesion type should be encouraged towards natural conception or assisted reproductive technology within 6 months after surgery.

1.13 Clinical Phenotypes and Genetic Regulation of Endometriosis in Indian Women

ECGRI is a large-scale, multi-site, case-control study covering representative Indian populations of eastern/north-eastern, northern, central, western and southern geographical zones of India. Endometriosis was classified as minimal, mild, moderate and severe disease [rASRM stages: I (n=153/476, 32.1%), II (n=53/476, 11.1%), III (n=93/476, 19.5%) and IV (n=177/476, 37.2%)]. The highest proportion of endometriosis cases in all the geographic zones was diagnosed with one subtype (range 46%-83%). The proportion of women diagnosed with two subtypes of endometriosis was highest in the north zone (39%) while lowest in the south zone (10%). All three subtypes of endometriosis were reported in 18% of patients in northeast and east zone, 12% in central zone, 9% in west zone, 7% in south zone and 2% in north zone. Pain symptoms such as dysmenorrhea, dyspareunia and chronic pelvic pain were significantly higher in endometriosis cases as compared to controls.

1.14 Damage Associated Molecular Patterns (DAMPs) and their Receptors in Endometrial Repair

Endometrium is an extremely dynamic hormone responsive tissue that continuously undergoes cyclical shedding and repair during a woman's reproductive life. Endometrial repair post menstruation is highly efficient and scar-free process. Impairment in the endometrial repair may lead to heavy menstrual bleeding or menorrhagia. Initial repair of endometrium is sex steroid-independent. We hypothesize that Damage-Associated Molecular Patterns (DAMPs) released during the menstruation may give cues to the immune cells to initiate the repair of endometrium. We have developed a rat model of endometrium breakdown and repair

to understand the role of DAMPs in the endometrial repair. Our preliminary analysis showed increased expression of alarmin HMGB1 and its receptor RAGE during the endometrial breakdown and repair in endometrium and uterine fluid. Studies are ongoing to identify the molecular signatures activated in response to DAMPs during endometrial repair.

1.15 Uterine Alarmins and their Relevance in Implantation

The project has been designed to understand the role of alarmins HMGB1 and S100A8 on implantation and to determine whether altered levels or loss of function of alarmins can cause immune modulation at the time of implantation. We created an excess of HMGB1 in the uterine fluid by intrauterine administration of recombinant HMGB1 (rHMGB1) in the uterine horns of pregnant rats on day 2.5 post coitum (p.c.) to determine whether excess of rHMGB1 in the uterine milieu alters the uterine immune cell repertoire during implantation, regulatory T cells (Tregs) from the uterine draining para-aortic lymph nodes (PALN). A trend ($p = 0.0592$) towards higher frequency of Treg cells (ratio of $CD4^+FOXP3^+CD25^+$ to total $CD4^+$ cells) was observed in the PALN of rHMGB1-treated animals as compared to that of sham control. Furthermore, a higher frequency of pan macrophage marker $CD68^+$ cells at the implantation site was observed in HMGB1-treated animals. However, the number of anti-inflammatory M2 macrophages (cells co-expressing $CD68$ and $CD163$) were found to be lower at the implantation sites of HMGB1-treated animals as compared to sham control animals. Flow cytometric analyses of uterine macrophages revealed reduced number of activated M2 macrophages ($CD163^+CD86^+MHCII^+$) in the endometrium of HMGB1-treated animals. Thus, these results suggest that excess of HMGB1 in the uterine microenvironment leads to altered immune profile during implantation, which may contribute to implantation failure. Experiments will be performed to determine whether reduced levels of HMGB1 also affect implantation and alter immune profile. These studies may help us understand the role of alarmins in implantation and implantation failure.

1.16 Identification and Preparation of Radiolabeled Modulator of hFSHR for Therapeutic Applications

Systematic review and data extraction of 161 experimentally validated FSHR LMW modulators from published literature. Using molecular docking experiments, the potential FSHR binding sites of these modulators were identified and these sites were then mapped to the FSHR modulating activity documented for the binders. Six novel peptidomimetics with potential FSHR modulating activity were identified and validated by measuring cAMP levels in HEK-rFSHR cells. Structural analysis of docked complexes revealed that all six peptidomimetics displayed H-bond interactions with Asp521 of FSHR.

2. MALE INFERTILITY AND ASSOCIATED REPRODUCTIVE DISORDERS

2.1 Functional Significance of Testis Specific Histone H2B Variant (TH2B) in Spermatozoa and Early Embryonic Development

Sperm retained histones and their variants are some of the significant epigenomic contributions of the father to the embryo. Two such histone variants, TH2A and TH2B, escape spermiogenic nuclear remodeling and are retained in the mature sperm. Importance of TH2A and TH2B in histone eviction during spermiogenesis is well acknowledged. However, their significance post spermatogenesis is not clear. We previously identified the genomic loci associated with TH2B in human sperm. In the reporting year, we analyzed the genes associated with TH2A(TAAGs) and TH2B(TBAGs) in mature mouse sperm. We observed a degree of conservation between human and mouse TBAGs that included genes crucial for embryo development. Additionally, we found that although TH2A and TH2B share the same bidirectional promoter, their genomic distributions are very diverse. TH2B is enriched at genomic coordinates important for spindle assembly and meiosis specific genes and embryo development was the most significant term amongst TBAGs. TH2A was highly enriched with mitochondrial function or mitochondrial encoded genes.

2.2 Microtubule (Mt) Dynamics and Sperm Function: Involvement of Tubulin Acetylation/Deacetylation

This study highlights the role of Microtubule (MT) acetylation-deacetylation and microtubule associated proteins (MAPs) in sperm motility. We investigated α -tubulin polymerization in rat- and human sperm flagella and its influence on sperm motility, using HDAC6 inhibitor- Tubastatin A and a depolymerizing agent, Nocodazole. We observed that Tubastatin A neutralized motility debilitating/depolymerizing effect of Nocodazole when incubated in combination. We also observed that HDAC6 demonstrated MAP-like behavior. In asthenozoospermic men, the levels of polymerized axoneme, MT stabilizing protein – SAXO1 and expression as well as activity of HDAC6, were reduced. Mass spectrometry analysis of MAP fractions - and HDAC6 interacting protein in human sperm, revealed ‘Tubulin complex’ and sperm anomalies like ‘Asthenozoospermia’, respectively, as significant terms by Gene Ontology (GO). In addition to MAPs, human homologs of a few MT inner proteins were identified. LFQ quantification identified the proteins differentially expressed in sperm of normozoospermic and asthenozoospermic men.

2.3 Unravelling Sperm Epigenetic Landscape Regulated by Estrogen Receptors in Adult Male Rats

Since several endocrine-disrupting compounds encountered in the environment are estrogenic in nature and are known to cause adverse effects on the reproductive health, it is important to understand the role of estrogen receptors in modulating the sperm epigenome and subsequently male fertility. The project aimed to elucidate changes in the sperm DNA methylation patterns after selective estrogen receptor agonist treatments in adult male rats, which could contribute towards decrease in fertility observed after these treatments. Whole genome bisulfite sequencing was initiated to decipher the DNA methylation changes in the sperm epigenome. Genomic feature distribution revealed that majority of the differentially methylated CpG sites lie within the intergenic regions, followed by intronic regions.

Small percentage of these sites were also located in the -10kb upstream region from the TSS, promoter regions, exon and 3' end regions of the genes. Further data analysis is ongoing.

2.4 Unravelling the Sperm Epigenetic Landscape in Infertile Men with Clinical Varicocele

Varicocele is one of the leading causes of male infertility. Elevated oxidative stress has been implicated as a key factor in varicocele associated male infertility. High oxidative stress during spermatogenesis may affect sperm mitochondrial functions and lead to epigenetic modifications in spermatozoa. Our present study suggests that infertile men with varicocele have significantly lower sperm count, motility, intact morphology and viability compared to healthy fertile control. Varicocele group shows a significantly lower mitochondrial membrane potential (MMP), high mitochondria copy number and higher intracellular reactive oxygen species than healthy fertile controls. Increased Sperm DNA fragmentation and low testosterone level were also detected in varicocele group. The methylation level of genes involved in mitochondrial functions such as UQCRC1, MIC60 and LETM1 was found to be altered in men with varicocele. Therefore, decreased MMP, high mitochondria copy number, increased iROS and altered methylation levels of mitochondrial genes indicate sperm mitochondrial dysfunction in men with varicocele. Further studies are underway to investigate the methylation landscape in spermatozoa of men with varicocele.

2.5 Development and Validation of a PCR Based Technology for Detection of Yq Microdeletions in the Diagnosis of Male Infertility

Herein we aim to develop and validate an assay for the detection of Yq microdeletions, as a major genetic cause of male infertility. Using previous datasets, we developed a multiplex PCR using 16 STS markers that can detect Yq microdeletions. Reproducibility, sensitivity and specificity were tested. The technology was transferred to the industry partner through ICMR. The assay will be validated at different centers across the country for its sensitivity and specificity.

2.6 Understanding the Molecular Mechanisms of Gonadal Development in the Mouse

The present study aims to understand the involvement of Lim homeobox gene Lhx2 in the XX and XY gonads. For the first time, we reported that Lhx2 is expressed by the germ cells in the developing ovaries. Using mice that lack Lhx2, we identified that in the developing XX gonads there is ectopic vascularization in the XX gonads. This was due to a disruption in the expression of angiogenesis-related genes. Our study highlights the role of germ cell suppression of vascularization of developing ovaries. Currently, the roles of Lhx2 in the developing male gonads are being explored.

3. RTI/ STIs/HIV/ MICROBICIDES

3.1 Development of a Multistrain Probiotic Lactobacillus Formulation Effective against Reproductive Tract Infections

We investigated 100 vaginal *Lactobacillus* isolates for their ability to produce exopolysaccharides. Two *Lactobacillus* isolates were shortlisted based on their ability to produce exopolysaccharides and biofilms. These two *Lactobacillus* isolates, identified as *L. salivarius* and *L. reuteri*, inhibited the growth of urogenital pathogens including *Candida*. The purified exopolysaccharides also exhibited anti-bacterial, anti-fungal and anti-cancer activities *in vitro*.

3.2 In-vitro Evaluation of Vaginal Lactobacilli Isolated from Indian Women for Potential Anti-Cervical Cancer Activity and Elucidating its Possible Mechanism of Action

Four *Lactobacillus* isolates among 327 vaginal isolates were identified to have potent anti-proliferative activities on HPV 16 and 18. The inhibitory effect of their metabolites on *in vitro* migratory ability of the cervical cancer cells (HeLa and SiHa cell lines) was observed. Further, antimicrobial susceptibility testing of the *Lactobacillus* isolates revealed that they were resistant to eight antifungals and seven antibiotics mainly comprising of quinolones and aminoglycosides. A few *Lactobacillus* isolates were subjected to whole-genome sequencing analysis which indicated differences in genome size, GC%, SNPs, INDELs, CRISPR cas genes, exopolysaccharide biosynthesis genes, presence of plasmids and phages. Comparative genomics analyses provided an insight into the characteristics of the *Lactobacillus* species which may help them adapt to the vaginal environment. The results revealed inter-species differences in numerous protein families suggesting that each species has experienced lineage-specific gene gain and loss to develop unique repertoire of protein families.

3.3 Role of *Gardnerella vaginalis* Membrane Vesicles in Pathogenesis of Bacterial Vaginosis

Gardnerella vaginalis is the predominant anaerobe associated with bacterial vaginosis. Our previous work demonstrated the cytotoxic potential of membrane vesicles (MVs) produced by *G. vaginalis*, towards vaginal epithelial cells. In continuation with these observations, studies on the effect of *G. vaginalis* MVs on epithelial cells lining other regions of the female reproductive tract such as the cervix (HeLa) and endometrium (HEC-1A) revealed significant variations. The extent of MV induced cytotoxicity was maximum in HEC-1A cells and least in HeLa cells. Likewise, induction of the pro-inflammatory cytokine, IL-8 was highest in HEC-1A and least in HeLa cells. The results thus suggest the possibility of *G. vaginalis* MVs induced pathogenesis in a distal location such as the endometrium, which is also the site of occurrence of polymicrobial biofilms containing *G. vaginalis*.

3.4 Molecular Characterization and Antifungal Resistance of *Candida glabrata*

A total of 19 *Candida glabrata* strains were retrieved from previously obtained vaginal isolates. Antifungal susceptibility testing was performed for four azole and two echinocandin drugs. Selective PCR amplification of six *Candida glabrata* housekeeping genes (FKS, LEU2, NMT1, TRP1, UGP1 and UR) was carried out followed by sequencing of the amplicons. Multi-locus sequence typing (MLST) of the isolates and characterization of the azole resistance genes is in progress to examine the correlation with the observed phenotypic resistance.

3.5 Three Dimensions of *Mycoplasma genitalium* Infection - Detection, Cure Rate and Co-Infections in Women Attending STI Clinics

This project is joint collaboration with Brihanmumbai Municipal Corporation, MDACS and Regional RTI/STI centre, Nair Hospital, Mumbai. The project focused on emerging STI pathogen *Mycoplasma genitalium*, its detection rate and coinfections among women clinically diagnosed with lower genital tract infections and attending STI Clinic, Nagpada. Total 193 women have been recruited and the prevalence of *Mycoplasma genitalium* was 7.2%. The mean age of the recruited women was 32.9±5.7 years, 84.8% have multiple sexual partners, 91.7% belong to the upper lower strata of socioeconomic class and 53.9% were using public or common toilet facility. Among coinfections, *Neisseria gonorrhea* was detected in 3.5% (n=8), *Chlamydia trachomatis* in 2.2% (n=5) and *Trichomonas vaginalis* in 1.31% (n=3).

3.6 Longitudinal Cohort Study to Evaluate the Effect of Various Contraception Methods on the Composition and Diversity of the Vaginal Microbiota

This project is a joint collaboration with Nowrosjee Wadia Maternity Hospital to map the baseline vaginal microbial (VMB) composition of women attending a community clinic in Mumbai and to map the changes in vaginal health status before and after using various contraception methods by diverse methods. Healthy sexually active women in the age group of 18-45 years visiting a community clinic in Mumbai for their contraceptive needs are being recruited as a part of a longitudinal contraception cohort study. A cohort of 79 women using various contraceptive methods: barrier, intrauterine devices, injectable contraception, LNG IUCD and control group have been formed and followed up. Evaluation of vaginal microbiome profile showed that the most abundant at phylum and class level were *Firmicutes* (60.8%) and *Bacilli* (55%). The predominant genus was *Lactobacillus* (51.10%) and at species level maximum were *Lactobacillus iners* (34.3%). Women with bacterial vaginosis had significantly higher abundance of *anaerococcus*, *peptoniphillus* and *Dialister*. There is evidence of restoration of VMB after 12 months of childbirth with significant decrease in *Bifidobacterium*. No specific genus showed up statistically with respect to age, menstrual cycle and sexual and hygienic practices.

3.7 Highly Dampened HIV-specific Cytolytic T cell Responses Define Viremic Nonprogression

The study aims to identify gut trafficking potential and virus specific functional attributes that could underlie the paradoxical virus-host equilibrium observed in Viremic Non Progressors (VNPs), a distinct group of HIV-1 infected individuals who remain asymptomatic for several years (>7 years) and have good preservation of CD4 count without ART treatment but display high viral replication. We show that Gag-specific IFN- γ response in CD4+ central memory (CM) compartment associates with viremic control while dampened Env-specific CD107a response in CD4+ effector memory (EM) characterizes VNPs. In CD8 compartment, VNPs tend to maintain lower proportion of Env-specific CD107a response while keeping higher proportion of MIP-1 β response similar to putative progressors (PuPs). Cytolytic activity is primarily limited to polyfunctional cells in Viremic Non-Progressors. Reduced frequency of $\alpha 4\beta 7$ expressing (gut homing) CD8 memory subsets suggested a reduced

gut pathology in VNPs. HIV-specific T cell responses in Viremic Non-progressors are modulated towards a dominant non-cytotoxic response enriched for MIP-1 β production with concomitantly dampened degranulation ability. Understanding the complex mechanism underlying protection in VNPs may allow immunotherapeutic interventions to achieve functional cures in the context of ART resistance.

3.8 Immune and Microbiome Correlates of TB Reactivation in PLHIV and a NHP Model

India bears a high burden of cases of TB/HIV coinfections. The present study seeks to elucidate both innate as well as adaptive immune correlates for their utility as prognostic markers for TB reactivation following HIV infection in tertiary health care setting based in Mumbai, India. Immunophenotyping of 101 participants was completed by flowcytometry. For 87 participants, IGRA reports were generated and shared with the clinical collaborators. Frequency of type 3 innate lymphoid cells (ILC3) was found to be significantly decreased in HIV+ LTBI+ and HIV+ LTBI- groups compared to both HIV-LTBI+ and HIV-LTBI- groups. A rebound in ILC3 frequency was observed in 6 months post ART follow up samples of HIV+LTBI+ individuals. ILC3 frequencies showed a significant positive correlation with absolute CD4 count in all study groups, though no significant correlation was observed with the viral load. Reduction of circulating ILC3s frequency in LTBI+ individuals with HIV may alter maintenance of protective lymphoid follicles within granulomas leading to an increased risk for latent TB reactivation. Increase in ILC3 frequency after 6 months of ART is indicative of restoration of gut barrier integrity and thereby reducing possibility of latent TB reactivation.

3.9 Harnessing T Cell Exhaustion for the Treatment of Immunopathology Associated with Excessive Immune Response to Inflammatory Agents

Infection with emerging or re-emerging pathogens results in self-injury and immunopathology due to excessive immune reaction. Available immunosuppressive drugs have undesired side effects and are unsafe during pregnancy. We investigated the effect of different exhaustion marker agonists (rTIM-3, rCTLA-4 and rPD-1) in reducing the expression of activation markers (CD25 and HLADR) on the surface of T cell subsets (CD4+, CD8+ and $\gamma\delta$ + T cells. A549 lung epithelial cell line was stimulated with synthetic viral mimic-Poly I:C and subsequently co-cultured with peripheral blood mononuclear cells (PBMCs). Treatment of PBMCs with combination of rTIM-3 and rCTLA-4 significantly decreases the expression of activation marker-HLA-DR on the surface of CD3+CD4+, CD3+CD8+ and CD3+ $\gamma\delta$ + T cells. In the same group, a significant upregulated expression of exhaustion marker-Tim-3 on the surface of these T cell subsets was also observed. We have also found poly I:C induced IFN- γ production from A549 cells.

3.10 Identification and Validation of Potential Drug and Drug-Targets for Infectious Diseases Using *in Silico* and Wet Lab Methods

Validation was done of two novel aspartyl protease inhibitor peptidomimetics that demonstrated higher inhibitory effects against *Candida* spp. in combination with fluconazole as compared to fluconazole alone. By *in vitro* assays identification and validation of two repurposed drugs showing potent antifungal activity was done.

Screening and identification of novel peptidomimetics was carried out against *Candida* SAP2, an important virulent factor, using docking and MD simulation studies.

4. MATERNAL AND CHILD HEALTH

4.1 Enhancing Male Participation in Interventions to Prevent Unintended Pregnancy

Gender equity in family planning interventions is posited to be an important means of supporting women's reproductive decision well as to improve modern contraceptive use. There is a need to educate males too on family planning methods, marital communication and joint decision making in family building. The CHARM2 intervention involves five sessions of family planning and gender equity counseling, two gender synchronized sessions delivered separately to husbands and wives by sex-matched health providers and a final session for the couple provided by either the male or female provider who delivers the individual sessions for the couple, whomever is available. Overall, the findings suggest that the intervention supports joint contraceptive decision-making, contraceptive use, marital communication over time in short-term effect and increased male engagement.

4.2 Understanding the Molecular Basis of Embryo Implantation

In this project, we aim to investigate the fundamental mechanisms by which embryos breach the endometrial epithelium and the control of decidualization of stromal cells. We observed that at the site of implantation, in the luminal epithelial cells there is a switch in the expression of markers associated with epithelial to mesenchymal transition (EMT). We observed that this EMT occurred due to the loss of HOXA10 and the activation of NLRP3. Mice treated with NLRP3 inhibitor had implantation failure and the luminal epithelial cells did not undergo EMT. Further, decidualization was also compromised in mice treated with NLRP3 inhibitor. We are currently dissecting the molecular targets of HOXA10 in endometrium and investigating how NLRP3 mediated inflammasomes control decidualization.

4.3 Genetic and Epigenetic Changes in Imprinted Genes in Male Partners of Women Experiencing Recurrent Spontaneous Abortions

The project aimed to elucidate the paternal epigenetic factors that could contribute to idiopathic recurrent spontaneous abortions (RSA). We found aberrant DNA methylation patterns in several imprinted genes involved in embryo development in spermatozoa of male partners in couples experiencing unexplained RSA. In order to determine whether the methylation levels of these imprinted genes can be used as a diagnostic marker to identify epigenetically abnormal spermatozoa, we used multiple logistic regression and combined the average methylation levels of five differentially methylated genes into a probability score (between 0-1). A threshold of 0.61 was set for the probability score with a specificity of 91.78% and sensitivity of 65%. This was further experimentally validated in an independent cohort of 38 control and 45 RSA spermatozoa samples. The validation results revealed that 97.36% of control samples were correctly classified below the threshold and a subset of RSA cases (40%) had abnormal methylation levels. We propose that the combined DNA methylation levels of these imprinted genes can be used as a diagnostic tool to identify

spermatozoa samples with epigenetic defects, which could contribute to the pathophysiology of RSA.

4.4 Idiopathic Recurrent Pregnancy Loss: Possible Association of Paternal Exposure to Endocrine Disruptors and Epigenetic Modifications in Sperm

Endocrine disruptors are known to affect sperm epigenome. Investigations are underway to explore the possible association between paternal exposure to endocrine disruptors and sperm epigenetic modifications of iRPL couples. Whole Genome Bisulfite Sequencing of sperm DNA samples was used to identify differentially methylated CpG (DMC) sites in sperm of iRPL cases compared to fertile control sperm. A total of 9497 DMCs with highest enrichment in intronic regions were obtained and 2087 differentially methylated genes were noted. DMCs within genes relevant to embryo and placenta development were selected to further validate their methylation levels in the study population by pyrosequencing. DMCs within genes *PPARG*, *KCNQ1*, *SETD2* and *MAP3K4* showed hypomethylated sub-populations within iRPL study population. Our study highlights the altered methylation landscape of iRPL sperm and their possible implications in embryo and placental development. The CpG sites that are hypomethylated specifically in the sperm of iRPL sub-population can be further assessed as predictive biomarkers.

4.5 Understanding the Role of Cannabinoid Receptors in Placental Calcium Transport

Placental calcification commonly increases with gestational age and becomes apparent after 36 weeks' gestation. The preterm placental calcification may hinder the uteroplacental blood flow and adverse fetal outcome. The gestation day 18.5 placentae were collected from gravid C57BL/6 females for explant culture and treated with cannabinoid receptor 1 (Cnr1) agonist N-arachidonyldopamine (NADA) or antagonist (AM251). To study the calcium deposition, the placentae were cultured with or without CaCl_2 in the presence of NADA or AM251. The expression of cannabinoid receptors and calcium transporters was upregulated. Alizarin Red S staining in placental tissues done to assess calcium deposit was found increased in Cnr1 agonist treated placental tissues. The findings suggest that Cnr1 may contribute towards placental calcification.

4.6 Detection of Infections Associated with Preterm Births

The aim of this study is to develop and validate a multiplex PCR assay for the detection of common bacterial species that are associated with preterm births. We shortlisted eight bacteria that are commonly found in the vagina of women with preterm delivery and a multiplex PCR is optimized. We are currently prospectively testing high vagina swabs of women who delivered preterm and term for validating the assay.

4.7 Maternal Near Miss Review and Corrective Measures at District and Women's Hospitals in Maharashtra

Government of India released operational guidelines for Maternal Near Miss - Review (MNM-R) in December 2014 designed for use by program managers at different levels of public health system. This information is used to bring health

system improvement aimed at reduction of maternal morbidity and mortality. MNM-R is complementary to Maternal Death Review. The objective was to implement these MNM-R guidelines developed by Government of India and take corrective measures at the District and Women's hospitals (Nasik, Parbhani, Ratnagiri, Akola and Nanded) in Maharashtra. The total number of MNM cases identified and forms filled in software at all District/Women's Hospital during April 2018-March 2022 were 460. Out of these, hemorrhage (64.6%) was the most common adverse event followed by hypertension (23%). MNM meetings were conducted at all the selected district/women's hospitals. After meeting, minutes were prepared, corrective measures were recommended based on three-delay model by the MNM Committee and corrective measures were taken by these hospitals accordingly. The findings of the District MNM study suggest that it is feasible to implement MNM-R guidelines at the district hospitals. However, there is need to revise the guidelines with respect to MNM facility based form and critical review of the criteria. Challenges at this level are lack of dedicated staff for this activity, inadequately filled case sheets leading to difficulty in identifying MNM cases, lower case load of MNM as many cases are referred out leading to lack of a separate committee for MNM. The study findings have been communicated to district and state government. These will be useful for implementation of the MNM-R guidelines of Government of India at the district hospitals in the country.

4.8 Implementation of Dakshata Program to Improve Quality of Institutional Deliveries in a Tribal Block of Maharashtra

Dakshata program aims to reduce maternal deaths resulting due to sub-optimal care provided during labour and delivery by providing competency based training to health providers. Despite a rise in institutional deliveries, districts with substantial tribal population still record higher maternal mortality. Objective of the present study was to document the enabling factors and challenges affecting implementation of Dakshata program at four government facilities with high delivery load - Dahanu, Jawhar, Kasa and Wada in Palghar district of Maharashtra. Assessment was carried out using checklists from program guidelines. Status of human and material resource, availability of supplies, adherence of staff to critical practices and compliance to standard protocols was recorded. To understand system bottlenecks, 17 key informant interviews were conducted. Shortage of staff and unavailability of specialist doctors makes management of obstetric complications difficult. Majority of the staff was untrained. Utilization of Safe Childbirth Checklist was poor. Supply of essentials was affected by poor coordination between district and state. Support and handholding from health authorities was required and maintaining records and data was deemed a secondary priority. Findings were disseminated to state health and maternal health care providers by a dissemination meeting. Strategies encouraging coherence between various levels of healthcare must be promoted for effective implementation of the Dakshata program to improve quality of care in the underserved tribal areas. A larger implementation study to address the gaps identified in the formative research is planned in the selected tribal area.

4.9 Population Based Birth Defect (BD) Surveillance in Linkage with Rashtriya Bal Swasthya Karyakram (RBSK) Programme in Rural Blocks of Palghar District in Maharashtra

This ongoing surveillance project is being implemented at MRHRU Dahanu to estimate prevalence of Birth Defects (BDs) in children upto 6 weeks age in seven rural blocks of Palghar district. At present, there is no database on BD except for data collected from screening done through RBSK Mobile Health teams. Therefore, the project has been linked with RBSK to utilize existing resources and in turn strengthen the BD component of RBSK. Through this project, a BD registry will be developed and maintained at PHC, RH and SDH. To avoid missed diagnosis at community level, trainings are being conducted for ASHAs to identify BDs during home based neonatal care (HBNC) visits. For training purpose, IEC materials like information booklet, flyer and video have been developed. A total of 1905 ASHAs were trained in total 38 PHCs of Palghar district. A total of 117 birth defects were registered at facility level. Thirty nine cases of birth defects were reported by ASHAs through Block Facilitators till March 2022. Birth defects were also verified through RBSK compiled data. From April 2021 to March 2022 - 67 birth defects were reported through RBSK. Total birth defect cases (facility, ASHAs, RBSK) from April 2021 to March 2022 after removing of duplicates was 155. Data analysis was conducted for the first quarter of data collection to measure prevalence, types, age and sex wise distribution of birth defects. Among the visible birth defects, cleft lip/ palate followed by clubfoot were commonly reported.

4.10 Development of a Non-Invasive Evaluation Protocol for Detection of Congenital Cytomegalovirus Infection in Newborns

Newborn screening is important for early diagnosis and management of congenital infections such as cytomegalovirus (CMV) infection, a leading cause of childhood mortality and morbidity. A hospital-based study was conducted for detection of CMV infection in 500 neonates by PCR, using a non-invasive sampling strategy. The results revealed that use of multiple, non-invasive samples such as saliva, urine and stool resulted in higher birth prevalence (16%) compared to that with only saliva samples (1.6%). Validation of this approach in studies with larger sample size will aid its eventual implementation in national programs aimed at comprehensive newborn healthcare management.

4.11 Assessment of Neonatal Screening Approaches for Sickle Cell Disease and the Effect of Early Intervention in Management of the Disease in Tribal Populations: Research cum Intervention Study

This ICMR Task Force study is implemented through seven centers across the country and NIRRCH is one of the centres. The study is being conducted at Dahanu block of Palghar District (SDH-Subdistrict Hospital, Dahanu and Kasa, PHCs). Sickle cell disease has the highest prevalence amongst tribal ethnic groups with an increased risk for severe morbidity during first 3 years of life. Currently, there is no national neonatal screening programme in India and children are identified only when they become symptomatic. The present study is undertaken to understand the magnitude of the sickle cell disease and to understand the barriers for undertaking such programme and to measure the benefit of early comprehensive care of affected babies.

It also aims to evaluate the genotypic and phenotypic correlation to understand role of genetic modifiers for disease severity. From April 1, 2021 to March 31, 2022, 3438 deliveries were conducted at both sites, out of which 2087 samples were screened by HPLC. A total of 172 (8.24%) heterozygous babies were detected. Ten (0.48%) babies were detected to have disease. Out of these, seven were found to have Sick Cell disease and three were HbS - Beta Thal Compound heterozygous. The babies are being followed up at 6 weeks for confirmation of diagnosis and for screening of family members as per the protocol followed by genetic counselling. Sick cell homozygous babies are started on pneumococcal vaccinations and prophylactic antibiotics from 3 month as per the standard of care and are being followed up to 3 years of life.

4.12 Community-based Screening and Management of Latent TB among Under-Five Children from Urban Slums in Mumbai

The diagnosis and treatment of Latent TB infection (LTBI) forms an important aspect of WHO's End TB Strategy. The present study is a community based study in urban slums in collaboration with a tertiary hospital and health post (MCGM), aiming to screen under-five children at risk for LTBI as per WHO guidelines. The study also aims to assess acceptability of testing and adherence to treatment of LTBI among parents. Total 369 eligible children have been enrolled and screened for LTBI using Tuberculin Skin Test (TST), CBC and IGRA by QuantiFERON-TB Gold Plus. It was observed that total number of IGRA positives was 46 (12.46%), out of which 39 (10.56%) were TST positives and 7 (1.89%) were TST negative. Total TST positives accounted to 79 (21.40%) and 39 (10.56%) were TST negative. IGRA results were indeterminate among 26 (7.04%) children. Active tuberculosis was ruled out amongst children suspected for LTBI. Children with LTBI completed their INH prophylaxis at BJ Wadia Hospital-TB Clinic as per the guidelines. Children with LTBI are being followed up at the Clinic as per the protocol.

4.13 Exploring Clinical and Therapeutic Relevance of Novel Biomarkers among the Children Presenting with Idiopathic and Incomplete Precocious Puberty at Tertiary Hospital, Mumbai

Precocious Puberty (PP) comprises of central, peripheral or incomplete variants. Central PP is most common and idiopathic central precocious puberty (ICPP) contributes to almost 90% in girls. Precocious Puberty is mainly associated with adverse outcomes such as risk of short stature, PCOS, Diabetes mellitus-1, metabolic disorders in later life and most detrimental effect is psychosocial stress. Currently ICPP poses significant diagnostic and therapeutic challenge. Few studies have shown that Kisspeptin, Neurokinin B, Neuropeptide Y and MKRN3 are involved in the PP pathophysiology. This project seeks to explore the clinical and therapeutic relevance of these potential novel biomarkers. The study will evaluate whether altered levels of these markers contribute to ICPP and incomplete PP. So far, 153 girls have been screened, out of which 36 girls have been enrolled as controls, four cases as ICPP and four as early puberty (PT). Circulating serum/plasma levels of Kisspeptin, Neurokinin B, Neuropeptide Y and MKRN3 have been evaluated from these study participants. Kisspeptin and Neuropeptide Y levels were marginally higher in ICPP as compared to controls. There was no difference in the circulating levels of

Neuropeptide Y in ICPP and in PT cases. Validation and standardization of kits for the estimation of Neurokinin B and MKRN3 is ongoing.

4.14 Role of Kisspeptin Mediated Signaling in Onset of Puberty

Inactivating mutations in the neuropeptide kisspeptin-1 (KISS1) or its cognate G-protein coupled receptor, the kisspeptin-1 receptor (KISS1R) can lead to hypogonadotropic hypogonadism (HH) or delayed puberty whereas activating mutations can cause precocious or early puberty. Identification and characterization of mutations in KISS1/KISS1R is therefore important in order to gain insights into the pathophysiology of pubertal disorders. Four naturally occurring heterozygous mutations R³⁸P, P⁴⁶Q, S¹²⁵L and R¹⁹⁸G in KISS1R, reported in HH, were generated *in vitro* using site directed mutagenesis. As compared to Wild type (WT) KISS1R, all the four mutant receptors exhibited low cell surface receptor expression. Consequently, KISS1 stimulated inositol phosphate production was also lower in cells expressing the mutant receptors as compared to those expressing WT KISS1R. Thus, these mutations caused an impairment in receptor function.

4.15 A Study on Depressive symptoms in Lower and Middle Socio-Economic Status Urban Post-Menopausal women with Osteoporosis and its Effect on Quality of Life

A pilot study was conducted to study the association of depressive symptoms among lower and middle-income urban post-menopausal osteoporotic women (50-75 years) and their quality of life. Women (n=100) were screened for depressive symptoms by Patient Health Questionnaire-9 (PHQ-9) and Quality of Life by using WHO-QOL BREF-26 questionnaire was undertaken. The preliminary analysis revealed depressive symptoms as follows - mild depressive symptoms (17%), moderate depressive symptoms (4%) and moderately severe depression (1%) with 78% of women having no symptoms of depression. Significant association of depressive symptoms with physical domain (61±13 SD v/s 86±11 SD), psychological domain (74±16 SD v/s 85±10 SD) was observed. Association of depressive symptoms with physical domain can be explained as 78% women had various musculoskeletal problems, which can affect the quality of life among the menopausal osteoporotic women.

5. GENETIC RESEARCH CENTER

5.1 Building and Analyzing Gene Network associated with Polycystic Ovary Syndrome

PCOS is one of the most common causes of anovulatory infertility. While several genes have been reported to be associated with PCOS, the genetic etiology is incomplete and not well understood. Network analysis and gene prioritization are powerful *insilico* approaches for the identification of novel candidate genes. Approximately, 2000 probable candidate genes expressed in the ovary were predicted to be associated with PCOS. The study also found ~ 300 genes encoding secretory proteins among the 2000 predicted candidates. Coagulation and complement cascade was identified as the most prominent pathway among the predicted secretory proteins

to be associated with PCOS. These predicted candidates are a useful resource that can be explored experimentally for their association with PCOS.

5.2 Identification and Characterization of Genetic Factors Associated with Multiple Morphological Abnormalities of Sperm Flagella

Male infertility can result either due to quantitative or qualitative defects of spermatogenesis. In recent years, morphological (motility and flagellar defects) are more commonly defined as a new syndromic phenotype characterized by multiple morphological abnormalities of the sperm flagella (MMAF). Individuals with MMAF show sperm morphology abnormalities, aberrant flagellar phenotypes (no flagella, short flagella, coiled flagella, bent flagella and/or irregular flagella) and seriously impaired sperm motility. Standards for the evaluation of MMAF differs from other conditions of teratozoospermia and asthenozoospermia (such as primary mitochondrial sheath defects and primary ciliary dyskinesia) and is primarily characterized by axonemal defects. Genomic studies of the MMAF phenotype in the past five years had shown that MMAF is a genetically heterogenous condition and ~18 genes were identified to induce MMAF and thus male infertility. However, the frequency of MMAF phenotype and the underlying genetic cause of MMAF among Indian infertile men is unknown. Hence, this study is primarily aimed at the identification and characterization of genetic causes associated with MMAF in infertile men from India. Three patients have been recruited till date.

5.3 Comprehensive Genetic Evaluation of Fetus in Antenatally Detected Abnormal Pregnancies with Fetal Malformations: Outcomes, Benefits and Limitations - a Pilot Study

Fetal malformations are the major causes of recurrent spontaneous abortions. Chromosomal abnormalities, microdeletion/duplication and single gene defects are the major causal agents. Hence, genetic evaluation of abortus material/fetus is very important. The study aims to identify genetic mutations associated with fetal malformations in an Indian cohort, evaluate the efficacy of the available genetic platforms and use the data generated for effective genetic diagnosis and possible management options. Karyotype analysis of the POC samples of twelve malformed fetuses identified Down's Syndrome (Trisomy 21) as one of the causes of fetal malformation.

5.4 Identification and Evaluation of Novel Metabolites with the Potential of Prenatal Diagnosis of Fetal Congenital Heart Diseases

This project proposes to perform untargeted metabolomics using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and nuclear magnetic resonance (NMR)-mass spectroscopy (MS) to measure all detectable metabolites present in different biological samples collected from women with congenital heart diseases (CHD) fetus (n=75) and age-matched healthy pregnant women with healthy fetus (n=75) in 11-24 weeks of gestation. The study may provide novel insights into the metabolomics of CHD, as the Indian context varies with unique dietary habits and geoethnic risk factors. The study is translatable and has therapeutic potential in the diagnosis and treatment of CHD. This may also help in conducting prenatal

counseling, timely obstetric management and postpartum diagnosis. The ethical approvals, recruitment of staff and design of the work plan are in process.

6. STEM CELL BIOLOGY

6.1 Functional Study of Voltage-Gated Calcium Channel Gene Mutations in Schizophrenia using Induced Pluripotent Stem Cells (iPSCs): A New Approach for Developing a Cellular Model

Schizophrenia is a complex neuropsychiatry disorder in which patients loses touch with reality. Genetic etiology of this disorder is not well established, however, the heritability as high as 80% makes it most heritable among common neuropsychiatric disorders. The aim of the project is to find to identify candidate genes to elucidate the genetics of Schizophrenia and further modelling it through iPSCs. Whole exome sequencing analysis revealed involvement of 6 coding variants in 5 genes. Among these variants, PIGQ gene has two pathogenic variants, one nonsense NP_683721.1:p.W634X and the other in-frame deletion NP_683721.1:p.E74_F80del. Four variants are found to be missense and their pathogenicity have been analysed using in-silico approach. Two missense variants (NP_065743.2:p.E377G in GALNT16 and NP_055383.1:p.P641S in GALNT5) have variable damaging score, however, the other two variants namely NP_891550.1:p.T957P in ADAMTS9 and NP_001036009.1:p.Y1500C in LTBP4 have highest damaging score. Further analysis has showed that the variant NP_891550.1:p.T957P in ADAMTS9 gene was found to be significant and was present in the thrombospondin repeat motif, one of the important motif of ADAMTS9 protein. Detailed molecular dynamics (MD) simulation study on this variant showed damaging effect on structural stability of the ADAMTS9 protein.

6.2 Genetic and Biochemical Characterization of Mitochondrial Oxidative Phosphorylation (OXPHOS) Disorders in Children

Oxidative phosphorylation (OXPHOS) disorder also known as mitochondrial disorders are a complex family of disorders with a vast array of clinical manifestations. OXPHOS mutations produce disease that affects a variety tissues, organs or organ systems, having more energy demands like muscles, nervous system, brain etc. Due to its clinical heterogeneity, large number of children remained undiagnosed. Moreover, lack of diagnostic facility is also a major limiting factor for confirmatory diagnosis in India. This research study is an attempt to develop a diagnostic method using a combinatorial approach of biochemical and genetic analysis. Muscle biopsy samples were collected for 30 controls and 2 patients. Biochemical enzymatic assays for complex I-IV and citrate synthase (CS) have been carried out in muscle homogenates of control samples; average activities would be used for determining enzyme deficiencies in patient's samples. Upon analysis of two patient samples, one sample found to have complex II deficiencies.

6.3 Investigating the Effects of Neonatal Exposure to Estrogen and DES on Mouse Testicular Stem Cells

We previously demonstrated that testicular dysgenesis syndrome has a stem cell basis and that various pathologies can be reversed by transplanting mesenchymal stromal cells and by treating with XAR (nano-formulation of resveratrol). In the

reporting year, detailed studies were undertaken to understand how testicular cancer initiates upon DES treatment. Sixty five percent of DES treated mice developed testicular cancer-like changes associated with increased expression of OCT-4, PCNA, Ki-67 and cancer stem cell marker CD166 along with minimal expression of PTEN, MVH, c-KIT and SOX-9. Excessive expression of OCT-4 in the VSELs initiated testicular cancer. Reduced 5-methyl cytosine expression suggested global hypomethylation and led to increased expression of *Igf-2* and *Dlk-1* along with reduced expression of *H19* and *Meg-3*, which pushed VSELs out of their quiescence and initiate cancer. Transcriptome analysis of FACS sorted SSEA-1 positive VSELs was undertaken by low-input RNAseq and showed dysregulation of transcripts and pathways associated with cancer and stemness after DES treatment.

6.4 Studies on Ovarian Stem Cells (OSCs)

We were able to track various events during oogenesis from very small embryonic like cells (VSELs) across estrus cycle, which leads to oocytes formation and primordial follicle assembly in adult mice ovaries under normal physiological conditions. Further evidence was accumulated in the reporting year to support this by BrdU uptake studies. BrdU positive stem cells differentiated into oocytes and BrdU incorporation was detected in the oocytes present in the primary, secondary and pre-antral follicles within 15 days of BrdU injection. This suggests that follicles form and mature on regular basis in adult ovaries. Our data are suggestive of a possibility that ovarian senescence/menopause occurs because of stem cells dysfunction. VSELs and OSCs exist along with increased numbers of germ cell nest (GCNs) arrested in pre-meiotic or early meiotic stage in aged ovaries and primordial follicle assembly is blocked possibly due to age-related changes in their microenvironment.

6.5 Investigating the Effects of Follicle Stimulating and Steroid Hormones on Uterine Stem Cells

Further evidence to suggest that VSELs are indeed the most primitive and yet elusive stem cells in the uterus was provided in the reporting year. VSELs enriched from GFP mice, upon transplantation in wild type mice showed their differentiation into epithelial cells lining both the lumen and the glands within the same estrus cycle. In addition, upon mechanical scratching, OCT-4 positive stem cells get activated and regenerate the endometrium. Further experiments were undertaken to delineate a possible role of VSELs in the initiation of endometrial cancer. Various 'uteropathies' including non-receptive uterus, hyperplasia, adenomyosis, endometriosis, endometrial cancer and *leiomyomata* are caused due to the dysfunctions of VSELs. We show that dysfunctional VSELs and epithelial progenitors migrate to the myometrium / perimetrium (adenomyosis) or ectopic sites in mice neonatally exposed to endocrine disruption to expand and differentiate into endometriotic tissue.

6.6 Neonatal Exposure to Estrogen and its Effects on Adult Mouse Prostate Stem Cell Biology

Studies have been undertaken to show the presence of VSELs and their characterization in adult mouse prostate. VSELs were found to express follicle stimulating hormone receptor (FSHR). Androgen deprivation by bilateral castration resulted in prostate atrophy, but VSELs survived and increased in numbers. FSH

treatment (5 IU/day for 2 days) resulted in increased numbers of VSELs along with up-regulation of alternately spliced FSHR isoform *Fshr-3* whereas canonical *Fshr-1* was not detected. On the other hand, DHT (50 µg/kg for 7 days) exerted a moderate effect compared to FSH. Prostate differentiation and function as well as prostate cancer growth are believed to be androgen-dependent. Our results presenting VSEL driven regeneration of atrophied prostate by FSH are surprising and question the current clinical practice of orchidectomy to manage prostate cancer patients. Although androgen deprivation results in prostate cancer atrophy but concomitant increased FSH levels are possibly responsible for resurgence and death of patients due to the highly aggressive castration-resistant prostate cancer.

6.7 Evaluating the Role of Hypoxia in Human Embryonic Stem Cells (KIND1) Differentiation towards Trophoblast Lineage

Fetal growth is supported by placenta and failure of proper placental development can compromise embryonic development and lead to complications like miscarriages, stillbirth, pre-term labour, pre-eclampsia and intra-uterine growth restriction. Early development of placental trophoblast cells is poorly studied due to limited sample availability and ethical concerns. Availability of a proper human model is highly warranted to investigate early trophoblast development. We have differentiated in-house derived human embryonic stem cell line (KIND1) into trophoblast lineage. The differentiated cells were characterized by expression of trophoblast specific markers namely, *Gata3*, *Tfap2a*, *Tfap2c*, *Cga* and *Cgb*. Further studies are underway to optimize this protocol to obtain detectable levels of hCG in spent media. We also plan to develop placental organoids to study the effects of hypoxia during differentiation.

7. NATIONAL CENTER FOR PRECLINICAL REPRODUCTIVE AND GENETIC TOXICOLOGY

7.1 An Endocrine Disrupter- Atrazine Induced Epigenetic Regulations in the Male Germ Line Following Perinatal Exposure

Atrazine (ATR), an endocrine disruptor, has been associated with altered endocrine and reproductive functioning in-vivo in animals exposed during the critical window of development. Thus, the present study investigates the effect of ATR exposure on F1-F2 male progeny exposed through gestation and lactation. F0 dams were administered with ATR at 2, 10, 70 and 100 mg/kg b. wt/day from gestation day 6 to postnatal day 21. The F1 male rats were assessed for sexual maturation and fertility on PND75. Delayed testicular descent was observed in 10, 70 and 100 mg/kg b. wt/day ATR dose with significantly lower serum testosterone, sperm count and motility with testicular defects. Expression of Androgen receptor (AR), Estrogen receptors (ER α and ER β), StAR, Aromatase and INSL-3 were upregulated at all doses indicating estrogenic/antiandrogenic activity of ATR. Fertility assessment revealed subfertility in F1 males with high pre- and post-implantation losses at 10, 70 and 100 mg/kg b. wt/day dose as compared to control. Further, F2 fetuses exhibited congenital disabilities viz. decreased weight, crown-rump length and anogenital distance with several other morphological deformities. To conclude, ATR exerted

estrogenic and/or anti-androgenic activity with fetotoxic effects through the male germline.

7.2 Deciphering the Molecular Mechanism of Triclosan on Hypothalamus Pituitary Gonadal Axis

The present study was undertaken to decipher the molecular mechanism of Triclosan (TCS), an antimicrobial agent, on hypothalamus-pituitary-gonadal axis and to investigate the effect of TCS exposure on male reproductive system through epigenetic mechanism. During the reporting year, studies were undertaken to observe the transgenerational effect upon perinatal exposure to TCS at different doses (0.1, 4, 40 and 150 mg/kg b. wt. /day). The F1 male and females from respective groups were mated with naïve female and males respectively. F2 animals were sacrificed at different time intervals. At day 75, the F2 male showed decrease in % motile and % progressive sperm in the treated groups as compared to control groups. There was a significant decrease in daily sperm production as well as organ weight in the treatment group as compared to control group. There was an increase in serum levels of estradiol and a decrease in testosterone in the treatment group as compared to control group.

7.3 Identification and Characterization of Sertoli and Leydig Cell Homing Peptides

Sertoli Cell Homing Peptides (SCHP) and Leydig Cell Homing Peptides (LCHP) were previously identified using phage display peptide library. The molecular target of SCHP2 was initially identified using affinity purification mass spectrometry (APMS). During the reporting period, the target of SCHP1 was identified using the same approach. The results show that the target of SCHP1 is HSPA2, HSPA8 or HSPA9 proteins. Cellular uptake study of the SCHP1 and SCHP2 was performed using TM4 cells and HEK293 cells using confocal microscopy. The results show the uptake of the SCHP1 and SCHP2 by TM4 cells but not by HEK293 cells, which confirms specific homing potential of the SCHP1 and SCHP2 peptides to Sertoli cells

7.4 Exploring the Therapeutic Potential of Peptides Targeting Lysophosphatidic Acid Receptors in Ovarian Cancer

In ovarian cancer, elevated levels of Lysophosphatidic acid (LPA) and aberrant expressions of LPAR 2 and 3 activate the downstream signaling cascades resulting in cell proliferation, invasion and migration causing metastasis. There are no potential drugs available that can target the LPARs in ovarian cancer. Targeting LPAR with LPAR binding peptide may disturb LPA-LPAR interaction and delay ovarian cancer metastasis. The present study aims to identify the peptides targeting LPAR using phage display library approach. In the preliminary study, differential expression of LPARs (1-6) proteins was evaluated in human ovarian cancer cell line (OVCAR3). LPAR3 was found to be overexpressed followed by LPAR2. We selected LPAR3 as a target for the identification of peptide targeting LPAR3 in ovarian cancer. Three rounds of *in vitro* panning was carried out using rLPAR3 as a bait by Ph.D-12 phage display library. Future plan is to deduce the 12 mer peptide sequences displayed by the M13 bacteriophages by Sanger sequencing/NGS. The most frequently occurred

peptide sequence will then be commercially synthesized and validated *in vitro* and *in vivo*.

7.5 Evaluation of Drugs-Cytochrome P450 Enzyme Interaction through Fluorometric High Throughput Screening Assays

Cytochrome P450 superfamily has been implicated as one of the most important drug-metabolizing enzymes because it metabolizes a wide range of pharmaceuticals. In the proposed study, ayurvedic formulations were studied for their herb drug interaction (HDI) against the key marker, human CYP isoforms CYP1A2, CYP2C9, CYP2D6, CYP2E1 and CYP3A4. During the drug development process, it is vital to include preclinical (*in vitro*) and clinical (*in vivo*) interaction studies. So these hundred formulations are validated by *in-vivo* and *in-vitro* studies for their HDI. The structure for 45 active principles of 30 traditional medicinal formulations was downloaded from the PubChem database. The molecular docking of 45 active principles with Cyp450 was performed using the CDOCKER docking optimization tool. A total of 10 conformations were generated for each active principle. Best conformation was selected based on their interaction with key residues (hydrogen bonding and pi-stacking), similar to their respective inhibitors and CDOCKER INTERACTION scores. Ricinoleic acid and Chlorogenic acid are the two main active principles that showed higher interaction scores and interactions with key residues similar to respective inhibitors in all four crystal structures of human cytochrome P50 isozymes.

8. REPRODUCTIVE CANCERS

8.1 PSP94 as an Adjunct Marker for Serum PSA for Differentiating between Benign Prostatic Hyperplasia (BPH) and Prostate Cancer (PCa)

Serum prostate specific antigen (PSA) test is used globally for prostate cancer screening, however its low specificity causes diagnostic dilemma and a number of patients have to undergo unnecessary biopsies. The objective of this study was to assess the utility of PSP94/PSA ratio (PP Index) in minimizing referrals for prostate biopsies in patients having lower urinary tract symptoms and with PSA levels between 4-20 ng/ml. Based on PSA alone, 100% of the participants in the PSA range of 4-20 ng/ml were recommended for biopsy whereas if PP Index with a cut off value of ≤ 5.26 had been used, ~50% of prostate biopsies could have been avoided. At a cut off of ≤ 5.26 , the PP Index had a sensitivity of 100% and specificity of 53.49%. PSP94 test when done in addition to PSA (PP Index) in patients having PSA levels between 4-20 ng/ml, it can better discriminate between prostate cancer and BPH.

8.2 Investigating the Key Elements in Estrogen Signaling and their Contribution to Prostate Cancer

Estrogens elicit a myriad of cellular functions through estrogen receptors (ERs) i.e., ER α / β mediated genomic and non-genomic signaling. Unlike genomic signaling, wherein ERs on binding to estrogen translocate to the nucleus to regulate transcription of genes, non-genomic signaling is initiated at the plasma membrane or extranuclear sites. In addition to ER α / β , GPR30, a non-conventional ER, is also reported to be present on the plasma membrane. We previously demonstrated the presence of ERs

on the plasma membranes of androgen-dependent and independent prostate cancer cell lines. Activation of these receptors using cell-impermeable estradiol led to increased invasion and migration *in vitro*. However, it is not clear whether estradiol induced effects are mediated through the conventional ERs present in plasma membrane or through GPR30. During the reporting year, studies were conducted to identify the role of GPR30 in prostate cancer. The expression of GPR30 was assessed at the transcript level in different prostate epithelial cell lines. GPR30 was found to be high in LNCaP (androgen-dependent cell line) followed by PC3 (androgen-independent cell line), RWPE (normal) and least was expressed in DU145 (androgen-independent cell line). GPR30 knockdown stable PC3 clones demonstrated increased migration and invasion in comparison to empty vector transfected stable clones. Further, *in vivo* tumorigenic ability of GPR30 knockdown clones is being assessed using NODSCID mice.

8.3 Deciphering the Mechanisms of Innate Immune Surveillance in Prostate Cancer for Immunotherapy Salient Achievement - *Ex vivo* Anti-Prostate Tumor Activity of a Recombinant Fragment of Human Surfactant Protein D (rfhSP-D): Induction of Immunogenic Cell Death and Immunomodulation

Surfactant protein D (SP-D) is emerging as a potent anti-tumoral innate immune defense molecule. Both early and late stages of prostate cancer in the transgenic mouse model (TRAMP) showed significantly decreased SP-D transcript and increased proteolytic degradation of SP-D protein suggesting impaired SP-D levels may contribute to PCa progression. Further, these studies provided a rationale to use an elastase inhibitor (Sivelestat) along with the exogenous supplementation with a recombinant fragment of human surfactant protein D (rfhSP-D) to sustain the functional efficacy in the protease enriched tumor micro-environment of murine tumor explants. The *ex vivo* study revealed that the combined therapy has a significant immunomodulatory potential and induced tumor macrophage polarization towards M1 and immunogenic cell death (ICD). The findings highlight that rfhSP-D plays an anti-tumorigenic role in PCa by transforming an immunologically unresponsive “cold tumor” into a responsive “hot” tumor.

8.4 Trop2 in Ovarian Cancer: Evaluating its Potential as Immunotherapeutic Target

Trop2 is an important therapeutic target for multiple solid cancers including ovarian cancer. Monoclonal anti-Trop2 antibody drug conjugate has got FDA approval. In an attempt to identify immunodominant epitopes of Trop2 for active immunization, we purified truncated Trop2ECD protein (Trop2-172) and used for immunization of rats. The anti-Trop2-172 sera could detect native Trop2 protein expressed by ovarian cancer cells by immunoblotting as well as flow cytometry. The immune sera when tested in ADCC (antibody directed cytotoxicity assay) exhibited cytotoxic effect against Trop2 positive cancer cells indicting their capability to identify and target Trop2 positive cancer cells.

8.5 Assessing Trop2 Expression and Anti-Trop2 Immune Status in Ovarian Cancer Patients

Ascitic fluid and sera from 35 ovarian cancer patients were subjected to exosome isolation and detection of Trop2 protein as well as its interaction partner claudin-7. Trop2 as well as claudin-7 were found to be present in the ascitic fluid derived exosomes, however, their levels varied. The levels of these proteins are being correlated with the Trop2 and claudin7 transcripts present in the cells present in the ascitic fluid. This data may help in utilizing these molecules as prognostic markers for ovarian cancer.

8.6 Expression of Natural Killer and Natural Killer T Cell Receptors and their Association with Ovarian Cancer

The study aimed to characterize the immune profile of chemo naïve primary debulking surgery (PDS) group, chemo treated interval debulking (IDS) group and during follow up of different cycles of chemotherapy of high grade serous epithelial (HGSOC) patients. One hundred and twenty serous epithelial ovarian cancer cases and fifty healthy individuals were enrolled as control (HC) to achieve these objectives. The study highlighted a reduction in the frequency of circulatory CD56^B NK, CD56^D NK, NKT-like and T cells with activating receptors and alteration in immune subsets with inhibitory receptors, indicating the differential immune profile of primary and recurrent ovarian cancer patients. Increased soluble MICA in the serum of cancer cases might act as "decoy" molecule and could be a probable mechanism of decrease in NKG2D positive subsets. An elevated level of serum cytokines IL-2, IL-5, IL-6, IL-10 and TNF- α observed in ovarian cancer patients may help to identify the cytokines associated with ovarian cancer progression and their possible use as biomarker. Clinical and immunological parameters were found to be associated with progression free survival of the disease.

8.7 A Study on Expression of miRNAs in Tissue, Tumor Derived Exosomes and Body Fluids in Ovarian Cancer

The study highlighted a differential expression of specific miRNAs such as miR-182; miR-433 and miR-145 in the serum of ovarian cancer patients in comparison with healthy controls, suggesting their possible use as prognostic marker in ovarian cancer.

8.8 Primary Screening of High Risk HPV DNA by a Low Cost Molecular HPV Test for Early Detection of Cervical Precancers and Cancers among Women in Urban and Rural Community of Maharashtra

Out of 1038 women recruited in the project, 680 women were from an urban site (Abhyudaya Nagar) and 353 women were from a rural site (Dahanu). HPV DNA positivity by HC2 test was seen in 5.45% women. While the HPV positivity by careHPV test was 4.08%. Most common genotype by geneXpert was HPV16 followed by 18 and other HR HPV Acetowhite lesion after application of 5% acetic acid (VIA test) was seen in 5.63% women. PAP results revealed ASCUS in 10 women, HSIL in 4 women and LSIL in 4 women. All the screen positives are being followed up in preventive oncology department, Tata Memorial hospital, Mumbai.

8.9 Identification of Circulating MicroRNA Signatures as Diagnostic Markers for Early Stage and Metastatic Breast Cancer

The study is being carried out to identify subtype-specific molecular profiles of differentially expressed miRNAs associated with early stage and metastatic breast cancer and to evaluate circulating microRNA signatures as biomarkers for determining the response to drug therapy and treatment outcome. The approvals of ICMR-NIRRH Ethics Committee, Saifee Hospital and Mumbai Oncocare Hospital ethical committees were obtained. So far, breast tissues and blood samples of 30 breast cancer patients and blood samples of 10 healthy controls have been collected. The expression of miR-20b, miR-141, miR-205, miR-143, miR-26a and miR-374 was evaluated in blood samples of patients by realtime PCR. The expression of miR-141 and miR-205 which have been associated with breast cancer in earlier studies was found to be significantly higher in blood samples of breast cancer patients compared to healthy controls. For identification of novel miRNAs and cancer-related miRNAs specific to the Indian population, samples have been sent for Next Generation Sequencing and the data is awaited.

8.10 Role of Toll-like Receptors and Toll-like Receptor Agonists in Modulating Response to Chemotherapy in Triple Negative Breast Cancer Patients

The objective of the study is to analyse the Toll like receptor (TLR) expression in triple negative and identify the therapeutic efficacy of relevant TLRs associated with progression and prognosis of TNBC. The study indicates that targeting TLR4/TL8 genes along with IFNG could be a potential therapeutic strategy for treating triple-negative breast cancer patients.

8.11 Deciphering the Placental-Breast Epithelial Cell Cross Talk in Pregnancy associated Breast Cancer

The study aims to understand the effect of placental-breast epithelial cell cross talk and its relevance in pathogenesis of pregnancy associated breast cancer. Preliminary studies indicate that placental cell secretions regulate the cell proliferation/migration/clonogenic abilities of breast cancer cells.

8.12 Improving Access for Screening of Common Cancers and Non-Communicable Diseases (NCDs) Among Women in Tribal Block of Maharashtra: Challenges in Implementation

The study is a joint collaboration between ICMR-NIRRH and District health system. In the first phase, training of master trainers, formative research using qualitative research tools like FGDs of eligible women and ASHAs, key informant's interview of CHOs and ANMs, facility survey was done to assess readiness of health system. Cervical (VIA and HPV testing), breast and oral cancer screenings were done. Also testing for common NCDs by doing blood pressure, RBS and Hb were done. Endocervical samples were tested for common RTIs. Barriers were transportation, time spent at the public health care facility and behaviour of the staff. ASHAs were found as the key connecting link between women and health system. ASHAs needed incentives for transport and some financial remuneration. Hence, ASHAs need to be incentivized to improve awareness and health seeking behaviour. There was lot of hesitancy among women to come for screening due to the fear of COVID. Health care facilities need to be upgraded in terms of infrastructure, equipment and trained manpower to implement screening services.

9. HEALTH TECHNOLOGY ASSESSMENT

9.1 HTA on Use of Tranexemic Acid in Management of PPH

The existing Indian guidelines on PPH management framed in 2015 recommends using tranexamic acid if oxytocin and other uterotonics fail to stop bleeding or if bleeding is partly due to trauma. The updated WHO recommendation has not yet reflected in Indian guidelines or the DAKSHATA checklist. For lack of guidance, Department of Health Research, Ministry of Health and Family Welfare, Government of India from Government of Maharashtra received a policy question regarding cost effectiveness of Tranexamic acid use in PPH management. A decision analytic model was built using a decision tree to determine cost-effectiveness of administering IV TXA within 3 hours of birth to existing management with uterotonics and supportive care to women with PPH. Results reveal that with an estimated annual cohort of 5,10,915 PPH cases in India, addition of IV TXA would result in a per-patient disaggregated societal cost of INR 6607 (USD 95) with discounted gain of 20.25 QALYs as compared to INR 6486 (USD 93) with discounted gain of 20.17 QALYs with existing standard care for PPH management. At an ICUR value of INR 1470 per QALY gained (USD 21), addition of IV TXA is cost-effective in Indian public health settings. The intervention is likely to prevent 389 maternal deaths, 177 surgeries and 128 ICU admissions per 100,000 PPH cases. Policy guidelines, training manuals and facility checklists should be updated to reflect this recommendation.

10. COVID-19 RESEARCH

10.1 Opposing Roles for sMAdCAM and IL-15 in COVID-19 Associated Cellular Immune Pathology

Using an integrated approach of monitoring inflammatory and mucosal migratory markers together with a key homeostatic cytokine and the gut microbiome in a cohort (n=130) of SARS-CoV-2 infected individuals we were able to show distinct depletion profiles, with relative sparing of CD8 effector memory and CD4+ regulatory T cells in hypoxemic disease within the lymphocyte compartment. An apparent increase in the frequency of intermediate monocytes characterized both mild as well as hypoxemic disease. IL-6 levels inversely correlated with those of sMAdCAM and both markers showed converse associations with observed lympho-depletion suggesting opposing roles in pathogenesis. Interestingly, IL-15, a key cytokine involved in lymphocyte activation and homeostasis, was detected in symptomatic individuals but not in healthy controls or asymptomatic cases. Further, plasma IL-15 levels negatively correlated with T, B and NK counts suggesting a compensatory production of this cytokine in response to the profound lymphopenia. Finally, higher levels of plasma IL-15 and IL-6, but not sMAdCAM, were associated with longer duration of hospitalization. Our results highlight key systemic and gut-associated parameters that need to be monitored and investigated further to optimally guide therapeutic and prophylactic interventions for COVID-19. Gut microbiome dysbiosis was observed in COVID-19 patients with symptomatic infection along with a putative protective microbiome signature in individuals with asymptomatic infection. These

were further found to be associated with systemic immune responses as well as clinical features of infection.

10.2 National Registry of Pregnant Women with COVID-19 in India

More than 8000 pregnant and postpartum women with COVID-19 are registered in the PregCovid registry. Data of 4203 pregnant women collected during the first wave of the COVID-19 pandemic (March 2020-January 2021) reported 6% pregnancy/foetal loss including stillbirths in Maharashtra. The most common complication was preterm delivery (16.3%) and hypertensive disorders in pregnancy (10.1%). The overall case fatality rate (CFR) in pregnant and post-partum women with COVID-19 was 0.8 %. During the second wave of the COVID-19 pandemic, five times higher severe COVID-19 cases, 8 times higher maternal deaths, 2 times higher stillbirth rate per 1000 births, 3 times higher spontaneous abortion rate per 1000 births, 3 times higher very spontaneous preterm births (28 to 32 weeks of gestation) were observed in pregnant and postpartum women admitted at BYL Nair Hospital, Mumbai. The evidence generated from PregCovid registry was useful for policy decision on COVID-19 vaccination in pregnant women in India.

10.3 Understanding Mother to Child Transmission of SARS-CoV-2

The study is aimed at identifying the determinants of congenital transmission of maternally acquired SARS-CoV-2. Maternal swabs, blood plasma, placental tissue and fetal cord blood plasma were collected from pregnant women infected with SARS-CoV-2. We identified a proportion of women who have a systemic infection with and without placental infection. A subset of these women mounted an immune response of antibodies against SARS-CoV-2 and transmitted it to the baby. Studies are ongoing to profile the placenta for the transcriptome changes to identify gene networks altered in response to SARS-CoV-2 infection.

10.4 Occurrence and Dynamics of SARS-CoV-2 Infection in Unvaccinated and Vaccinated Population of Mumbai, India

A retrospective analysis of the occurrence and dynamics of SARS-CoV-2 infections and their correlation with vaccination status was carried out. The RT-PCR cycle threshold (Ct) data of confirmatory genes of SARS-CoV-2 was retrieved from the in-house database for samples collected between December 28, 2020 and August 30, 2021. A total of 42,415 samples suspected of SARS-CoV-2 infection, received from unvaccinated cases (96.88%) and vaccinated cases (3.12%) were analyzed. A lower incidence of SARS-CoV-2 infection in fully vaccinated cases (5.07%) compared to partially vaccinated cases (6.5%) and unvaccinated cases (13.453%) was recorded. The vaccine efficacy (VE) in clearing the SARS-CoV-2 infection was highly significant after the first dose of vaccination. Similarly, the SARS-CoV-2 infection was significantly controlled within 30 days after first dose of vaccination through 60 days after second dose of vaccination. While Covaxin and Covishield vaccination offered protection against SARS-CoV-2 infection, the emergence of new infections persisted despite vaccination. A high viral load was observed in women and senior citizens in comparison to men and individuals with age <60 respectively. In addition, the viral

loads in partially vaccinated individuals were observed to be high as compared to individuals who had taken the second dose and those who were unvaccinated.

11. MISCELLANEOUS

11.1 ICMR National Snakebite Project on Capacity Building of Health Systems on Prevention and Management of Snakebite Envenomation including its Complications

India is the snakebite capital of the world with about 60000 annual deaths occur due to snakebite envenomation (SBE). Poor farmers, shepherds, tribal and migrant population are largely affected by SBE. Based on the model of prevention and management of snakebites developed by NIRRH, Indian Council of Medical Research (ICMR)-National Task Force Expert Group for 'Research on Snakebite in India' recommended upscale of the Dahanu model to a Health System Research project using a similar multi-sectoral approach for attaining the 2030 WHO goal of reducing snakebite deaths by 50%. The national snakebite project is an interventional study being conducted in Shahapur and Aheri blocks of Maharashtra and Khordha and Kasipur blocks of Odisha. As of 31 March 2022, retrospective data of about 504 snakebite cases has been collected and 28 community FGDs have been conducted in the study blocks.

1. FEMALE INFERTILITY AND ASSOCIATED REPRODUCTIVE DISORDERS

1.1 PON1 Expression, Activity and its Relationship with Oocyte and Embryo Quality in Women with PCOS undergoing Assisted Reproductive Techniques (Partly Funded by Board of Research in Nuclear Sciences)

Principal : **Srabani**
Investigator : **Mukherjee**
Principal Co- : D Modi
Investigator
Project : A Naigaonkar,
Associates Sushma Khavale,
Gayatri Shinde
Collaborator : Indira Hinduja, PD
Hinduja Hospital,
Mumbai
Duration : 2014-2023

Polycystic Ovary Syndrome (PCOS) is a multifactorial disorder and leading cause of an ovulatory infertility. The women with PCOS are often infertile; hence opt for assisted reproductive techniques (ART) to achieve pregnancy but their oocyte quality is poor. Role of oxidative stress (OS) is well established in PCOS pathophysiology and it can influence oocyte/embryo quality and ART outcome. One of the aims of this project is to study OS parameters in oocyte microenvironment, i.e. follicular fluid (FF) and granulosa cells (GCs), as these two are pivotal in oocyte development and can be viewed as ideal surrogates for reflection of events inside the ovarian follicle. Skewed activities of antioxidant enzymes and their transcript levels in FF and GCs respectively were observed in women with PCOS (Annual report 2017-18 to 2019-20). During reporting year, our investigations demonstrated higher intracellular OS levels in GCs of PCOS women, compared to controls (Fig. 1).

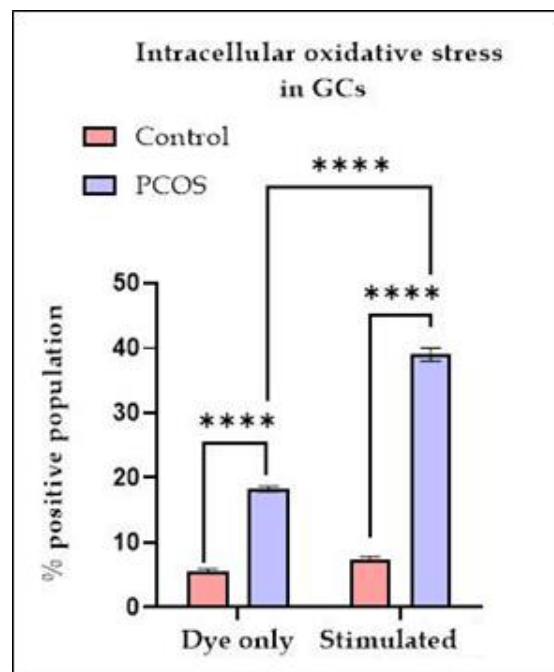


Figure 1: Intracellular oxidative stress levels measured in GCs of controls (n = 5) and women with PCOS (n = 5), represented as mean $\pm$ SEM, ****p<0.05 is significant.

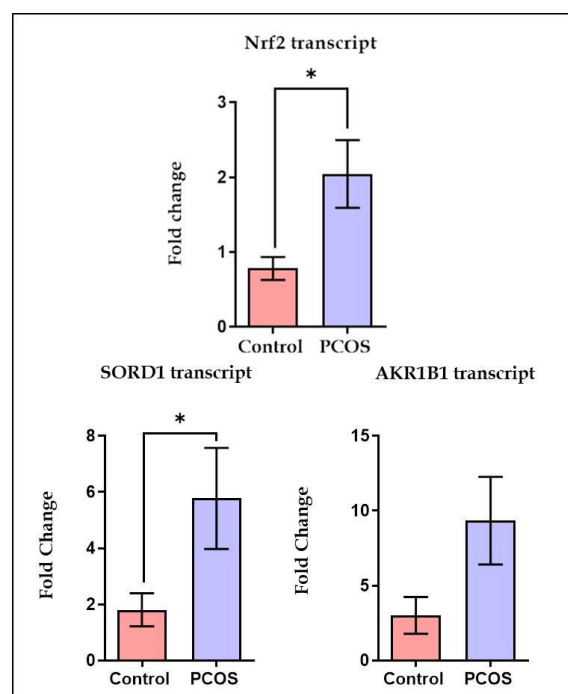


Figure 2: Comparison of transcript levels of Nuclear factor erythroid 2-related factor 2 (Nrf2), sorbitol dehydrogenase (SORD1) and aldose reductase (AKR1B1) in granulosa cells of control (n=11) and

PCOS (n=12) women, represented as mean $\pm$ SEM, *p<0.05

Treatment of GCs with oxidizing agent (stimulated) increased oxidative stress in PCOS but not in control indicating an impairment of anti-oxidant machinery. The transcript levels of Nuclear factor erythroid 2-related factor 2 (Nrf2), a master redox regulator, were found to be upregulated in GCs from women with PCOS (Fig. 2). This upregulation may be a result of the cellular compensatory mechanism in response to the increased OS in GCs in PCOS. Expression of enzymes involved in polyol pathway was also studied, as it is known to be associated with oxidative stress and insulin resistance, hallmark features of PCOS. Aldose reductase (AKR1B1) transcript showed an increasing trend whereas Sorbitol dehydrogenase (SORD1) transcript was significantly higher in GCs from PCOS women indicating a hyper-activated polyol pathway which in turn can further increase the oxidative stress by changing NADPH/NADP ratio (Fig. 2). These data indicate that the redox homeostasis is compromised in follicular microenvironment of PCOS women. Hence, targeting redox metabolism may be one of the worthy strategies to improve oocyte/embryo quality, IVF prognosis and outcome in women with PCOS in ART settings.

1.2 Understanding Follicular Angiogenesis in Women with Polycystic Ovary Syndrome (Partly Funded by Department of Biotechnology)

Principal Investigator : **Srabani Mukherjee**
 Project Associates : Krutika Patil, PP More, V Khedekar
 Collaborators : Indira Hinduja, PD Hinduja Hospital, Mumbai
 J Shah, Mumbai Fertility Clinic, Mumbai

Duration : 2015-2022

Angiogenesis is crucial for the growth and maturation of ovarian follicles, ovulation, and development of corpus luteum (CL). In the growing follicle, the complex interaction between several pro- and anti-angiogenic factors regulates the development of capillary network surrounding the follicle. Hence, any alteration of angiogenic factors/process in the ovary may result in aberrant folliculogenesis leading to the development of ovarian disorders. Intense vascularization is essential for the CL formation and subsequent progesterone secretion. Luteal phase inefficiency and frequent miscarriages have been reported in women with PCOS. We previously reported altered expression of some angiogenesis-associated genes in granulosa lutein cells (GLCs) of women with PCOS. However, there may be many more angiogenesis-associated genes dysregulated in PCOS. The literature search, and in-silico analysis, alluded to differential expression of a total of 99 angiogenesis-related genes in the ovary of women with PCOS as reported on the past years. The present study attempted to identify the miRNAs involved in regulation of differentially expressed angiogenesis associated genes in PCOS. miRNAs targeting the dysregulated angiogenesis-associated genes (n=99) were fetched using experimentally validated miRNA databases and enlisted 428 miRNA-mRNA interactions. Only those miRNAs were included that were either validated with strong experimental evidence or a low-throughput technique. A similar literature search was carried out to identify the miRNAs expressed in the ovary of women with PCOS. The miRNAs common to both the lists were selected. The miRNAs, reported to be expressed in the ovary of women with PCOS as well as known to regulate the differentially expressed angiogenesis-related genes were focussed upon. Pathway analysis of these 'common miRNAs' revealed their enrichment in the pathways

involved in angiogenesis viz., extracellular matrix receptor interaction, PI3K/Akt, HIF-1, VEGF, and Notch signaling pathway. The maximum number of altered genes and miRNAs were enriched in PI3K/Akt pathway, from which 4 miRNA-mRNA interactions were shortlisted. Selected four genes namely, VEGFA, FGFR1, PDGF receptor alpha (PDGFRA), and FN1 are known to play a role in angiogenesis and reported to be dysregulated in PCOS. The miRNAs interacting with these genes viz., miR-218-5p, miR-214-3p, miR-20a-5p, and miR-140-3p were validated using ddPCR and were observed to be up-regulated in the GLCs of women with PCOS compared to controls (Fig. 3).

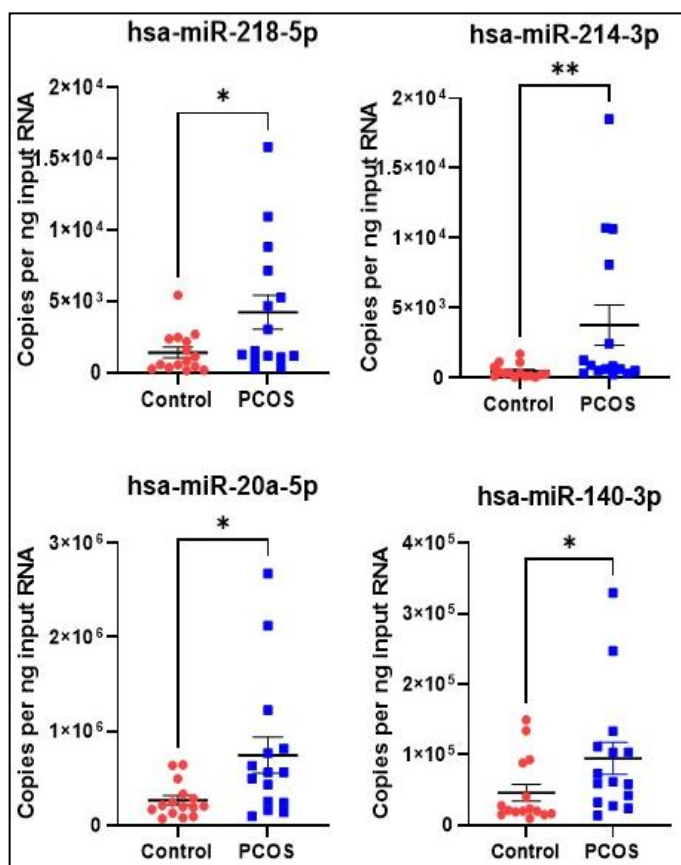


Figure 3: Absolute quantification of miRNAs obtained from GLCs of women with PCOS and controls using digital droplet PCR

Altogether this study in highlighted the compromised follicular angiogenesis in women with PCOS and generated a list of potential

angiogenesis-related targets and miRNAs regulating them. Data generated from this analysis is an important resource for researchers evaluating the mechanism of altered angiogenesis in the PCOS ovary. This information will augment research on potential therapeutic options in PCOS management in the future.

1.3 Unraveling Pathogenetic Mechanisms of Polycystic Ovary Syndrome by Whole Exome Sequencing (Partly Funded by Department of Science and Technology)

Principal Investigator : **Srabani Mukherjee**
 Project Associates : Mahalakshmi Bhat, Medini Samant, Roshan Dadachanji, Sushma Khavale, Gayatri Shinde, Nanda Joshi, Pallavi Shukla
 Collaborators : Anushree Patil, Beena Joshi
 Duration : 2017-2023

Polycystic Ovary Syndrome (PCOS) is a multigenic disorder. Numerous loci of interest for this polygenic disorder have been reported using Genome-Wide Association studies (GWAS). We performed whole exome sequencing to delineate genetic predisposition profile of Indian women with PCOS and identify novel causative genetic variants in our well-characterized PCOS cohort. Exome sequencing was carried out in 25 PCOS and 25 control samples. Variant annotation from sequencing data is a crucial step which links the sequence variants with changes in phenotype. VCF files were used for annotating and prioritizing the variants using Human genome assembly GRCh37. Annotation tools like Exomiser 13.0 and Annovar were used to annotate and prioritize the variants which were subsequently categorized into types of variants (Fig. 4). These were further screened based on the minor allele

frequency (MAF) and pathogenicity. The pathogenicity scores were generated through various tools like Polyphen, SIFT, Mutation taster. From the preliminary analysis, we have been able to shortlist both polymorphisms and rare variants in a few genes with a possible role in the pathophysiology of PCOS. These genes mainly influence important pathways such as gonadotropin imbalance, steroidogenesis and androgen excess, insulin resistance, oxidative stress, lipid metabolism, oocyte development. Few genes are also involved in regulation of transcription and epigenetic mechanisms. Further, loci of already established genes like AR, MTHFR, THADA, IRS2, PON1, FSHR, KISS1 and GWAS studies in the Chinese and Caucasian population were also found in our study population, indicating that some of these could be important biomarkers for PCOS etiology.

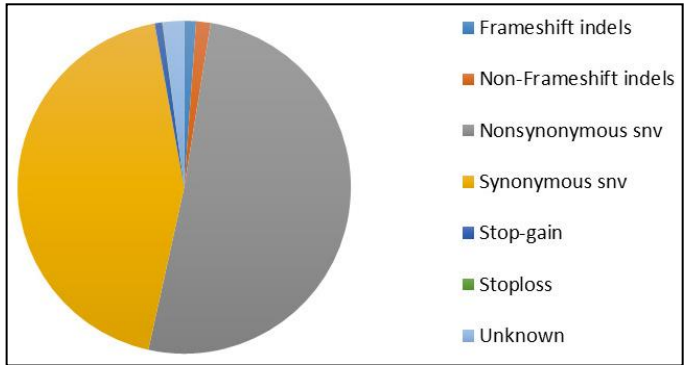


Figure 4: Classes of variants as seen in the women with PCOS: Out of 2,56,374 Variants from the data, 50.4% non-synonymous variants, 44.6% synonymous variants, 1.2% frameshift indels, 1% non-frameshift indels, 1% unknown variants and 1.8% stopgain/stoploss were found.

Known and novel variants in a few interesting genes which have been previously linked to pathogenetic mechanisms of PCOS were observed to be occurring at a higher frequency in the PCOS as compared to controls. These included WWTR1, a transcriptional coactivator which acts as a downstream regulatory target in the Hippo signaling pathway; insulin receptor

(INSR), a key player for initiation of insulin signaling pathway; kisspeptin (KISS1), which stimulates gonadotropin-releasing hormone (GnRH)-induced gonadotropin secretion; and ghrelin (GHL), a powerful appetite stimulant. Additionally, few rare missense variants of PCSK6, NOBOX and FSHR variants have been observed and validated in our study (Fig. 5).

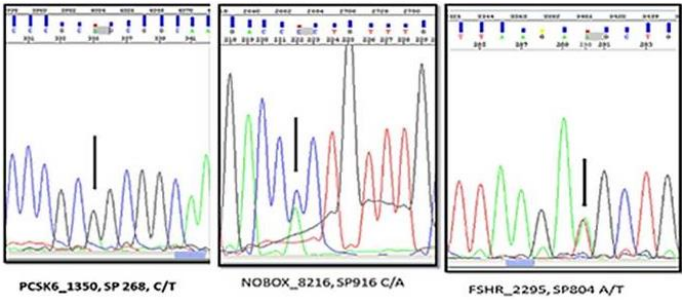


Figure 5: Electropherogram of Sanger sequencing analysis of PCSK6, NOBOX and FSHR genes.

Further extensive analysis in larger sample size will help to elucidate the genetic component underlying PCOS pathophysiology by identifying functional polymorphism associated with PCOS risk and its related traits.

1.4 Identification of Differentially Expressed Genes in Lean and Obese PCOS and Validation using Rat Animal Models *(Funded by Department of Health Research)*

Principal Investigator	: Susan Thomas
Project Associates	: A Kanojia, Kaushiki Prabhudesai
Co-investigator	: V Dighe
Duration	: 2019-2022

Polycystic Ovary Syndrome (PCOS) is a multifactorial endocrinopathy prevalent among reproductive age women. The interplay between genetic and environmental factors contributes to the development of PCOS; though the exact mechanism remains elusive. Obesity is a known

risk factor for PCOS related metabolic and reproductive anomalies. The project aims to identify the genes that are expressed differentially in lean and obese PCOS. Initially, female rats were fed with low fat diet (LFD: 6% fat) and high fat diet (HFD: 22% fat) to determine if HFD can induce PCOS in rats. The animals were sacrificed post 17 weeks of feeding. The establishment of obesity was confirmed by adiposity index and body weight. The animals belonging to obese group showed altered lipid and glucose profile suggestive of development of dyslipidemia and hyperglycemia respectively. Microarray data analysis identified 125 genes and retinol pathway to be commonly dysregulated in liver and ovary of obese female rats. Many of these dysregulated genes were associated with hepatic and ovarian disorders.

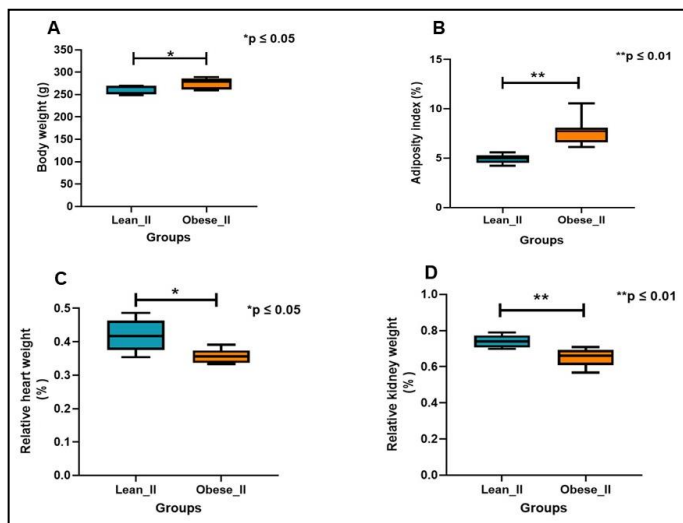


Figure 6: Effect of HFD on A) body weight, B) adiposity index, C) relative weight of heart and D) relative weight of kidney of female rats at the end of 31st week of feeding: Box and whisker plot representation of A) body weight and B) % adiposity index, relative weight of C) heart and D) kidney of animals fed with 6% fat (Lean_II) and 22% fat (Obese_II) for 31 weeks. The data was found to have normal distribution using Shapiro-Wilk test; and was analysed for significance using Student's unpaired t-test. * and ** indicate statistical significance between Lean_II and Obese_II at $p \leq 0.05$ and $p \leq 0.01$ respectively.

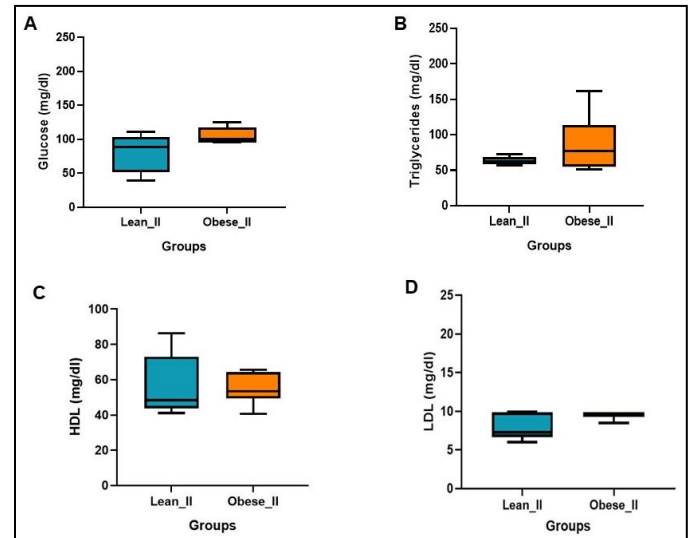


Figure 7: Effect of HFD on serum A) glucose B) triglycerides C) HDL and D) LDL levels at the end of 31st week of feeding: Box and whisker plot representation of A) glucose B) triglycerides C) HDL and D) LDL levels of animals fed with 6% fat (Lean_II) and 22% fat (Obese_II) for 31 weeks. The normality of the data was assessed using Shapiro-Wilk test. The normally distributed data was further analysed for significance using Student's unpaired t-test.

To assess the implication of increased feeding period on obesity associated gene dysregulation, a second study was conducted wherein female rats were fed with HFD for 31 weeks. The animals fed with HFD showed significant increase in body weight and adiposity index at the end of 31st week (Fig. 6 A and B); these animals also displayed a significant decrease in heart and kidney weights as compared to lean animals. Obese animals had elevated serum levels of glucose, triglycerides, LDL and reduced levels of HDL as compared to the lean group, suggesting altered glucose metabolism and lipid profile (Fig. 7 A, B, C and D). The most significantly dysregulated genes obtained from microarray data of first study are being validated through qRT-PCR using ovaries and liver samples of lean and obese rats from the second study.

1.5 Investigation of Epigenetic Alterations in Estradiol-Treated Cystic Ovarian Murine Model

Principal : **Srabani Mukherjee**
 Investigator
 Project : Gayatri Shinde, Sushma
 Associates : Khavale, PP More
 Duration : 2017-2024

In addition to genetic factors, epigenetic modifications play a key role in PCOS pathogenesis. Epigenetic effects of Endocrine Disrupting Chemicals (EDCs) that imitate or antagonise the actions of estrogens on the hypothalamo-pituitary-ovarian axis have been well described in animal models of PCOS. PCOS is also characterised by functional impairment of various tissues such as ovaries, adrenal glands, and adipose tissue, as well as hypothalamo-pituitary axis. Investigations of the epigenetic profiles of these tissues is not possible in humans. Therefore, we used a cystic ovarian murine model developed in laboratory to explore the epigenetic changes elicited by estrogen in various tissues. Our earlier study demonstrated that after 12 weeks of treatment, neonatal estrogenization of Swiss mice starting from postnatal day 5 results in altered ovarian morphology with cystic follicles (Annual report 2014-2015; pp. 6). Hormonal profiles revealed altered gonadotropin ratio (increased LH: FSH), decreased P₄ and E₂ levels, thereby mimicking characteristics of PCOS. We observed decreased transcript levels of Dnmt1, Dnmt3a, Dnmt3l and increased transcript levels of Tet1 and Tet3 at 12 weeks in the ovaries of estrogen treated (E2T) animals which suggests epigenetic alterations in estrogenized animals (Annual report 2020-2021; pp. 7-8). During the reporting year, epigenetic profile of hypothalamo-pituitary (HP) tissue was investigated in estrogenized animals. No significant changes were observed in % 5mC levels in E2T animals as compared to control after 12 week of treatment (Fig. 8).

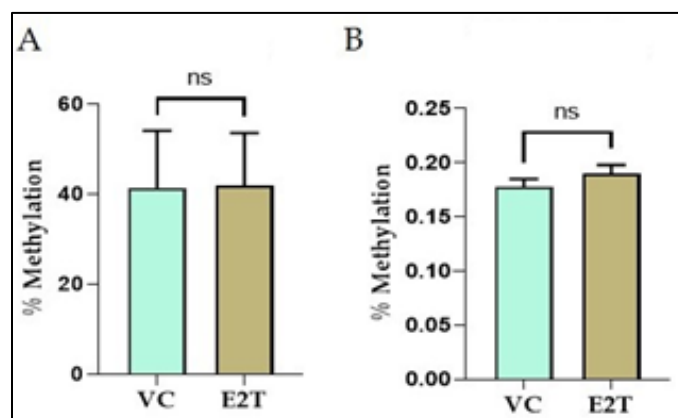


Figure 8: Global DNA 5-methylcytosine (% 5mC) levels assessed in Hypothalamus-Pituitary tissue of vehicle-treated control (VC) and E2-treated (E2T) adult mice at 12 weeks post-treatment. A) represents assessment of % 5mC by Flow cytometry (FCM) and B) represents percent methylation assessed by ELISA.

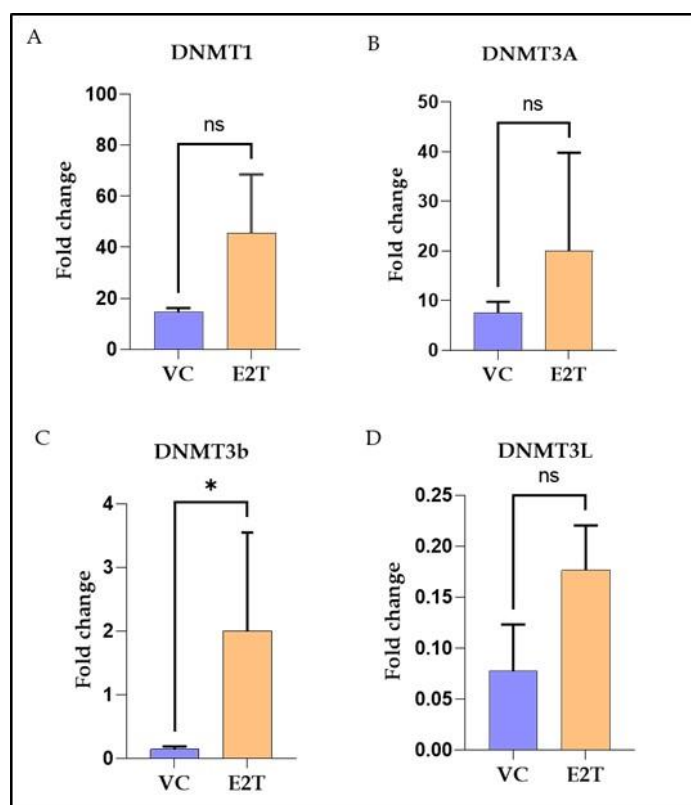


Figure 9: Transcript levels of DNA Methyl Transferase (Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l) in the Hypothalamus-Pituitary tissue of vehicle-treated controls (VC, n=4) and E2-treated adult mice (E2T, n=4) at 12 weeks post treatment. Fold change was evaluated with respect to GAPDH as a housekeeping gene.

Further, the study was extended to perform qPCR analysis for gene transcripts encoding the DNA methyltransferase (DNMTs) family of enzymes that catalyze 5mC modifications. The study revealed the upregulation of the DNMT3b only in E2T group compared to control (Fig. 9). These preliminary findings along with lowered estradiol levels (Annual Report 2020-2021; pp7-8) in E2T animals suggest that neonatal estrogenization may alter epigenetic machinery at hypothalamo-pituitary-ovarian axis. Further, epigenetic demethylation machinery will be explored by assessing transcript expression of ten-eleven translocation enzymes (TETs) and global 5hmc levels in E2T animals.

1.6 Exploring the Epigenetic Alterations Regulating miRNA Expression in Women with Polycystic Ovary Syndrome

Principal Investigator : **Srabani Mukherjee**

Project Associates : Snehal Bhingardev, Gayatri Shinde

Collaborators : Sadhana Desai, V Mangoli, Richa Jagtap

Duration : 2020-2025

Several evidence indicate that epigenetic alterations play a role in PCOS pathophysiology. These changes include DNA methylation, histone modifications, miRNA driven gene silencing and RNA methylation modifications. Epigenetic dysregulation of genes involved in important processes associated with follicular development may contribute to ovarian defects observed in women with PCOS. Our earlier study on genome-wide DNA methylome profiling led to identification of differential methylation of several miRNAs coding genes in PCOS women. This suggests that the epigenetic dysregulation of miRNAs may contribute to ovarian defects in PCOS. Therefore, it is

important to investigate the role of DNA methylation in regulating miRNA expression, their target gene expression and their significance in the pathophysiology of PCOS. The study aims to investigate whether the differentially methylated miRNA genes (DMmiRs) identified in methylome analysis exhibit differential expression in concurrence with altered DNA methylation in granulosa cells of women with PCOS. The study also aims to investigate the expression of genes targeted by DMmiRs and their effects on some of the downstream pathways. The expression levels of miR-10b, miR-127, miR-193a were studied in granulosa cells of PCOS and controls. Transcript levels of miR-10b, miR-127 were decreased, and of miR-193a increased in the granulosa cells of women with PCOS compared to control (Fig. 10). Methylation analysis by pyrosequencing showed hypermethylation of miR-10b and miR-127 in PCOS compared to controls (Fig. 11). Thus, the findings show differential expression and differential methylation status of miRNAs in women with PCOS compared to controls.

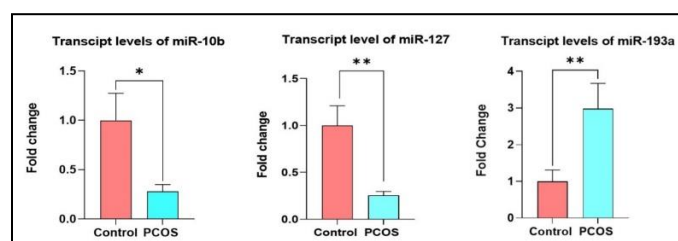


Figure 10: Quantitative real-time PCR analysis of miR-10b, miR-127, miR-193a in GCs of women with PCOS and controls.

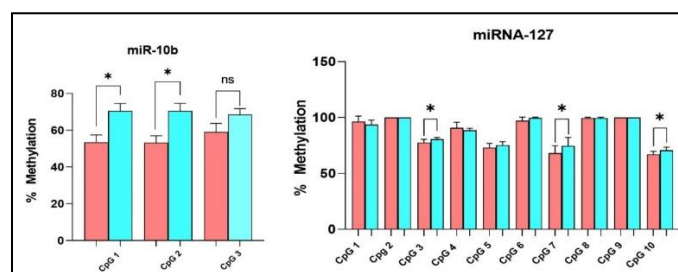


Figure 11: Methylation profiling of miR-10b and miR-127 was carried out using pyrosequencing. Data are presented as 'mean $\pm$ SD'. Data are analyzed using the Mann-Whitney U test.

1.7 Assessing the Coagulation and Fibrinolytic System as Contributors of Thrombotic State in Polycystic Ovary Syndrome

Principal Investigator : **Srabani Mukherjee**

Project Associates : Roshan Dadachanji,
Gayatri Shinde,
Sushma Khavale,
Nanda Joshi

Collaborators : Anushree Patil
B Kulkarni, NIIH,
Mumbai

Duration : 2021-2026

PCOS is not only the leading cause of anovulatory infertility but is also associated with increased incidence of long term comorbidities including type II diabetes (T2D), dyslipidemia, hypertension, metabolic syndrome and cardiovascular disease (CVD). Women with PCOS exhibit an array of potential biochemical and anatomical CVD risk factors. However, only a few studies have shown positive correlation of cardiovascular disease with PCOS. The increased risk of cardiovascular disease may be due to altered coagulation and fibrinolysis, leaning towards a hypercoagulable prothrombotic state. Recent meta-analyses have revealed elevated risk of CVD and cerebrovascular disease (CeVD) events including myocardial infarction, ischemic heart disease, stroke, and venous thromboembolism but not mortality-related outcomes in women with PCOS. Common treatment regimens used in PCOS including oral contraceptive pills (OCPs) have also been reported to increase propensity to thrombosis while addition of metformin alleviates this risk. It is noteworthy that coagulation and fibrinolysis are integral in key ovarian processes such as ovulation, granulosa cell luteinization, ECM remodelling and corpus luteum function. Thus, investigating

systemic and ovarian dysregulation of the hemostatic system will expand our knowledge of underlying mechanisms of cardiovascular events and ovarian dysfunctions which contribute to PCOS pathophysiology. Screening coagulation assays including prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time (TT), are most frequently utilized to ascertain presence of any derangements in intrinsic and extrinsic pathways of coagulation and determine the fibrinogen activity by assessing the time taken for formation of fibrin clot, respectively. During the reporting year, preliminary analysis did not indicate significant differences between controls and women with PCOS in any of the screening assays (Fig. 12). Two important components of the fibrinolytic cascade were studied. Tissue plasminogen activator (tPA) is an important fibrinolytic factor involved in clot breakdown and its inhibitor is plasminogen activator inhibitor-1 (PAI-1). However, initial findings indicate that plasma levels of both PAI-1 (Fig. 13A) and tPA (Fig. 13B) are comparable between women with PCOS and controls, although both parameters show a trend towards increased levels in women with PCOS. Recruitment of more participants and data collection is ongoing.

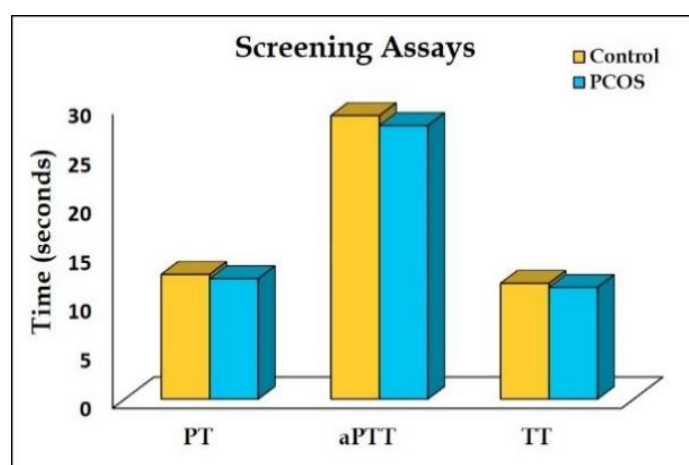


Figure 12: Comparison of coagulation screening assay times between controls (n= 16) and women with PCOS (n= 17). PT: Prothrombin Time, aPTT: activated Partial Thromboplastin Time and TT: Thrombin Time.

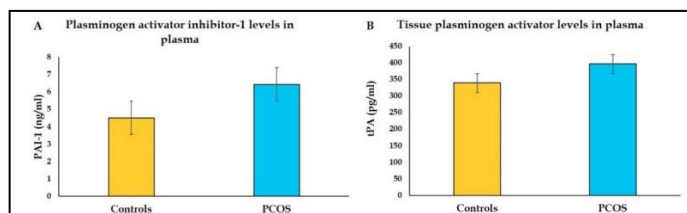


Figure 13: Comparison of plasma PAI-1 (A) and tPA (B) levels between controls (n= 16) and women with PCOS (n= 17).

1.8 Analysis of Mitochondrial DNA Sequence Variants in Polycystic Ovary Syndrome Women with Insulin Resistance

Principal : Pallavi Shukla
 Investigator
 Project : Srabani Mukherjee, Anushree
 Associates Patil, Beena Joshi
 Duration : 2018-2022

Polycystic Ovary Syndrome (PCOS) is a common cause of infertility among women of reproductive age. Seventy women with PCOS and 50 healthy control women were investigated for the mitochondrial DNS copy number (mtDNA) and mtDNA copy number was significantly reduced in women with PCOS compared to control women ($p=0.001$) (Fig. 14A).

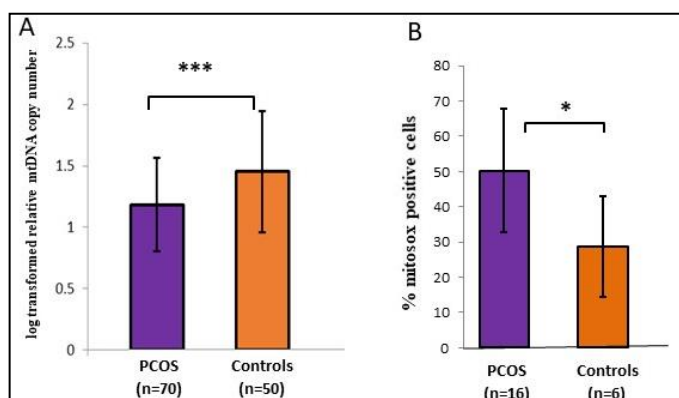


Figure 14: mtDNA copy number was significantly reduced in women with PCOS compared to healthy women ($p= 0.001$). (B) Mitochondrial ROS production was significantly increased in women with PCOS compared to healthy women ($p=0.0177$).

Investigation of mitochondrial ROS in PCOS participants (n=16) and healthy participants (n=6) using a fluorescent probe, MitoSOX, a red mitochondrial superoxide indicator by flow cytometry analysis suggest that mitochondrial ROS production was significantly increased in women with PCOS, compared to healthy women ($p=0.0177$) (Fig. 14B). This suggests mitochondrial dysfunction in women with PCOS and its contribution to oxidative stress in women with PCOS.

1.9 Evaluation of Prevalence, Regional Phenotypic Variation, Comorbidities, Risk Factors and the Variations in Response to Different Therapeutic Modalities among Indian Women with Polycystic Ovary Syndrome (PCOS): A Multicenter Study across India (ICMR TASK Force Study)

Principal : Beena Joshi
 Investigator
 Project : Saharmeen Sheikh, Gazala
 Associates Hasan, Rita Parab, Himali
 Vadan, Shramika Thakur
 Collaborators : Hemangini Thakkar,
 Anushree Patil, Srabani
 Mukherjee, Shahina
 Begum, A Lila
 Duration : 2018-2022

This is an ICMR Task Force Study to estimate community based prevalence of PCOS across nine states in India and we were one of the sites in the Western Region of India. The study, which began in 2018, was greatly affected due to COVID pandemic and hence extension was sought to continue screening and meet the expected sample size both in urban and rural areas. Over the one year period, we saw a lot of drop outs among those who had agreed to participate in pre-covid times. There were a number of participants who had migrated out or got pregnant or crossed their age as mandated in

inclusion criteria. Certain strategies were changed to improve enrollment such as having multiple options of accessing USG after ensuring quality in reporting, conducting clinical examinations and blood collections at places closer to the homes of participants etc. We completed screening 1924 cases and enrolled 1087 cases of which 145 were probable cases and 182 known PCOS cases. Complete work up was done for a total of 243 cases which included 91 probable and 13 known cases and rest were controls. Hormonal investigations were carried out at SKIMS, Srinagar and reports were awaited. Data has been submitted to ICMR and coordinating unit at SKIMS Srinagar for compilation. The study is ongoing.

1.10 Building and Analyzing Gene Network for Polycystic Ovary Syndrome (Partly Funded by DST-WOSA)

Principal Investigator : **Shaini M Joseph**
 Principal Co-Investigator : **Smita D Mahale (Mentor)**
 Collaborator : **Srabani Mukherjee**
 Duration : 2019-2022

Polycystic Ovary Syndrome (PCOS) is one of the most common causes of anovulatory subfertility/infertility in women of reproductive age. While several genes have been identified for their association with PCOS, its genetic etiology remains unclear. This project aimed at predicting novel candidate genes for PCOS by integrating information from existing literature, gene expression data, and functional information on known genes. The use of gene prioritization and network analysis led to identification of 13514 genes with a probable association with PCOS. Further, filtering based on a score >0.5 or number of evidences >2 reduced the number of genes to 8683. Among

these genes, 380 secretory proteins were identified. Pathway enrichment of these genes encoding secretory proteins identified Complement and Coagulation cascade, ECM-receptor interaction pathway, Melanoma, Protein digestion and absorption and TGF-beta signalling pathway as the top five most enriched pathways. Three genes Protein S (PROS1), Kininogen 1 (KNG1) and Vitronectin (VTN) were selected from the Complement and Coagulation cascade for experimentally validation in follicular fluid. The levels of PROS1 and KNG1 were found to be dysregulated in women with PCOS as compared to controls. The levels of VTN remained unchanged in women with PCOS as compared to controls.

1.11 Studies to Evaluate the Effect of Metformin on Endometrial Functions

Principal Investigator : **U Chaudhari**
 Project Associates : **M Imran, Geetanjali Sachdeva, SM Metkari, RR Katkam**
 Duration : 2018-2023

Metformin, an oral anti-diabetic drug, is an effective therapeutic agent for treatment of reproductive disorders including PCOS. This study was undertaken to evaluate the direct effects of metformin on endometrium. Our previous observations demonstrated proliferative effects of low dose metformin on Ishikawa and HEC-1A cells lines (Annual report 2017-18, pp. 18). In addition, microarray analysis of metformin-treated HEC-1A cells revealed differential regulation of pathways such as mitochondrial translational elongation, cell adhesion, cell cycle, cell division, and ribosomal processing. During the reporting year, validation of selected differentially expressed genes (DEGs) was performed using qRT-PCR. Based on their fold change values and relevance in cell proliferation and mitochondrial functions,

8 DEGs were chosen. Of these, 5 genes (MRPS34, EGR1, GDF15, ODC1, and DDIT4) showed a significant up or downregulation as revealed by microarray studies. On the other hand, expression of 3 genes (CYCS, EDF1, TP53) showed a similar pattern as that of the microarray analysis, but was not statistically significantly different between control and treated groups (Fig. 15). Microarray analysis also revealed higher expression of mitochondrial function related genes in cells treated with lower dose (50 μ M) as compared to high dose (10,000 μ M) of metformin. Therefore, succinate

dehydrogenase (SDHD), which is a mitochondrial inner membrane protein and also a marker of overall mitochondrial activity, was analysed. SDHD expression was found to be significantly higher in 50 μ M (low metformin) treated Ishikawa and HEC-1A cells ($p < 0.05$, $p < 0.05$ respectively) compared to untreated controls (Fig. 16). The results obtained so far indicate dose-dependent effects of metformin on gene expression. Enhanced expression of SDHD in low-dose metformin treated endometrial epithelial cells indicated augmentation of mitochondrial functions.

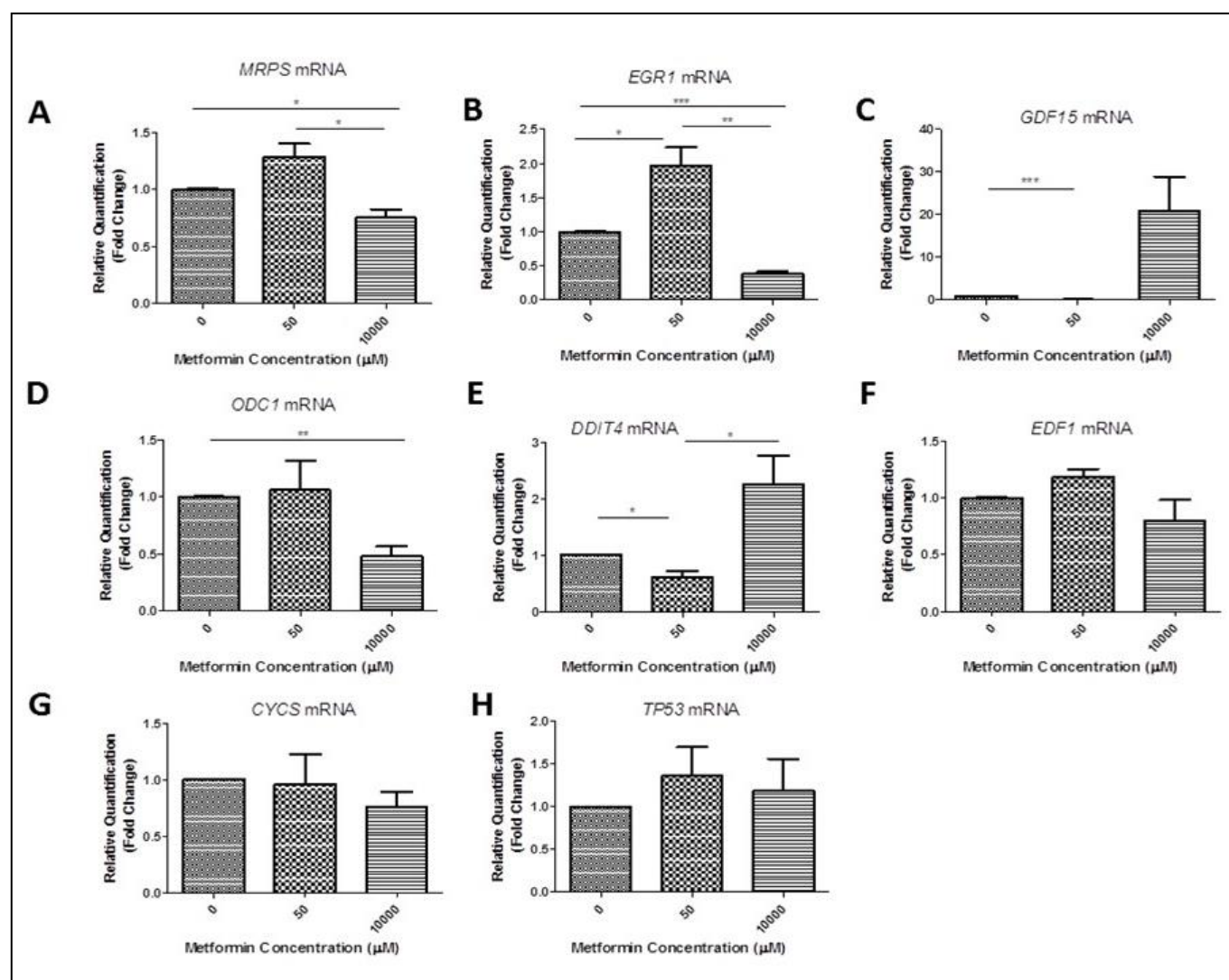


Figure 15: Validation of selected DEGs in metformin treated HEC-1A cells using qRT-PCR. Graphs represent the relative quantities of gene transcripts - MRPS (A), EGR1 (B), GDF15 (C), ODC1 (D), DDIT4 (E), EDF1 (F), CYCS(G), TP53(H) upon treatment with low (50 μ M) or high (10,000 μ M) metformin. (*p<0.05, **p< 0.01, ***p<0.001).

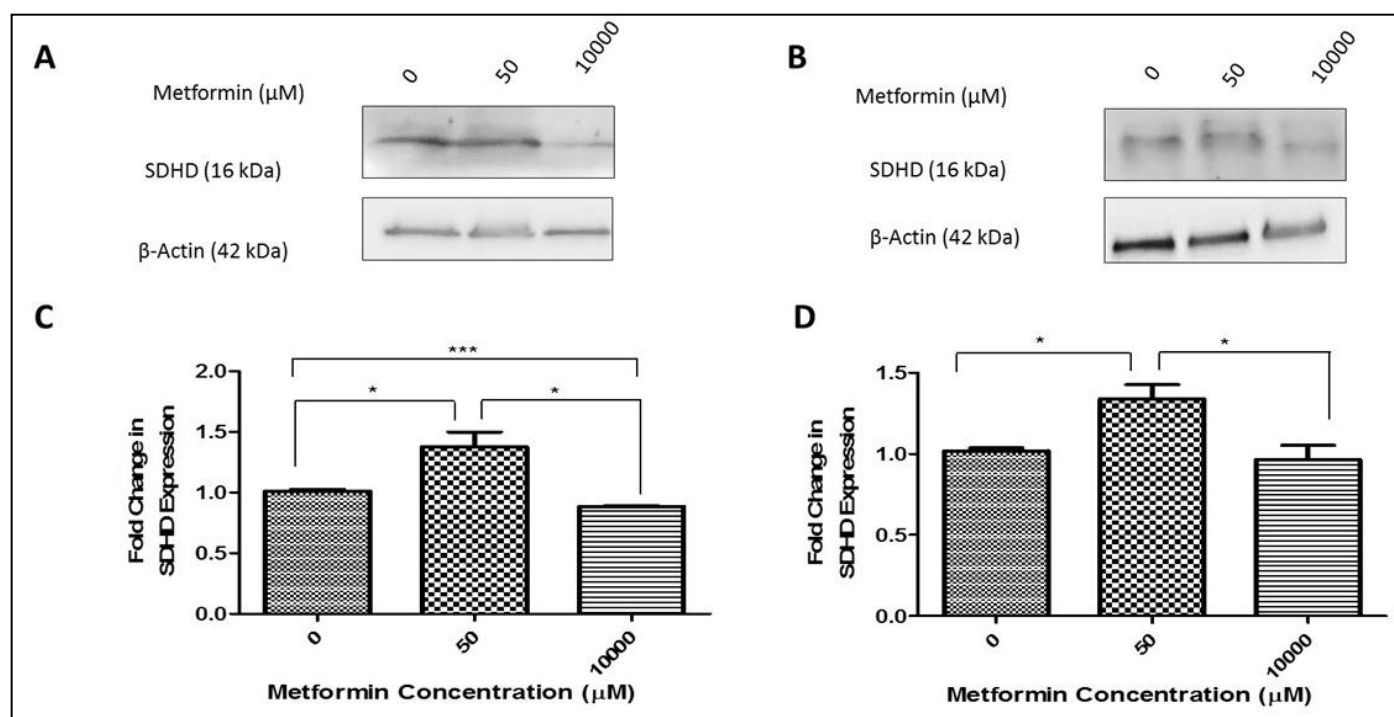


Figure 16: Succinate dehydrogenase (SDHD) expression in metformin treated Ishikawa and HEC-1A cells. Panels (A and B) represent immunoreactive bands of SDHD and β -Actin (internal control) in Ishikawa and HEC-1A cells respectively. Graphs indicate the densitometric analysis of SDHD/ β -Actin in Ishikawa (C) and HEC-1A cells (D) (* $p < 0.05$, *** $p < 0.001$).

1.12 Damage Associated Molecular Patterns (DAMPs) and their Receptors in Endometrial Repair *(Funded by Science and Engineering Research Board)*

Principal : **U Chaudhari**
Investigator
Principal Co- : Geetanjali Sachdeva
Investigator
Project : A Khandvilkar, SM Metkari,
Associates RR Katkam
Collaborators : Vandana Bansal, Deepti Tandon
Duration : 2019 – 2022

Heavy Menstrual Bleeding (HMB) or Menorrhagia is defined as menstrual blood flow > 80 ml or bleeding for > 7 days. It is one of the most common gynaecological problems and in India, it accounts for around 16% of gynaecological visits. Menorrhagia is caused due to altered or inadequate endometrial repair

post menstruation. It is established that early endometrial repair which occurs simultaneously during menstruation is hormone-independent. Early events of endometrial repair occurring simultaneously during menstruation are not completely understood. We hypothesized that damage associated molecules released during the process of endometrial breakdown facilitate the initial events of endometrial repair. During the previous year, we reported the development and characterization of a rat model of endometrial breakdown and repair (Annual report 2019-2020, p. 18). Ovariectomized rats were sequentially administered estrogen and progesterone and sacrificed at estradiol (E2) treatment and post-progesterone withdrawal at different time points; 0h (T0), 8h (T8), 24h (T24) and 48h (T48). Endometrial breakdown is initiated after progesterone withdrawal and repair of endometrium takes place within 48 hours. The endometrial tissues from these

groups were assessed for HMGB1 expression. Uterine fluid samples from these animals were checked for HMGB1 by ELISA. HMGB1 expression in endometrial glands was found to be significantly higher in estrogen dominant phase (E2 treated) as compared to progesterone dominant phase (T0). Progesterone withdrawal significantly increased glandular HMGB1 expression at T8 ($p<0.001$) compared to T0 (Fig. 17). A similar trend of HMGB1 expression was also observed in endometrial luminal epithelium and stroma (data not shown). Further, secreted HMGB1 levels were found to be significantly higher in the uterine fluid of E2 treated rats as compared to T0 group ($p<0.05$) (Fig. 18). Preliminary observation indicates estrogen increases and progesterone decreases the expression of HMGB1. The expression and secretion of HMGB1 increases immediately upon post progesterone withdrawal and levels and during the endometrial repair phase (24 to 48 hrs), HMGB1 levels were showed decrease trend. Further studies are ongoing to understand the role of inflammatory cells in the endometrial repair.

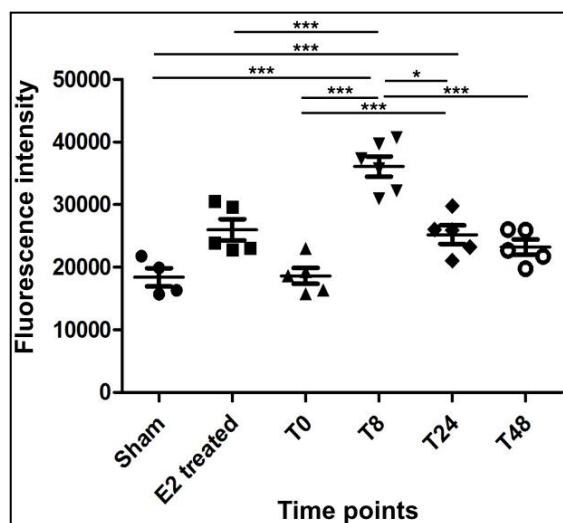


Figure 1: Graphs showing mean fluorescence intensity (MFI) of HMGB1 immunostaining in endometrial glands of different groups ($n=5$, except sham) of a rat model of endometrial breakdown and repair viz. sham operated ($n=4$), estradiol (E2) treated and post-progesterone withdrawal at 0h (T0),

8h (T8), 24h (T24) and 48h (T48). Data expressed in mean $\pm$ SEM (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

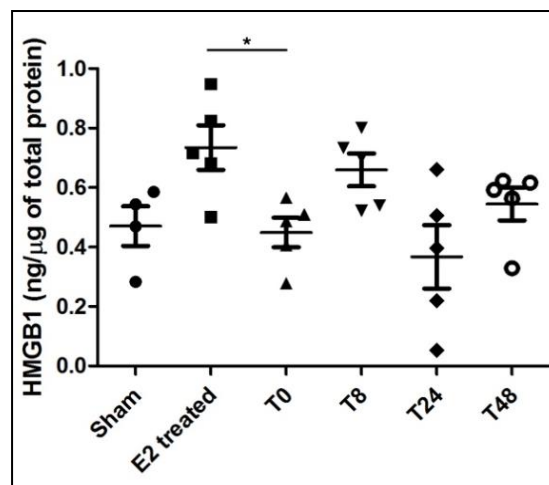


Figure 2: Graph to depict the concentration of HMGB1 (ng/ μ g of total protein) in the uterine fluid collected from different groups of rat model of endometrial breakdown and repair viz. sham operated, estradiol (E2) treated and post-progesterone withdrawal at 0h (T0), 8h (T8), 24h (T24) and 48h (T48). Data expressed in mean $\pm$ SEM (* $p<0.05$).

1.13 Determining the Role of HOXA10 in the Pathogenesis of Endometriosis

Principal : DN Modi
Investigator
Project : Anuradha Mishra, Nancy
Associates Ashary, Richa Sharma, S Metkari
Collaborators : M K Jolly, Indian Institute of Science, Bangalore
S Majumdar, NIAB, Hyderabad
Duration : 2021-2024

Endometriosis affects almost 10% of reproductive-age females and is observed in about 50% of infertile females. However, the cause of endometriosis is an enigma. HOXA10 is a transcription factor and required for implantation and decidualization in adult endometrium. Reduced expression of HOXA10 is reported in ectopic endometrium of women

with endometriosis. We had developed a mouse model where endometriosis was induced surgically by implanting uterine fragments with intestinal mesentery and ectopic lesions were collected. In these animals, there were altered levels of estrogen receptors while the levels of inflammation-associated genes (*Esr2*, *Ifng*, *Tnf* and *Il1b*) increased. There was no change in the levels of cytokeratin and vimentin in the ectopic endometrium, there was no N-Cadherin expression, collagen deposition, or α -SMA positive myofibroblasts suggesting that the mouse model for endometriosis is characterized by inflammation and cell proliferation but does not recapitulate epithelial-to-mesenchymal transition (EMT) and fibrosis like that seen in humans. To investigate the role of HOXA10, we surgically induced endometriosis in HOXA10 hypomorphs. We analyzed the expression of markers associated with EMT and fibrosis in ectopic lesions of mice hypomorphs for HOXA10. The results revealed that unlike that in controls, the ectopic lesions of mice with reduced HOXA10 (hypomorphs) expressed EMT markers mainly N-cadherin and TWIST2. There was intense staining of fibrotic marker α -SMA. Together these results suggest that loss of HOXA10 is a cause of EMT and fibrosis in endometriosis. Studies are ongoing to dissect these phenotypes in detail and identify the transcription factors associated with these responses.

1.14 Pregnancy Outcomes in Indian Women with Endometriosis and Biomarker Correlation: A Cohort Study *(Funded by Department of Science and Technology)*

Principal Investigator : **R Gajbhiye**
 Project Associates : Geetanjali Sachdeva, P Kuppusamy, Shagufta Khan, Aishwarya Bhurke, Sowmini C, Aarti

Collaborators : Srivastava, Mintumani
 Sarma, Amiya Majumdar
 PD Mahapatra, Spectrum
 Clinic & Endoscopy
 Research Institute, Kolkata
 P Das, Guwahati Medical
 College, Guwahati,
 Sreekumari R, SATH
 Medical College,
 Thiruvanthapuram
 B John, Credence Hospital,
 Trivandrum,
 Uma Singh, King George
 Medical University,
 Lucknow
 Ketki Kulkarni, Nowrosjee
 Wadia Maternity Hospital,
 Mumbai
 Duration : 2017-2022

Endometriosis is defined as the presence of endometrium-like tissue outside the uterine cavity. Retrospective studies indicated that women with endometriosis are at risk of pregnancy complications. Limited data is available on pregnancy outcomes in Indian women with endometriosis. The objectives of the present study are i) to study the progression of pregnancy in women with endometriosis in terms of pregnancy complications and outcome; ii) to estimate the serum levels of markers [anti-endometrial antibodies against Stomatin like protein 2 (SLP2), Tropomodulin 3 (TMOD3) and Tropomyosin 3 (TPM3)], placental growth factor (PlGF), and soluble fms-like tyrosine kinase 1 (sFlt-1) in sera of women with endometriosis and subsequently on becoming pregnant during 1st, 2nd, 3rd trimester and 6 weeks post-delivery; iii) to correlate the serum levels of markers with the type of endometriosis and pregnancy complications; and iv) to study the association between type of endometriosis (peritoneal, ovarian and deep infiltrating endometriosis) and pregnancy complications. In a cohort of 204 women with endometriosis associated

infertility, we observed deep infiltrating endometriosis (DIE) as the most common lesion (40%), followed by 31% ovarian endometrioma (OMA) and 29% superficial peritoneal endometriosis (SUP). Women recruited in eastern India had the highest proportion of SUP (41%). OMA was most commonly seen in the southern Indian population (40%), followed by the north Indian population (33%). The highest proportion of DIE was observed in the northern region of India (83%). The study demonstrated women with SUP and only one type of endometriotic lesion were more likely to conceive post-surgery. Therefore, we recommend that infertile women with endometriosis, younger than 35 years of age with SUP or with only one lesion type should be encouraged towards natural conception or assisted reproductive technology within 6 months after surgery.

1.15 Clinical Phenotypes and Genetic Regulation of Endometriosis in Indian Women *(Funded by Wellcome Trust DBT India Alliance)*

Principal Investigator	: R Gajbhiye
Supervisor	: Smita Mahale
Mentor	: G Montgomery, University of Queensland (UQ), Australia
Project Associates	: Komal Khade, Ashwini Patel, Kiran Kharsodiya, Aarti Kushwaha, Nafifa Rahman, Sheetal Dubey, Akshatha Shetty, Nadha Abdul Razack, Tabassum Khan
Collaborators	: Gita Mishra, UQ, Australia R Dikshit, Tata Memorial Center, Mumbai
Duration	: 2019-2024

Endometriosis is a chronic, estrogen-dependent, inflammatory condition associated with pelvic

pain, subfertility, dysmenorrhea, and dyspareunia, affecting ~ 10% of women of reproductive age. It affects an estimated 247 million women worldwide and 42 million women in India. The Endometriosis Clinical and Genetic Research in India (ECGRI www.ecgri.in) study aims to identify the clinical phenotypes and genetic risks associated with endometriosis in the Indian population. The objectives of this study are: i) to identify and characterize endometriosis subtypes [superficial peritoneal endometriosis (SUP), cystic ovarian endometriosis or endometrioma (OMA), and deep infiltrating endometriosis (DIE)] in different geographical regions of India; ii) to identify the clinical, epidemiological, environmental, lifestyle factors associated with different endometriosis subtypes in India; and iii) to investigate the genetic risks associated with endometriosis in the Indian population. The details of the collaborating centres and geographical locations of ECGRI study sites are shown in Table 1 and Fig. 19.

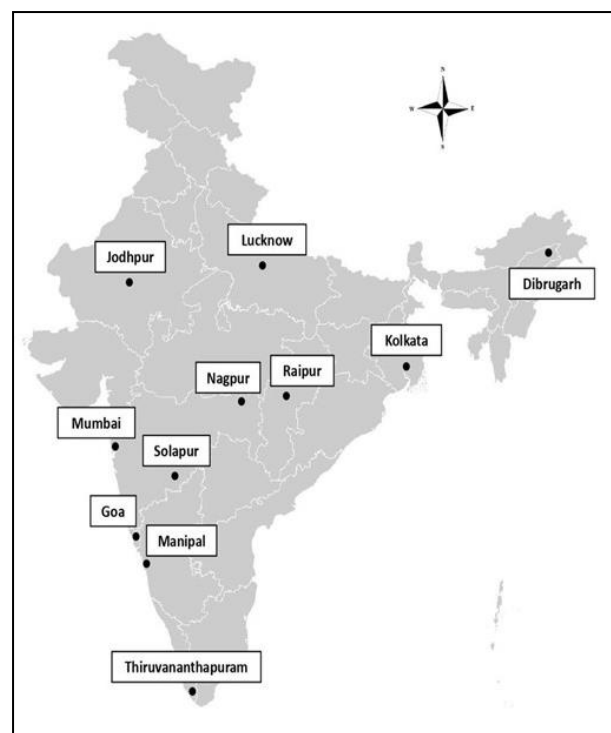


Figure 19: Network of ECGRI [6 Geographical Zones, 9 States, 11 Cities, 16 Study Sites, 17 Clinical Collaborators]

Table 1: ECGRI Study Sites and Collaborators

Zone	City , State	Study site	Collaborator
North-East	Dibrugarh, Assam	Assam Medical College	P Phukan
East	Kolkata, West Bengal	Spectrum Clinic	PD Mahapatra
North	Lucknow, UP	King George's Medical University Morpheus Fertility Clinic	Uma Singh, Aarti Srivastava Sunita Chandra
	Jodhpur, Rajasthan	AIIMS	S Shekhar
Central	Nagpur, Maharashtra	Government Medical College Omega Hospital	A Humane C Shembekar
	Raipur, Chhattisgarh	AIIMS Sai Baba Nursing Home	Nilaj Bagde Swati Mahobia
West	Mumbai, Maharashtra	Sanjeevani Endoscopy Centre Nowrosjee Wadia Maternity Hospital Worli Women's Hospital	Neeta Warty Ketki Kulkarni N Sardeshpande
	Solapur, Maharashtra	Naval Hospital	M Shah
	Panjim, Goa	Dr Kedar Hospital	K Padte
South	Manipal, Karnataka	Kasturba Medical College	M Pai
	Trivandrum, Kerala	Credence Hospital	B John

Recruitment of study participants was impacted by COVID-19 pandemic. During the reporting year, 410 endometriosis cases and 373 hospital controls were recruited. Endometriosis was classified as minimal, mild, moderate and severe disease [rASRM stages: I (n=153/476, 32.1%), II (n=53/476, 11.1%), III (n=93/476, 19.5%) and IV (n=177/476, 37.2%)]. The women were also categorized by lesion type (SUP, OMA, DIE), and lesion types (One type, Two types, Three types). All 3 sub-phenotypes of endometriosis: superficial peritoneal (SUP), ovarian endometrioma (OMA), and deep infiltrating endometriosis (DIE) were observed in Indian women. Isolated and/or overlap of subtypes was seen in Indian women. Distribution of isolated lesion types reported in our study cohort was as follows: 40.5% OMA, 12.8% SUP and 2.4% DIE (Fig. 20). Highest proportion of endometriosis cases in all the geographic zones was diagnosed with one subtype (46%-83%). Proportion of women diagnosed with two subtypes of endometriosis was highest in North zone (39%) while lowest in South zone (10%). All 3 subtypes were reported in 18% of patients in Northeast & East zones, 12% in central zone, 9% in west zone, 7% in south zone and 2% in north zone. Data was analysed for comorbidities.

Preliminary analysis shows positive association of endometriosis with thyroid disorders [OR 2.4 (1.6-3.6)] and polycystic ovary syndrome [OR 3.7 (1.0-13.3)]. Odds of having high blood pressure in women with endometriosis were about 2.2 times higher than the hospital controls [OR 2.2 (1.0-4.8)]. As more cases and controls become available for analysis, the magnitude and direction of association between comorbidities and endometriosis is likely to change.

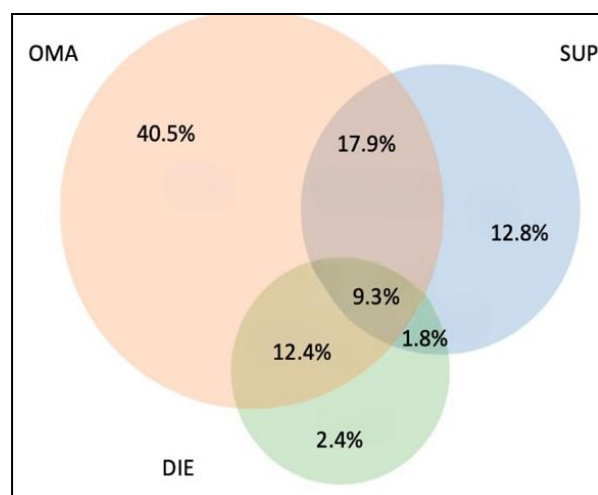


Figure 20: Distribution of endometriosis cases as per endometriosis lesion type. OMA is any patient with OMA ± SUP, but no DIE. SUP is any patient with SUP and no other lesion type. DIE is any patient with DIE and any other lesion type.

Table 2: Sociodemographic, clinical and surgical characteristics of Indian women with endometriosis cases and hospital controls

Characteristics		Cases	Controls	P value
Demographics		(n=491)	(n=487)	
Age (years), Mean ± SD		32.9 ± 6.9	31.7 ± 7.0	<0.01 ^a
BMI (kg/m²), Mean ± SD		24.0 ± 4.0	24.6 ± 4.2	0.03 ^a
BMI (kg/m²) categories (parenthesis indicate percentage)	Underweight (≤18.4)	43 (8.8)	22 (4.5)	<0.01 ^b
	Normal (18.5-22.9)	154 (31.4)	172 (35.3)	
	Overweight (23-24.9)	112 (22.8)	82 (16.8)	
	Obese (≥25)	182 (37.1)	211 (43.3)	
Education level	Up to Higher Secondary	117 (23.8)	168 (34.5)	<0.001 ^b
	Graduate and above	374 (76.2)	319 (65.5)	
Employment	Government/Private job	113 (23.0)	241 (49.5)	<0.001 ^b
	Self employed	40 (8.1)	53 (10.9)	
	Unemployed	313 (63.7)	139 (28.5)	
	Student	25 (5.1)	54 (11.1)	
Annual family income (in Rs.)	≤100,000	155 (31.6)	171 (35.1)	0.01 ^b
	100,000 - 500,000	231 (47.0)	246 (50.5)	
	≥500,000	105 (21.4)	70 (14.4)	
Marital status**	Married	431 (87.8)	308 (63.2)	<0.001 ^b
	Unmarried	52 (10.6)	161 (33.1)	
	Widowed/Divorced	8 (1.6)	18 (3.7)	
Area of residence	Urban	267 (54.4)	299 (61.4)	0.03 ^b
	Rural	224 (45.6)	188 (38.6)	
Clinical				
Age at Menarche (in years) [Cases n=489]	≤12	128 (26.1)	117 (24.0)	0.44 ^b
	>12	361 (73.5)	370 (76.0)	
Regularity of menses	Regular (n=455)	366 (80.4)	447 (95.7)	<0.001 ^b
	Irregular (n=467)	89 (19.6)	20 (4.3)	
Menstrual Bleeding Duration (in days), Mean ± SD		4.4 ± 2.2	4.2 ± 1.6	0.1 ^a
Menstrual Cycle Length (in days) [Cases n=451 Controls n=464]	<24	21 (4.7)	10 (2.2)	<0.0001 ^b
	24-31	327 (72.5)	392 (84.5)	
	>31	64 (14.2)	55 (11.9)	
	Too irregular	39 (8.6)	7 (1.5)	
Pain symptoms				
Dysmenorrhea	Yes	417 (84.9)	218 (44.8)	<0.0001 ^b
	No	74 (15.1)	269 (55.2)	
Dyspareunia	Yes	93 (18.9)	22 (4.5)	<0.0001 ^b
	No	328 (66.8)	331 (68.0)	
	No intercourse yet	46 (9.4)	124 (25.5)	
	Do not wish to answer	24 (4.9)	10 (2.1)	
Chronic Pelvic Pain	Yes	145 (29.5)	11 (2.3)	<0.001 ^b
	No	346 (70.5)	476 (97.7)	
Surgical				
Endometriosis subtypes (n=485)	SUP	63 (13.0)	-	-
	OMA	199 (41.0)	-	-
	DIE	12 (2.5)	-	-
	SUP + OMA	88 (18.1)	-	-
	OMA + DIE	61 (12.6)	-	-

	SUP + DIE	9 (1.9)	-	-
	SUP + OMA + DIE	46 (9.5)	-	-
	Unknown subtype	2 (0.4)	-	-
	Scar endometriosis	5 (1.0)	-	-
Endometriosis severity (n=476)	rAFS stage I	153 (32.1)	-	-
	rAFS stage II	53 (11.1)	-	-
	rAFS stage III	93 (19.5)	-	-
	rAFS stage IV	177 (37.2)	-	-
EFI score [#] (range 0-10)		6.0 ± 2.4	-	-
Mean ± SD (n=439)				

Abbreviations: BMI, Body Mass Index; rAFS, Revised American Fertility Society; EFI, Endometriosis Fertility Index

^a Student's t Test

^b Pearson's Chi Square Test

[#] EFI score for married, divorced or widowed women with endometriosis

1.16 Investigating the Contribution of DNA Damage, Repair and Demethylation in Pathogenesis of Endometriosis (Partly funded by Department of Biotechnology)

Principal Investigator : **Geetanjali Sachdeva**

Principal Co-Investigator : R Gajbhiye

Project Associates : Itti Munshi, Asma Rojewale, U Chaudhari, RR Katkam

Collaborators : A Mangeshkar (Jaslok Hospital, Breach Candy Hospital, ACI Cumballa Hill Hospital and Mangeshkar's Clinic), Pratima Thamke (MGM Hospital, Kamothe, Mumbai)

Duration : 2019-2023

Endometriosis is a condition in which endometrium-like cells are found outside the uterus. Previous reports suggest presence of higher oxidative stress, inflammation and proliferation in the eutopic endometrium from women with endometriosis. These altogether can lead to DNA damage in the eutopic endometrium from women with endometriosis. During the reporting period, paired eutopic and

ectopic endometrium were collected from women with (n=37) and without endometriosis (n=16). Histological dating on Hematoxylin and Eosin (H&E) stained endometrial tissue sections was carried out using Noyes criteria and the last menstrual period. In the endometriosis group 25 samples were histologically dated as "proliferative phase" and 12 were in "secretory phase". Fifteen participants in endometriosis group were infertile (13 with primary infertility; 2 with secondary infertility). All participants in the control group were fertile. Ten samples represented proliferative phase and five represented secretory phase at the histological level. Our previous study (Bane et al. 2022) demonstrated that the eutopic endometrium from women with endometriosis encounters higher DNA damage than its control counterparts. In addition, higher expression of GADD45 (Growth Arrest and DNA Damage Inducible) family proteins, GADD45a and GADD45g, was reported in proliferative phase eutopic endometrium from women with endometriosis compared to those without the disease. GADD45a is reported to be involved in DNA repair and demethylation, cell cycle and apoptosis. The present study was initiated to explore if higher DNA damage observed in the eutopic endometrium of women with endometriosis is associated with alterations in the DNA methylation machinery. MBD4 (Methyl-CpG Binding Domain 4), a glycosylase

involved in BER (Base Excision Repair) pathway is reported to be involved in active demethylation along with GADD45a and AID (Activation-Induced Cytidine Deaminase). Initial data revealed higher expression of MBD4 in the eutopic endometrium from women with endometriosis during proliferative and secretory phases compared to women without endometriosis (Fig. 21). Higher expression of MBD4 protein in eutopic endometrium from women with endometriosis, in response to DNA damage, could be because of increased protein stability as no significant change was observed in the levels of endometrial MBD4 transcript in women with and without endometriosis. Further the expression of GADD45a and MBD4 was found elevated in response to H₂O₂ induced oxidative DNA damage in t-HESC cell line (Fig 22). These investigations collectively revealed that DNA damage is associated with higher expression of MBD4 and GADD45a in endometrial cells from women with endometriosis. Further, studies are being conducted to assess the targets of MBD4. The study is anticipated to provide insights into the mechanisms by which the endometrium acquires differential gene expression signatures in women with endometriosis.

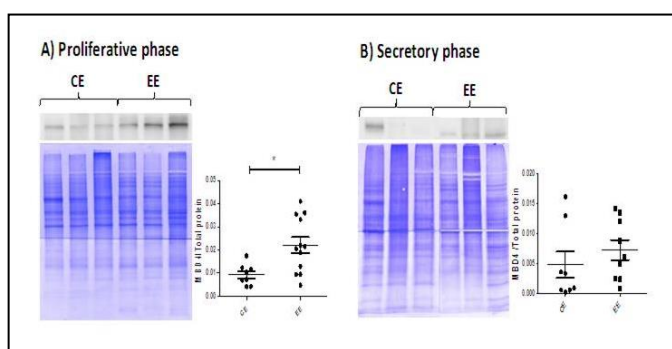


Figure 21: Expression of MBD4 protein in eutopic endometrium from women with and without endometriosis in (A) proliferative phase (EE, n=12; CE, n=8) and (B) secretory phase (EE, n=9; CE, n=8) of menstrual cycle.

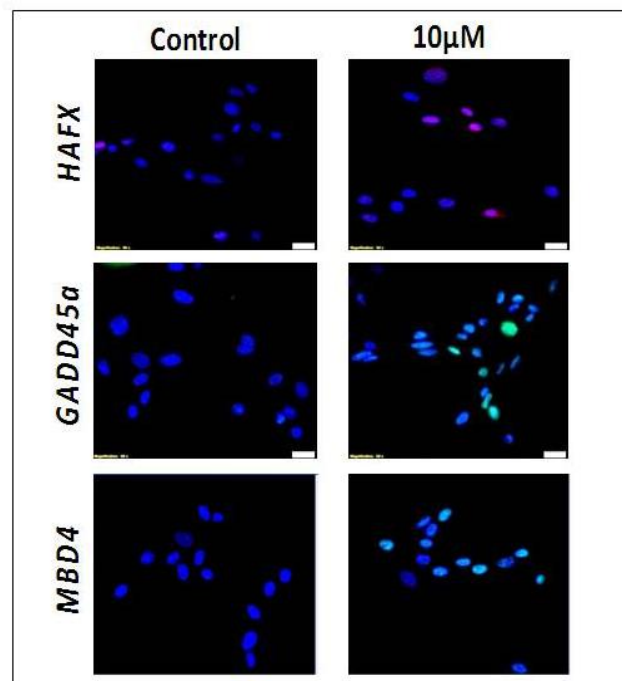


Figure 22: Expression of HAFX (red), GADD45a (green) and MBD4 (green) on treatment with H₂O₂ (10 μM, 8hr) in t-HESC cell line.

1.17 Uterine Alarmins and their Relevance in Implantation *(Partly Funded by Science and Engineering Research Board)*

Principal Investigator : **Geetanjali Sachdeva**
Principal Co-Investigator : S Adiga, Kasturba Medical College (KMC), Manipal
Project Associates : U Chaudhari, V Patel, SM Metkari, RR Katkam, Rithika Rajendran, Sheetal Singhania
Collaborators : P Narayan, Anjali M, Kasturba Medical College (KMC), Manipal
Duration : 2019-2022

Alarmins or Damage-Associated Molecular Patterns (DAMPs) are a diverse family of endogenous molecules, which upon stress or stimuli are released from the cells and initiate an inflammatory reaction by interacting with

Pattern Recognition Receptors (PRRs) such as Toll-Like Receptors (TLRs) and nucleotide-binding and oligomerization domain (NOD)-Like Receptors (NLRs) in sterile inflammation. Our laboratory had previously demonstrated the presence of HMGB1 and S100A8 in the uterine fluid of healthy women, wherein the levels of HMGB1 were found to be higher in the pre-receptive phase as compared to the receptive phase (Annual report 2010-11, pp. 25-26). The uterine fluid and tissue levels of HMGB1 were found to be the lowest in metestrous (receptive) phase, whereas highest levels were found in the diestrous phase in rats. Further excess of HMGB1 created in the uterus during the implantation period, resulted in implantation failure (Annual report 2013-14, pp. 23-24). These observations suggested that excess of extracellular HMGB1 was detrimental to implantation.

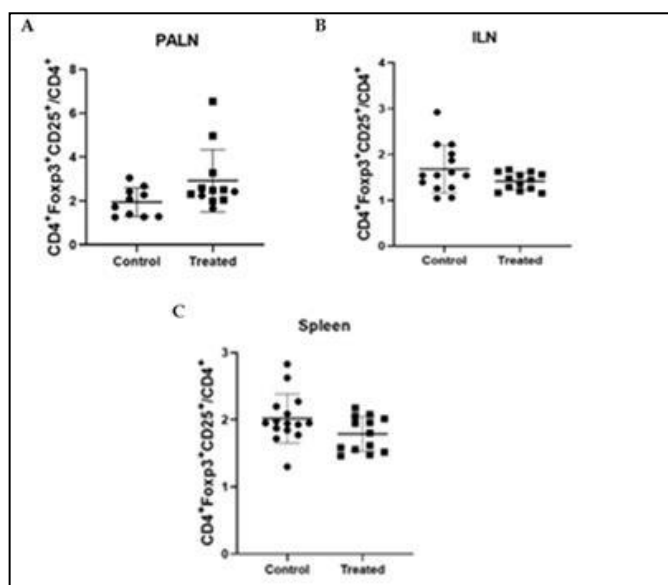


Figure 23: Effect of excess of HMGB1 on the frequency of CD4⁺FOXP3⁺CD25⁺ Treg cells in PALN (panel A), ILN (panel B) and spleen (panel C) during implantation as determined by flow cytometry of lymphocytes isolated on day 4.5 p.c. from sham (n=10) and rHMGB1-treated (n=12) animals.

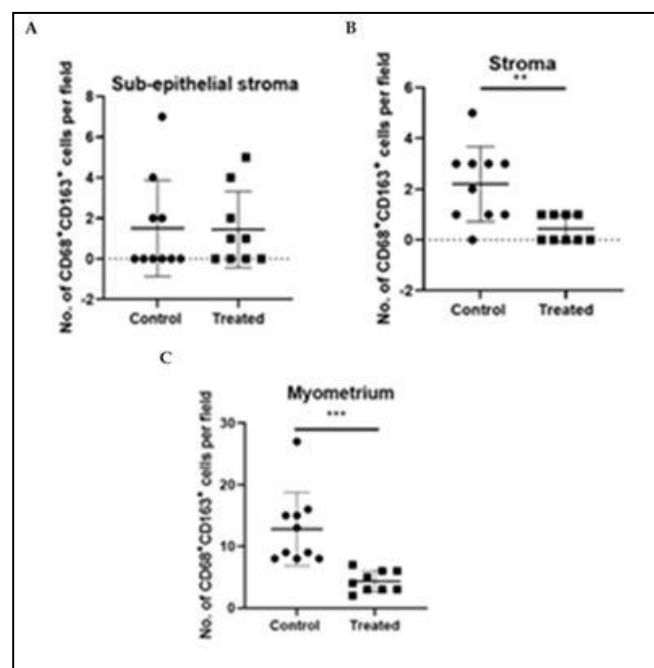


Figure 24: Effect of excess of HMGB1 on the distribution of CD68⁺CD163⁺ M2 macrophages in uterine sub-epithelial stroma (panel A), stroma (panel B) and myometrium (panel C) at implantation site of sham (n=10) and rHMGB1-treated (n=9) animals determined by immunofluorescence. * in panels B (p= 0.0036) and C (p= 0.0007) indicates statistical significance of the difference between the two groups.

The present study was conceptualised to understand whether altered levels of the alarmins HMGB1 and S100A8 modulate the immune profile of the endometrium during implantation. Towards this, 0.8µg/15µL of recombinant HMGB1 (rHMGB1) was administered to female Wistar rats on day 2.5 p.c. Rats were sacrificed on day 4.5 p.c. and lymphocytes were isolated from their para-aortic lymph nodes (PALN), iliac lymph nodes (ILN) and spleen and assessed for regulatory T (Treg) cell frequency. A trend (p = 0.0655) towards higher frequency of Treg cells (ratio of CD4⁺FOXP3⁺CD25⁺ to total CD4⁺ cells) was observed in the PALN of rHMGB1-treated animals as compared to that of sham control (Fig. 23A). Interestingly, the percentage of Treg cells was found reduced in ILN (Fig. 23B; p = 0.0985) and spleen (Fig. 23C; p = 0.0692) of

rHMGB1-treated animals. Further, reduced numbers of CD68 and CD163 double positive M2 macrophages in the stromal (Fig. 24B; $p = 0.0036$) and myometrial (Fig. 24C; $p = 0.0007$) compartments were observed in HMGB1-treated rats as compared to sham control rats. Further effect of reduced levels/function of HMGB1 in the uterine milieu on the uterine immune repertoire and embryo implantation will be studied. Efforts will also be taken to determine whether dysregulated levels of HMGB1 and S100A8 in the uterine fluid are associated with idiopathic recurrent implantation failure (iRIF).

1.18 Deciphering the Immunomodulatory Roles of Homeobox A10 in the Endometrium during Embryo Implantation (Partly Funded by Department of Biotechnology)

Principal Investigator : **DN Modi**
 Project Associates : Richa Sharma, Nancy Ashary, Anuradha Mishra
 Collaborator : S Chauhan, Institute of Life Science, Bhubhaneswar
 Duration : 2021-2024

Endometrial immunomodulation is a key event for the successful implantation of the semi-allogenic blastocyst and the continuation of pregnancy. Previous *in vitro* studies from our laboratory have shown that with loss of HOXA10, there is a burst in the expression of pro-inflammatory cytokines including IL-1 β , suggesting it may be a key regulator of the inflammatory process. Herein, we aim to investigate the involvement of HOXA10 and the regulation of inflammatory processes during embryo implantation and decidualization. We observed that during implantation, there is an activation of the cytokine IL1 β expressed at the

implantation site mainly in the decidua and not at the inter-implantation site. IL1 β is processed by inflammasomes which are oligomeric multi-protein complexes of NLRP3 and ASC. We found the existence of NLRP3 and ASC positive inflammasomes at the implantation site with peak expression on day 6 of mouse pregnancy. To investigate the role of NLRP3 in embryo implantation, we treated the animals with MCC950, which is a chemical inhibitor for NLRP3. Histological analysis revealed that on day 5 of pregnancy, the embryos in the control animals were well positioned in the implantation cup and oriented antimesometrially, whereas in the treated animals the embryos were loosely attached to the epithelium or were seen floating in the uterine lumen. The embryos were misoriented and the implantation cup had not correctly formed. By day 6, in controls, the embryos were embedded in the endometrial bed and the surrounding stroma had decidualized. In the MCC590 treated group, in most animals, there were no clear implantation sites. In the ones that had some embryos implanted, the stroma appeared abnormal and there was a failure of stromal cells to decidualize. These results revealed that NLRP3-mediated inflammation is required for embryo implantation and stromal cell decidualization. Studies are ongoing to determine if HOXA10 regulates NLRP3 and subsequent inflammation during early implantation.

1.19 Investigating the Role of Epithelial to Mesenchymal Transition in the Process of Embryo Implantation

Principal Investigator : **DN Modi**
 Project Associates : Nancy Ashary, Richa Sharma

Collaborator : MK Jolly, Indian Institute of Science, Bangalore

Duration : 2020-2023

Embryo implantation involves three crucial stages: apposition, adhesion, and invasion. Invasion of the embryo requires displacement of luminal epithelial (LE) cells so that the embryo can breach the epithelial barrier and be embedded in the stroma. The molecular mechanisms that regulate embryo attachment and invasion are poorly understood. Previously demonstrated that during the process of embryo implantation there is Epithelial to Mesenchymal Transition (EMT) in the Luminal Epithelium (LU), which is associated with the gain of EMT transcription factor such as SNAIL, SLUG, and TWIST in the luminal epithelium during embryo implantation. Using double immunofluorescence we observed that the EMT in the LE is not a complete transition but a hybrid state co-expressing both E-Cad and N-Cad. This hybrid state seems to be acquired due to activation of the hybrid EMT-associated transcription factor ZEB2 and TWIST2. During implantation, HOXA10 expression disappeared in the LE. To test if loss of HOXA10 is associated with EMT in the LE, we investigated the uteri of mice hypomorphic for HOXA10 and compared it with control mice. In the non-pregnant animals, LE was strongly positive for epithelial cell marker E-cad and negative for the mesenchymal marker N-cad. However, in the HOXA10 hypomorphs, the LE was low in E cad and was positive for N-cad. These observations suggest that loss of HOXA10 was sufficient to drive hybrid EMT in the LE cells of the endometrium. In the pregnant control animals, the LE was intact and polarised on day 4 when the embryos began apposition. However, in HOXA10 hypomorphs, the LE cells were in the hybrid EMT state, had prematurely started sloughing and gaps were created in the LE barrier. Together our results suggest that loss of

HOXA10 is necessary for inducing EMT in the LE. Studies are ongoing to identify the downstream targets of HOXA10.

1.20 Understanding the Biosensing Ability of Endometrial Cells to Embryonic Aneuploidy: a Pilot Study

Principal Investigator : Nupur Mukherjee

Principal Co-Investigator : D Modi

Collaborator : K Chaitanya, Oasis Center for Reproductive Medicine, Hyderabad

Duration : 2021-2024

Embryo implantation is a crucial step for a successful pregnancy and implantation failure, the leading cause of infertility. The successful implantation of the blastocyst depends on adequate interactions between the embryo and the uterus. The rate at which embryos successfully implant has been suggested to be on account of two factors: (i) the quality of the embryo and the (ii) receptiveness of the uterus. Previous studies have reported the frequency of chromosomal abnormalities to be higher in embryos from patients experiencing multiple IVF failures. There is accumulating evidence showing that IVF culture medium also has an impact on pre and post-implantation development thereby implicating the role of embryo-derived factors and signaling cross-talk for successful implantation and pregnancy outcome. Several lines of evidence support the notion that aneuploidy is common, in early-stage mammalian embryos. The occurrence of mitotic errors is highest during the first three cleavages after fertilization, and as a result about three-quarters of human preimplantation embryos show aneuploidies and are chromosomally mosaic. 73% of all human

preimplantation embryos after IVF are mosaic, 22% are diploid and 5% contain other abnormalities. It is well known that many aneuploid embryos are morphologically normal and can form high-grade blastocysts. It is not clear if the endometrium executes its biosensor capability to select euploid blastocysts for implantation. The mechanisms by which the endometrial cells differentiate between morphologically good and poor quality embryos are well known, however, the molecular pathways that operate to differentiate between the euploid and aneuploidic embryos are hitherto unexplored. The aim of the study is to determine if the endometrial cells respond differently to euploid and aneuploid blastocysts.

For the study, spent blastocyst culture medium from euploid, and aneuploid embryos will be collected from clinical collaborator's lab where routine testing of IVF embryos (cultured till 5-day blastocysts stage) is done for Preimplantation Genetic Testing for Aneuploidies (PGT-A) through NGS. Thus

collected blastocyst culture medium from euploid/aneuploidy embryos will then be co-cultured with endometrial cells (decidualized stromal cells and epithelial cells) and further assessed for changes in endometrial cell characteristics (cell proliferation/migration/alterations in transcriptome profile). The study will help to determine if the endometrial cells can differentially sense euploid vs aneuploidy blastocysts and identify novel genes and signaling pathways involved in the biosensing functions of the endometrium, which will eventually help in the understanding of the causes of repeated implantation failures and miscarriages.

Approval from NIRRCH Scientific review committee was received on 13/9/2021 and the study has been submitted to NIRRCH ethics committee for clinical studies for approval. Ethics approval from Clinical collaborator's institute ethics committee (Oasis Ethics Committee) was received on 15/12/2021 for initiating the study.

2. MALE INFERTILITY AND ASSOCIATED REPRODUCTIVE DISORDERS

2.1 Design and Development of a Microfluidic Chip for Sperm Selection/Sorting Based on Chemotaxis (*Partly Funded by DBT*)

Principal Investigator : **Priyanka Parte**

Project Associates : Shraddha Gandhi, Smita Yevate, D Gaikwad, MT More

Collaborator : V Gundabala, Indian Institute of Technology Bombay

Duration : 2021-2024

Infertility among young couples is on the rise in India as well as globally. Consequently, there is a marked increase in the need for Assisted Reproductive Techniques (ART). In-Vitro fertilization is one of the most expensive types of ART having 10-30% success rate depending upon several factors. Despite being an expensive process, the desire to become biological parents is overwhelming enough to drain infertile couples emotionally and financially apart from the psychological stress due to the repeated attempts. Although recent advances have improved the success rate of fertilization, the take home baby rate still remains low due to implantation failure. Several evidences are available implying the role of sperm not just in fertilization but also in implantation. Selection of sperm is therefore imminent to the success of IVF. This will help bring down the cost associated with IVF by reducing the repetitive IVF attempts and also enhance the prevalent IVF success rate. Our group has earlier reported a microfluidic device for studying sperm chemotaxis (Annual report 2018-2019, pp. 25-27). Using this device N-Formyl L aspartate was identified as a novel

sperm chemoattractant (Annual report 2020-2021, pp. 33-34).

The present study is aimed at developing a microfluidic device to sort the good quality sperm based on chemotaxis. COMSOL Multiphysics 4.4 (FEM simulation) software is being used to test and optimize the device design. Data obtained from this simulation can reliably predict the utility of the device for its desired purpose, minimizing the development time as well as the cost of fabrication. Finite element simulations were carried out by solving the mass and momentum conservation equations. Incompressible-fluid Navier-Stokes equation was used to model the fluid motion in the channel while convection-diffusion equation will be used to determine the concentration profile. After simulation, device design was drawn in CleWin4 software and printed on a photomask that serves as framework for the design. The device design was printed on silicon wafer using soft lithography and further prepared using Polydimethylsiloxane (PDMS). The device will be tested for its efficacy using mouse sperm and if necessary, the design may have to be fine-tuned.

2.2 Investigation of Potential Chemotactic Metabolites in the Follicular Fluid

Principal Investigator : **Priyanka Parte**

Project Associates : Durva Panchal, MT More

Collaborator : Priyanka Vora, Nowrosjee Wadia Maternity Hospital, Mumbai

Duration : 2020-2024

In mammals, sperm chemotaxis is an important guidance mechanism directing sperm to the egg. Recent studies by our group have reported N Formyl L Aspartate (NFA) to be a sperm chemoattractant at 0.01M concentration. Further studies to understand the mechanism of chemotaxis mediated by NFA investigated the response of sperm to gradients ranging from 0.001 to 0.02 M NFA using microfluidics-based chemotaxis assays. Whilst the percentage of sperm moving towards higher concentrations and their velocities increased concomitant to the gradient concentrations, it was highly significant at gradient concentration of 0.01 M NFA. A significant increase in the percentage of sperm with VSL range of 81-160 and >160 $\mu\text{m}/\text{sec}$ was noted with 0.01 M NFA. At 0.02 M NFA, there was a drop (Fig. 25A-C). This bell-shaped

dose response curve is characteristic of a chemoattractant. NFA has been patented as a β -2-AR agonist. The presence of cognate receptors for NFA on rat sperm was therefore investigated using β -2-AR specific antibody, which detected a distinct band at approximately 72 kDa (Fig. 26A). The expression of β -2-AR did not differ between capacitating and non-capacitating sperm. Localization by immunofluorescence showed that β -2-AR are localized on the head, and in the mid-piece region of the sperm, with fainter fluorescence along the principal piece and end piece. Loss of the positive signal on permeabilization of sperm membrane indicated its presence on the sperm surface (Fig. 26B). This observation suggested that β -2-AR may be the putative receptor for NFA.

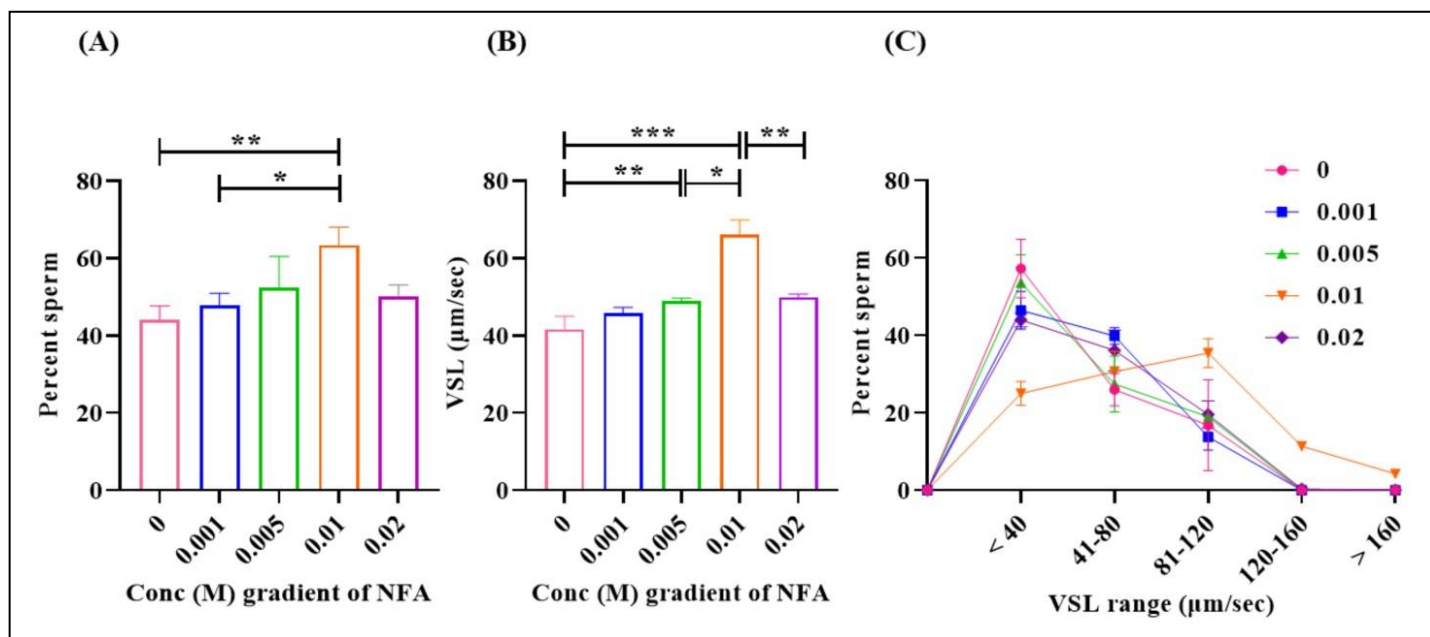


Figure 25: Bell-shaped dose response of sperm to NFA Chemotaxis was studied in the microfluidics device at gradient concentrations of 0, 0.001, 0.005, 0.01 and 0.02 M NFA after capacitating sperm for 2.5 h. Sperm directionality represented as percentage of sperm entering the transverse channel and moving towards the ascending concentration gradient of NFA (A), Sperm VSL (B), and Frequency distribution of sperm VSL in response to increasing gradients of NFA (C). Each column represents Mean $\pm$ SEM for the respective gradient tested (**p < 0.01; *p < 0.05).

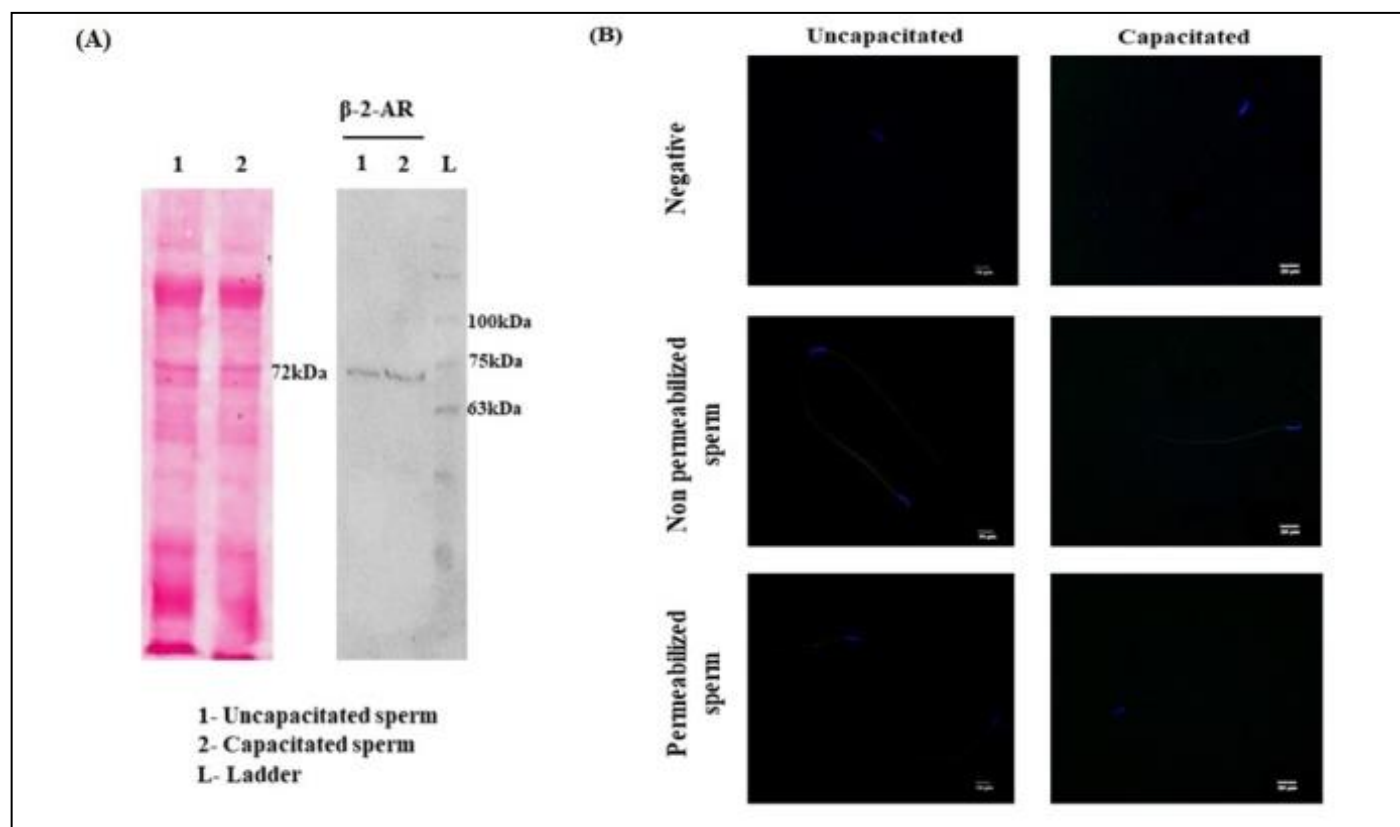


Figure 26: Expression and localization of β -2-AR in sperm lysates of uncapacitated and capacitated sperm by western blot analysis, using antibodies specific for β -2-AR (A). Immunofluorescence localization shows the presence of β -2-AR (green) in uncapacitated and capacitated intact and permeabilized sperm (B).

2.3 Microtubule (MT) Dynamics and Sperm Function: Involvement of Tubulin Acetylation/Deacetylation (Partly Funded by ICMR Medical Innovation)

Principal Investigator : **Priyanka Parte**
 Project Associates : Veena Dalvi, Smita Yevate
 Collaborators : R Gajbhiye, Kedar Ganla, Ankur Fertility Centre, Mumbai
 Duration : 2018-2021

The present study aimed to determine the status of microtubule (MT) dynamics in sperm, ascertain the role of α tubulin acetylation/deacetylation in this phenomenon, and its implication to sperm motility. Towards

this, Microtubule Associated Protein (MAP) like characteristics of HDAC6 and role of HDAC6 in MT stability of sperm flagellar axoneme were demonstrated. Furthermore, the MAPs were identified in human sperm. The list of proteins identified in MAP fraction was analysed with already known proteins interacting with HDAC6 and Tau (Biogrid database). Few of the proteins found to be common amongst them were reported previously (Annual report 2020-2021, pp. 37-38). In this reporting year, HDAC6 Interacting Proteins (HIPs) were identified by coimmunoprecipitation from human sperm lysates using HDAC6 specific monoclonal antibody. Immunoprecipitated eluates of normozoospermic men were pooled and subjected to LC-MS/MS analysis. Enrichment in Biological Processes, Molecular Function and other relevant categories was analysed using ShinyGo 0.76 Gene ontology tool. The

interacting partners identified were predominantly involved in cytoskeletal organization, cytoskeletal protein binding, cell projection organization and structural molecule activity. Some of these are known for their role in post-translational modifications, developmental biology, ciliary landscape and situs inversus.

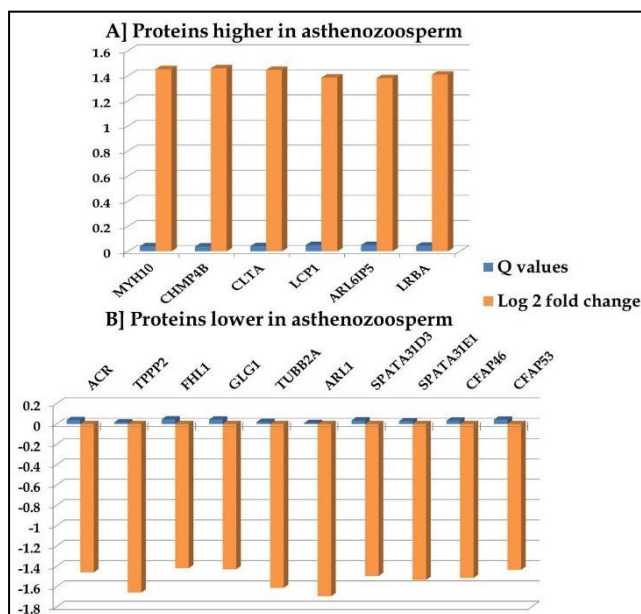


Figure 27: Label free quantification of identified proteins in asthenozoosperm. Crucial proteins identified as MAPs by 'MAP analyzer' were observed to be differentially expressed in asthenozoosperm. Q values and Log 2 fold change values are plotted.

Analysis of the MAP fraction identified 315 proteins and that of HIPs identified 1681 proteins. GO analysis identified 90 and 567 proteins from these respective groups to be enriched in motility and reproductive processes. 82 proteins were present in the 'MAP fraction' as well as in HIPs. The protein lists of MAP and HIPs were processed in 'MAP analyzer' and those identified as MAPs by MAP analyser were further quantified in normozoosperm and asthenozoosperm by high-throughput Label free quantification with data dependent acquisition. The expression of ACR, TPPP2, FHL1, GLG1, TUBB2A, ARL1,

SPATA31D3, SPATA31E1, CFAP46, CFAP53 were observed to be lower in asthenozoosperm as compared to normal sperm. The expression of MYH10, CHMP4B, CLTA, LCP1, ARL6IP5, LRBA were higher in asthenozoosperm as compared to normozoosperm. Overall, the work carried out in this project suggested that the threshold levels of MT acetylation/deacetylation along with microtubule associated proteins (MAPs) are important for MT dynamicity. This study also identified novel MAPs like ACR, FHL1, GLG1, ARL1, ARL6IP5, LRBA, LCP, CLTA, CHMP4B, SPATA31D3, SPATA31E1 which are hitherto unreported. The study also reports changes in expression of few MAPs in asthenozoosperm.

2.4 Functional Significance of Testis Specific Histone H2B variant (TH2B) in Sperm and Early Embryonic Development

Principal : **Priyanka Parte**
Investigator
Principal Co- : Nafisa Balasinor
Investigator
Project : A Patankar, Isha Singh,
Associates DVS Sudhakar, D
Gaikwad, M More
Collaborators R Gajbhiye, Suchitra Surve
Duration : 2018-2024

Spermatogenesis involves unique chromatin organization in which majority of DNA is compacted with protamines while histones are retained at loci important for embryo development. Sperm chromatin compaction abnormalities have been correlated with adverse IVF and ICSI outcomes. Our previous highthroughput phosphoproteomics study reported lower Testis Specific Histone 2B (TSH2B) in asthenozoospermic men (AS). These observations however remained to be validated. In the previous period, TSH2B associated genes in fertile human sperm by ChIP-qPCR were validated and their expression

was studied in sperm of infertile men (Annual report 2020-21, pp. 39-41). In the reporting period, to correlate human TSH2B ChIP-seq data with mouse model, ChIP-seq analysis was done to understand the epigenomics of the retained TH2B and TH2A, using murine caudal sperm. Our observations in mice indicate that contrary to existing belief, TH2A and TH2B have relatively fewer overlapping genomic regions (Fig. 28A). TH2A is majorly found in intergenic region, whereas TH2B is mainly in

promoter proximal and exonic sequences (Fig. 28B). Genomic distribution revealed 35% of TH2B peaks within $\pm 5\text{kb}$ of TSS. TH2B was enriched at spindle assembly and meiosis specific genes, and embryo development was the most significant term amongst TH2B associated genes (TBAGs) (Fig. 28C). This is important as TH2A/TH2B DKO mice have defective cohesin release. TH2A was enriched with mitochondrial function genes (Fig. 28D).

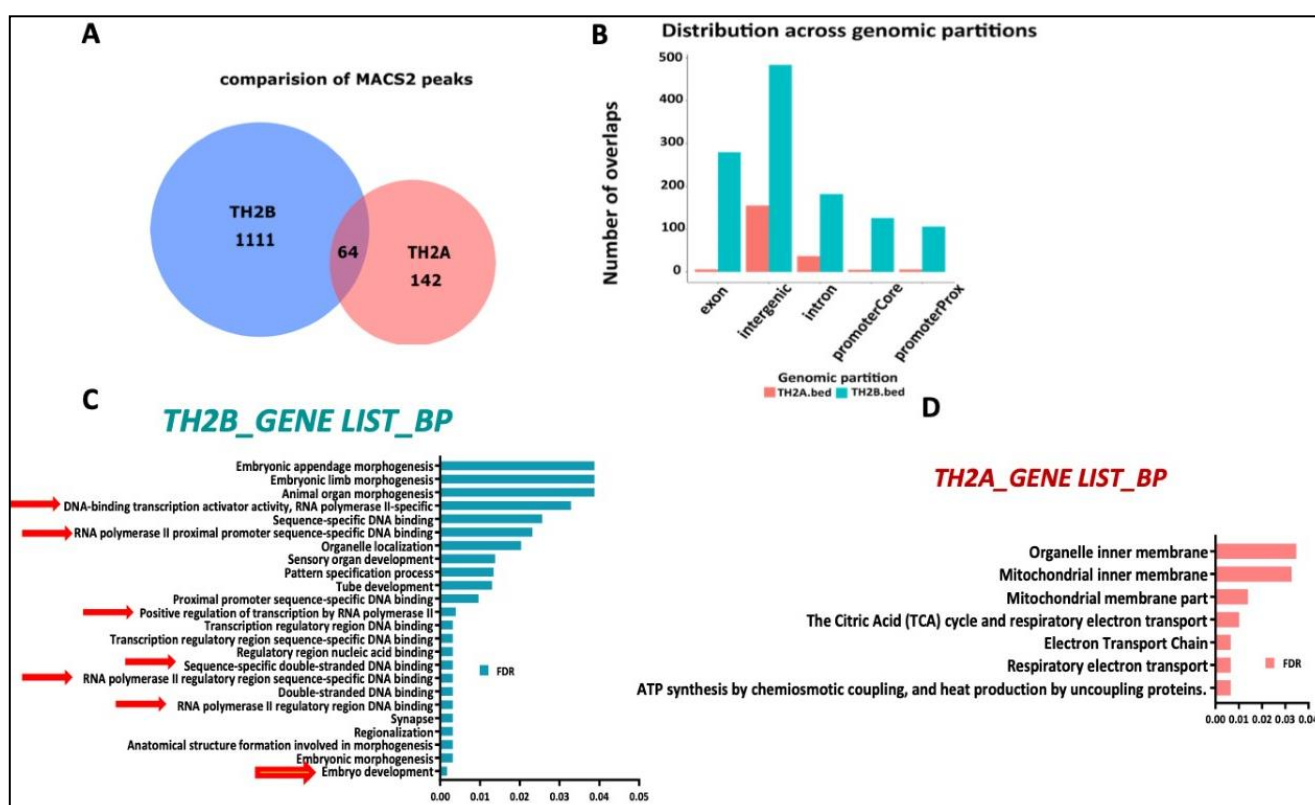


Figure 28: A: Overlap between TH2A and TH2B peaks; B: Distribution across genomic partitions; Gene Ontology of TH2B- (C) and TH2A associated genes (D)

Presumably, TH2A is associated with mitochondrial DNA inserted in nuclear DNA. Also, there was 26% evolutionary conservation between human and murine TBAGs including genes crucial for embryogenesis. Most importantly, heterogeneity in the epigenetic landscape of TH2A and TH2B was seen. Chromatin compaction (Histone and Protamine status), levels of TSH2B and its phosphorylated form in infertile men were also investigated.

Histone and protamine content in sperm of 54 fertile (N) and 64 infertile men was assessed by Aniline Blue (AB) and CMA3 staining, respectively. AB staining positively correlates with the level of histones present in a cell while CMA3 staining negatively correlates with the protamine levels. Samples from AS-, OS-, and OAS men showed higher percentage of AB- and CMA3 positive sperm indicating higher histones and lower protamines in sperm

($p < 0.001$; Fig. 29A & B). Phosphorylated TSH2B (pTSH2B) levels in sperm of AS men were studied by pan-phospho immunoprecipitation followed by western blotting. TSH2B and Total H2B levels were observed to be significantly lower in sperm of OA and OAS men ($p < 0.001$; Fig. 29C). In AS men, not TSH2B per se but pTSH2B levels were reduced ($p < 0.05$; Fig. 29D). Lower levels of TSH2B in OA- and OAS men and of pTSH2B in AS men can be correlated with inefficient meiosis and reduced sperm motility, respectively.

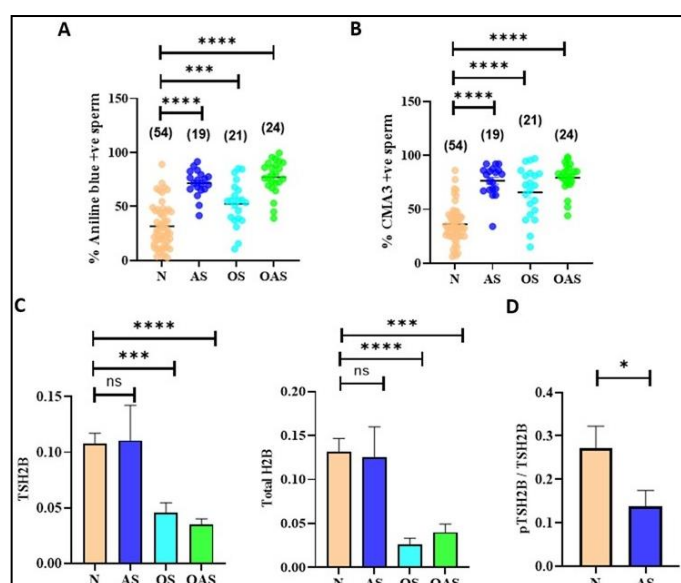


Figure 29: Chromatin compaction, total H2B, TSH2B and phosphorylated TSH2B in sperm of fertile and infertile individuals. The dot plots show percent of aniline blue (A) and CMA3 (B) positive sperm from semen samples of Fertile (N) and Asthenozoospermic (AS), Oligozoospermic (OS) and Oligoasthenozoospermic (OAS) infertile men. Numbers in parenthesis indicate the number of participants in that group. Bar plot shows densitometric analyses of the bands for TSH2B, total H2B (C). Pan-phospho immunoprecipitated proteins from fertile and AS individuals were subjected to Western blot analysis for TSH2B. Bar plot represents the ratio of pTSH2B: TSH2B in fertile and AS infertile men (D). pTSH2B was determined by normalizing band intensity of pTSH2B to its respective input. Data are presented as Mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

2.5 Identification of New Male Infertility Genes in Obstructive Azoospermic Men with Congenital Bilateral Absence of Vas Deferens (Funded by Department of Biotechnology)

Principal : **R Gajbhiye**
Investigator
Project : DVS Sudhakar, Shagufta
Associates Khan, V Dighe
Collaborators : R Shah, V Kulkarni
Duration : 2019-2022

Congenital bilateral absence of the vas deferens (CBAVD) affects 2-3% of all male infertility cases and responsible for 25 % of cases of obstructive azoospermia. The objectives of the study are: i) to identify and characterize new male infertility genes in obstructive azoospermic men with CBAVD; ii) to identify the genes involved in CBAVD men having unilateral renal anomalies; and iii) to investigate the role of Adhesion G Protein-Coupled Receptor G2 (ADGRG2) gene and other new male infertility genes (identified from objective 1 and 2) in Indian men with CBAVD. We performed whole exome sequencing (WES) in 16 men of which, 9 were men with CBAVD, 4 were men with CBAVD and unilateral renal agenesis (URA), 3 were men with congenital bilateral absence of vas deferens (CUAVD) and 1 individual with CUAVD and URA phenotype. On WES analysis, a total of 40 rare, pathogenic, LoF, missense, splice site single nucleotide variants and small frameshift indels were prioritised from 40 genes for validation. We have selected hybridisation based targeted re-sequencing approach with high mean depth (300x) for sequencing of the 40 potential candidate genes. Target panel (206kB) was designed using Agilent Sure design software and target sequencing is ongoing.

2.6 Role of Complex N Glycans and Functional Significance of Basigin (Glycoprotein Possessing N Glycans) in the Testicular Germ Cells for Spermatogenesis (Funded by DST-INSPIRE)

Principal Investigator : **Barnali Biswas**
 Mentor : Taruna Madan
 Project Associates : Rupashree Salvi,
 RD Shinde
 Duration : 2018-2023

N-linked glycosylation is a prime post-translational modification that plays important role in sustaining spermatogenesis and male fertility. Glycosyltransferases are key components of N-linked glycosylation machinery that govern protein folding and orientation of protein. Dysregulation or loss of glycosyltransferases can lead to differential cell

surface protein expression and ER stress. Previously (Annual report 2020-2021 p. 42-43), down regulation of IGF-1R transcript and increased expression of integrins was reported in Lec1 cells (N-acetylglucosaminyltransferase I, MGAT1 deficient CHO cells) and Lec4 cells (N-acetylglucosaminyltransferase V, MGAT5 deficient CHO cells) compared with wild type Chinese Hamster Ovary cells (CHO cells, W5) as control. This can be due to a defect in processing of the receptor. The reduction in IGF1-R was confirmed by Gene Set Enrichment Analysis (GSEA), which showed significant enrichment of IGF1-R signaling in control cells. Western blotting analysis confirmed significant reduction of IGF-1R levels in Lec4 cells as compared to W5. The cells were induced to overexpress IGF-1R with IGF-1R3, a ligand of IGF1R. After induction, the IGF1-R expression was increased in W5 but not in the Lec4 cells (Fig. 30).

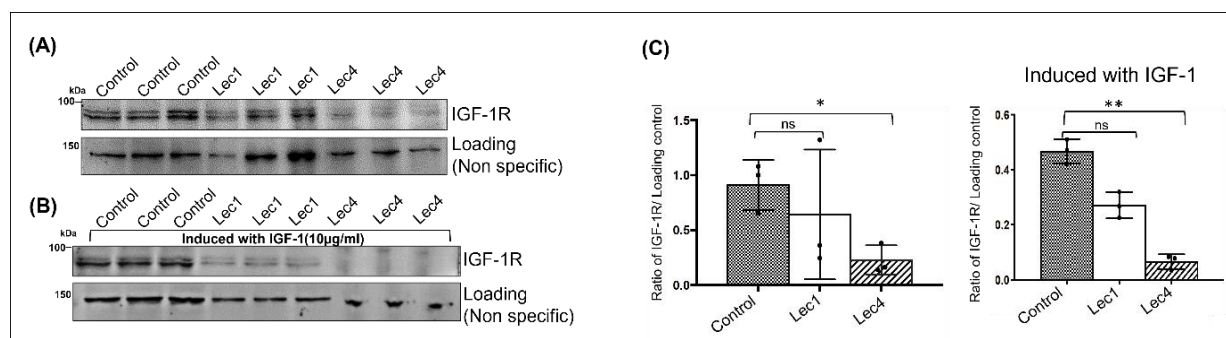


Figure 30: Insulin-like growth factor-1 receptor (IGF-1R) transcript and protein levels expression in W5, Lec1 and Lec4 cells. A) Cells incubated in serum free media for 12 h, Protein lysates were analysed by Western blot for IGF-1R and nonspecific band is used as loading control. B) Cells were stimulated with IGF-1R ligand (IGF-1R3) for 10 minutes. C) Protein lysate were analysed by WB for IGF-1R and nonspecific band used as loading control. Signals were quantified by densitometry, normalised to nonspecific band and expressed as ratio of IGF1R to nonspecific band for control cells. Data correspond to mean $\pm$ SEM from three independent experiments. *P value=0.05 and **P value <0.05-0.01.

To decipher the plausible mechanisms that cause downregulation of IGF-1R expression in N-glycosylation deficient cells, it was hypothesised that IQGAP1 could be involved in IGF-1R signalling. *In-silico* docking studies suggested that the IQ domain of IQGAP1 directly binds to Kinase domain of IGF1R (Fig. 31). The IQGAP1 interaction might be essential

for proper orientation of IGF1-R intracellular domain to maintain the kinase activity of the receptor. To summarise, in the absence of N-glycosylation, the amount of IGF-1R present on the cell surface is low, IGF1 induces minimal synthesis of IGF-1R and that IQGAP1 is likely a binding partner for IGF-1R.

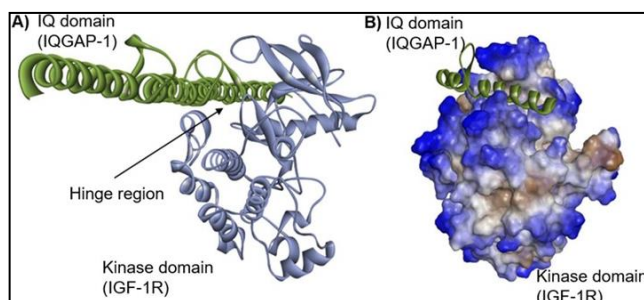


Figure 31: Docked pose of IQGAP1 and IGF1R through their IQ (modelled) and kinase (PDB ID: 1JQH) domains respectively (A) represents interaction of IQ domain (pale green) and kinase domain (pale blue) in cartoon view, and (B) represents interaction of IQ domain (pale green) with hydrophobic surface (brown hydrophobic and blue hydrophilic) of kinase domain.

2.7 Identification and Characterization of Sertoli and Leydig Cell Homing Peptides

Principal : VD Dighe
Investigator
Principal Co- : Taruna Madan
Investigator
Project : Yugandhara Jirwankar,
Associates : S Jadhav, P Salunke
Duration : 2020-2023

Conventional treatment and delivery methods of drugs for testicular disorders face limitations such as low efficacy due to the need for high dose and high dosing frequency, systemic toxicities, the problem of feedback inhibition,

etc. All these issues can be solved with the help of selective targeting of drugs to the testis. Homing peptides are the potential ligands for targeted drug delivery and diagnosis and can act as therapeutic molecules. In this study, efforts are being carried out to identify Sertoli cell and Leydig cell homing peptides using phage display peptide library and characterization of a few selected peptides. Initially, Sertoli Cell Homing Peptides (SCHP) were identified successfully using in-vitro and in-vivo biopanning followed by the Sanger sequencing method. Two of these peptides, named SCHP1 and SCHP2 were shown to be specific to Sertoli cells, compared to a scrambled version of them. During the reporting period, Sertoli Cell Surface Homing Peptides (SCSHPs) and Sertoli Cell Penetrating Peptides (SCPPs) were identified using next-generation amplicon sequencing. Phage pools from the third round (last round of in-vitro panning) and the sixth round (last round of in-vivo panning) from the SCSHP and SCPP biopanning were subjected to high throughput sequencing. The number of unique peptides normalized with a total number of good reads for both the SCSHP and SCPP panning experiments decreased in the 6th round compared to the 3rd round (Fig. 32A and B), indicating enrichment of a few peptides from the 3rd round to 6th round. Only 1551 peptides were found common in all the four phage pools sequenced (Fig. 32C).

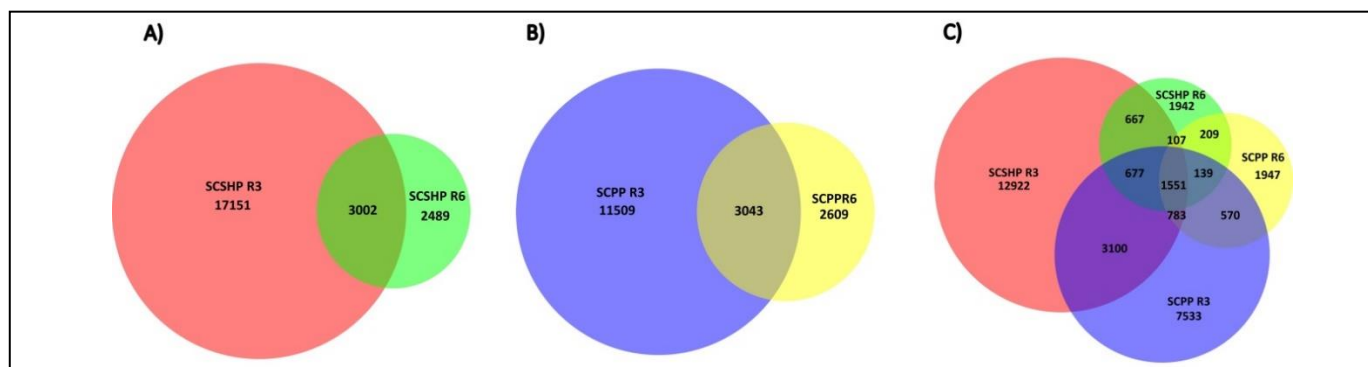


Figure 32: Venn diagram showing common peptides obtained in phage pool of round 3 and round 6 of SCSHP (A), common peptides obtained in phage pool of round 3 and round 6 of SCPP (B), and comparisons between all four phage pools sequenced (C).

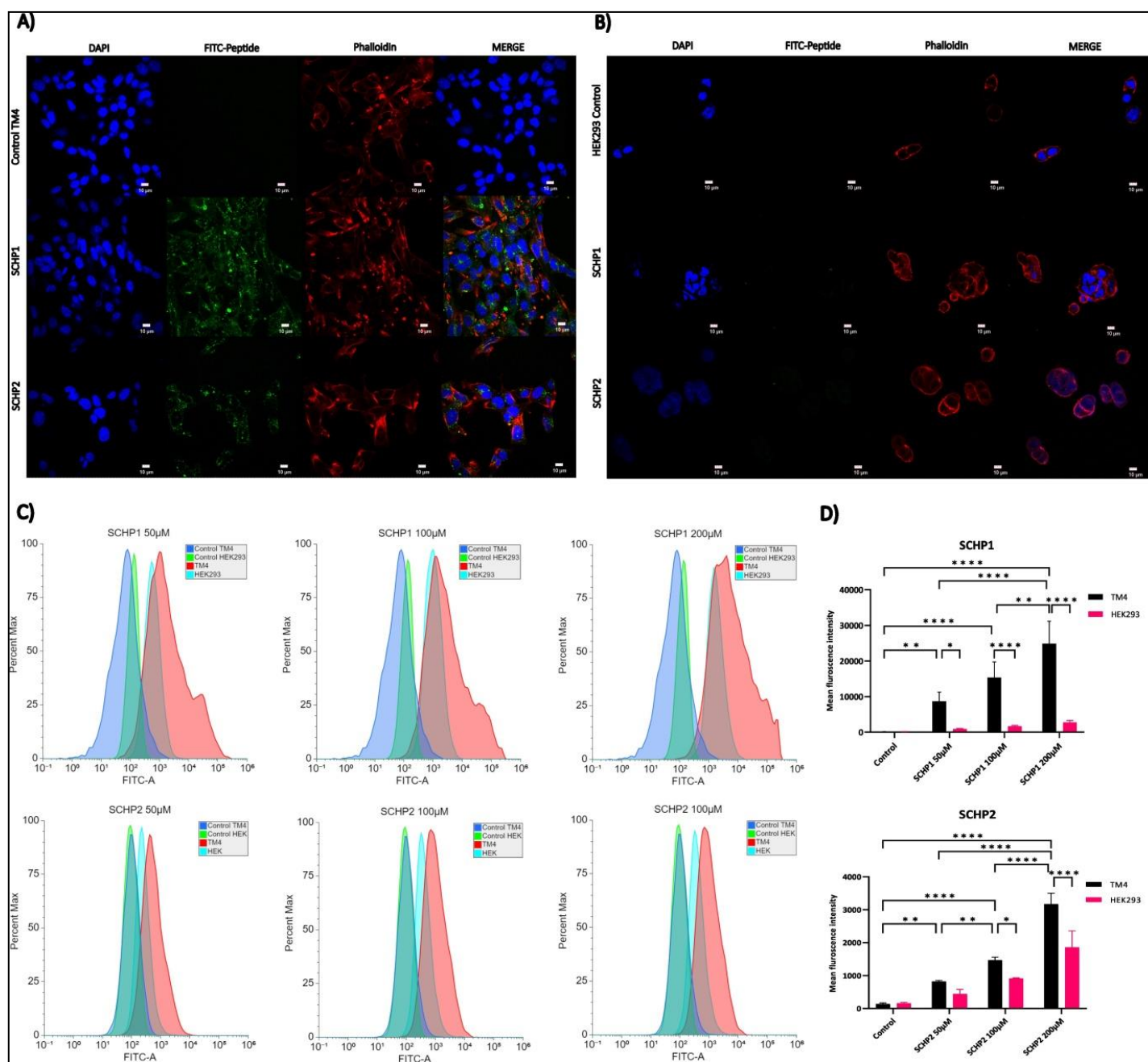


Figure 33: FITC labeled SCHP1 and SCHP2 uptake by TM4 cells (A) and HEK293 cells (B); cells were counterstained with Phalloidin rhodamine for cytoskeleton and with DAPI for nucleus. C. Histograms of uptake of SCHP1 and SCHP2 by TM4 and HEK293 cells with flow cytometry, D. Quantitation and statistical analysis of the uptake of SCHP1 and SCHP2 by TM4 and HEK293 cells by flow cytometry.

Peptides SCHP1 and SCHP2 binding was observed in TM4 cells but not in HEK293 cells (Fig. 33A and B). The uptake of SCHP1 and SCHP2 by TM4 cells was significantly higher than control TM4 cells at all the three concentrations tested. Further, uptake of both the peptides was increased significantly at

200 μ M compared to 50 μ M and 100 μ M. SCHP1 uptake by HEK293 cells was significantly lower than TM4 cells at all three concentrations, but there was no significant difference compared to control HEK293 cells. SCHP2 uptake by TM4 cells was also significantly higher than HEK293 cells at 100 μ M and 200 μ M concentrations (Fig.

33C and D). The results indicate that the SCHP1 peptide can efficiently target mouse Sertoli cells (TM4) in-vitro even at lower concentrations, but higher concentrations of SCHP2 are needed for targeting other cell lines.

2.8 Molecular Mechanisms Involved in Prolactin and Dopamine Signaling in Male Reproduction *(Funded by Science and Engineering Research Board)*

Principal : **Nafisa H Balasinor**
Investigator
Project Associates : Sanketa Raut,
Kushaan Khambata
Duration : 2020-2023

Hyperprolactinemia is prevalent in up to 11% of the infertile males. It leads to deleterious effects on male fertility altering sperm quality. Prolactin and Dopamine Receptor (PRLR and D2R) are localized on various testicular cells, including spermatogonia, spermatocytes, and spermatids. The aim of this study is to delineate the mechanisms by which prolactin and dopamine affects male reproduction. In the previous year, serum prolactin and dopamine levels along with expression of PRLR and D2R in rat testes at different developmental ages were analysed. Serum prolactin showed a significant increase in the pubertal group as compared to pre-pubertal and adult groups; whereas, serum dopamine was highest in the pre-pubertal group. Expression of PRLR in the testes showed a significant increase in the pubertal and adult group as compared to pre-pubertal group. Expression of D2R on the other hand, also increased significantly in adult age group as compared to pre-pubertal (Annual report 2021-22, pp. 46-47). During the reporting year, downstream signaling after PRLR activation was studied using *in vitro* seminiferous tubule culture (STC). A significant up-regulation in p-STAT5 levels 30 mins after PRLR stimulation suggesting that PRLR

activates JAK2/STAT5 cascade in the testicular germ cells. Similar studies are underway for MAPK/ERK and PI3K/AKT pathways. To identify genes regulated by prolactin, microarray was performed after treating STC for 24 hours by 100ng recombinant Prl. A total of 692 differentially expressed genes (DEGs) were found to be significantly altered. Of these, 405 were up-regulated and 287 were down-regulated. Gene ontology of DEGs performed using GSEA showed cell cycle, chromosome organization, and RNA metabolic process to be significantly enriched under biological processes. The top genes up-regulated were LOC102555091, Atp6v1e2, Fam228b; and down-regulated genes are LOC102550885, LOC102552809, Nectin1.

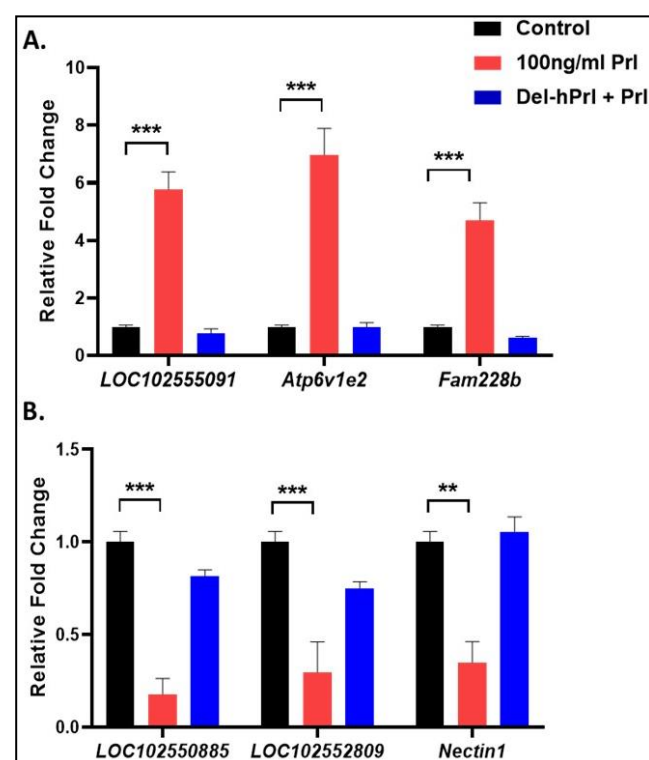


Figure 34: qRT-PCR validation of top regulated genes in microarray analysis after treatment with agonist 100ng/ml Prl (red bars) with and without (or in presence or absence of) antagonist Del-hPrl (blue bars). Only medium is used as control. Values are represented as mean $\pm$ SEM; N = 6; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

To validate the results of microarray, qPCR was performed. *In vitro* STC was treated with Prl

(100ng/ml) or PRLR antagonist (Del-hPRL 1ug/ml) along with Prl (100ng/ml). All the genes followed similar trend in gene alteration as in microarray and the effects were reversed by antagonist treatment (Fig. 34). Since cell cycle was a significantly altered process, and is crucial in spermatogenesis, ten genes were selected for validation. The results obtained showed that six genes (Ccnal, Ccnb1, Ccnb2, Cdc25a, Cdc27, Plk1) were significantly up-regulated, whereas four genes (Ccar2, Nudc, Tuba1c, Tubb2a) were significantly down-regulated by Prl (100ng/ml) treatment (Fig. 35).

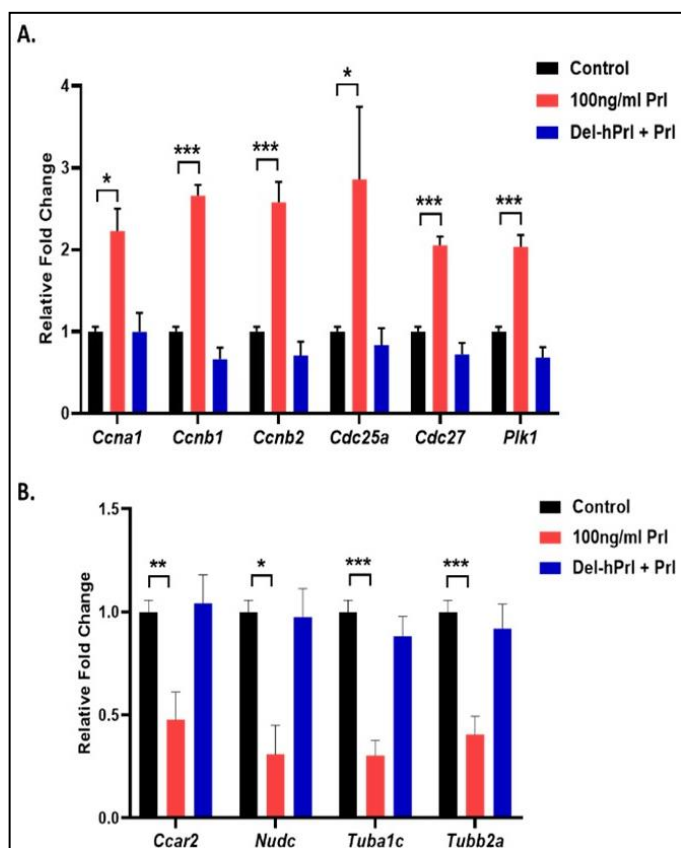


Figure 35: Expression of genes (A) up-regulated and (B) down-regulated in seminiferous tubule *in-vitro* culture 24 hours after treatment with agonist 100ng/ml Prl (red bars) with or without antagonist Del-hPrl (blue bars). Only medium is used as control. Values are represented as mean $\pm$ SEM; N = 6; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

This result corroborated the results obtained by microarray. Additionally, STAT5 binding

motifs were obtained in the respective promoter region of these 10 genes (Table 3). All the genes showed no significant change in their expression after addition of the antagonist when compared to controls, suggesting a direct regulation by prolactin. *In vitro* studies for D2R are on-going. The results obtained so far suggest that prolactin and its receptor may play a crucial role in the process of spermatogenesis.

Table 3: List of genes involved in cell cycle process obtained by microarray after prolactin treatment along with STAT5 binding motif sequence in the promoter region

Gene Symbol	Gene Name	Score	Sequence
Ccnal	Cyclin A1	0.956	TTCCTA GAA
Ccnb1	Cyclin B1	0.876	CTACGG GAA
Ccnb2	Cyclin B2	0.982	TTCCCA GAA
Cdc25a	Cell division cycle 25a	0.859	CTATGG GAA
Cdc27	Cell division cycle 27	0.956	TTCCTA GAA
Plk1	Polo-like kinase 1	0.966	TTCCTG TAA
Ccar2	Cell cycle and apoptosis regulator 2	0.87	TTCTGG CAA
Nudc	Nuclear distribution C, dynein complex regulator	0.897	TTCCCC TCA
Tuba1c	Tubulin alpha 1C	0.938	TTCTGA GAA
Tubb2a	Tubulin beta 2A	0.866	TTCTGG GAG

2.9 Validation of a Novel Assay for Detection of Y Chromosome Microdeletions and Development of an Algorithm to Predict Clinical Outcomes in Male Infertility (Funded by Department of Health Research under Young Scientist Scheme)

Principal Investigator : **Stacy Colaco**
 Project Mentor : DN Modi
 Project Associates : A Sengupta,
 Priyanka Narad,
 Amity University,
 Noida
 Duration : 2020-2023

Defects in the male partner account for the cause of infertility in 50% of infertile couples. Male infertility is caused by several factors of acquired or genetic origin. Microdeletions in the long arm of the human Y chromosome (YCMD) is one of the leading genetic causes of male infertility. The detection of YCMD requires STS-PCR technique and the European Academy of Andrology [EAA] recommends six STS markers, which remain the gold standard for YCMD screening. However, we have earlier shown that these six markers miss out almost 48% of cases in Indian sub-continent leading to a very high rate of misdiagnosis. We developed a 17 STS-marker panel India-specific YCMD diagnostic assay in a multiplex PCR format. The current project involves external validation of the assay in laboratories and the development of a machine learning-based algorithm that will predict clinical outcomes in YCMD bearing males should they opt for assisted reproduction. Through ICMR, the technology developed and laboratory validated was transferred to APS LifeTech (Pune, India) on Feb 17 2022. The company has since then validated the test in their lab and is now in process of scaling it up. Eight different centers are participating in the external and independent evaluation of the assay. To date, the centers have completed testing on about 400 patients and the numbers are expected to be completed by end of 2023. Along with Amity University, Noida, we developed a tool termed as 'FertilitY Predictor' that allows the prediction of outcomes in men with YCMD and who may

opt for IVF. Random forest model allowed the best prediction for successful sperm retrieval embryo quality and live birth rates. The assay and 'FertilitY Predictor' will aid clinicians in improving the quality of services to infertile couples opting for assisted reproduction.

2.10 Cross Talk of Sertoli Cells and N-Glycosylation Deficient Germ Cells: Impact on Male Fertility *(Funded by Department of Biotechnology)*

Principal Investigator : **Barnali Biswas**
 Co-Investigator : Taruna Madan
 Project Associates : Krupanshi
 Brahmbhatt, RD
 Shinde
 Collaborator : Pamela Stanley,
 Albert Einstein
 College of Medicine,
 USA
 Duration : 2019- 2022

N-glycans are involved in molecular interactions and are essential for fertility. Mgat-1, the N acetyl glucosaminyl transferase, is integral to spermatogenesis and initiates the synthesis of complex N glycans. Previously (Annual report 2020-2021, pp. 43-44), global N-glycosylation deficiency induced in spermatogonial cells (GC-1) by tunicamycin, significantly inhibited survival of the germ cells. Further, lack of complex N-glycans in GC1 cells led to altered junctional protein complex and expression of basigin or CD147 - a transmembrane glycoprotein. To investigate the reason for infertility of Mgat1-/- male mice, we examined the morphology of murine seminiferous tubules in different age groups by H and E staining. Compared with the control mice, a remarkable decrease in the number of elongating and elongated spermatids was observed at 35 days post-partum in Mgat1-null mice (Fig. 36), suggesting that possible

abnormality occurred during the spermiogenesis. Transmission Electron Microscope (TEM) analysis of testicular tissues showed a normal condensing sperm nucleus in elongating spermatids of control mice (Fig. 37A), but irregular-shaped nucleus coupled with malformed acrosome in spermatids of *Mgat1*^{-/-} mice. In addition, fragmentation of the nucleus was observed in the Sertoli cells (Fig. 37B).

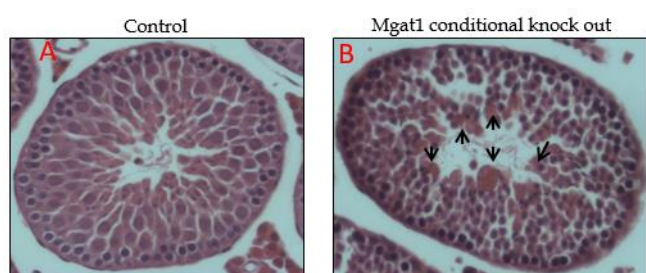


Figure 36: H & E Staining of testis from (A) Control mice showing seminiferous tubules with presence of spermatids and spermatozoa (B) *Mgat1* conditional knockout mice testis showing lack of matured elongated spermatids and intercellular gaps.

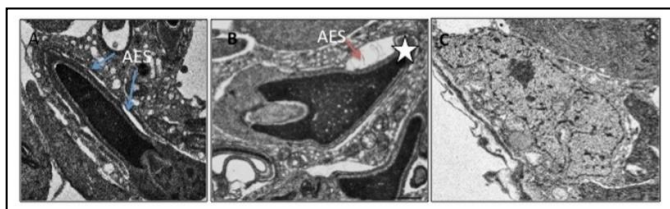


Figure 37: Transmission Electron Microscopy images for (A) Control testis showing elongated spermatids and Apical ectoplasmic specialisation (AES) marked with blue arrows (B) *Mgat1* conditional knockout testis showing abnormality in the head portion of elongated spermatids (white star) and vacuolar AES marked with red arrow (C) fragmented Sertoli cell architecture.

Together, these results support a role for *Mgat1* in the germ cells in communicating with Sertoli cells during late spermatid differentiation. Differentially expressed cell-surface proteins of Spermatogonia under N-glycosylation defect induced by Tunicamycin treatment (0.3 µg/ml, 5 hours) were identified by proteomic analyses using Orbitrap LC MS. A total of 314 proteins

were found to be up regulated while 27 proteins were down regulated. Treated GC1 cells experienced an enhanced unfolded protein response and induction of genes that encode endoplasmic reticulum chaperones such as the binding protein (BiP or GRP78). Several junctional proteins were differentially regulated. The proteins regulating cellular stress response, cell cycle, oxidative phosphorylation were upregulated, whereas proteins involved in keratinization and apoptotic cleavage of cell adhesion proteins were found down regulated (Table 4). To validate the above findings from treated GC1 cells, germ cells isolated from *Mgat1* null mice were subjected to Western blotting analysis. However, expression of important glycoproteins such as Basigin, EGFR and Integrins were not altered.

Table 4: Reactome analysis and gene ontology showing the most significant biological pathways affected by N-glycosylation inhibitor Tunicamycin in GC-1 cells (red-upregulation, blue-downregulation)

Reactome term	Gene count	p Value
R-MMU-2262752- Cellular stress response	22	3.37E-04
R-MMU-1640170- Cell cycle regulating protein	21	0.005114
R-MMU-6805567- Keratinization	4	7.88E-05
R-MMU-351906- Apoptotic cleavage of cell adhesion proteins	1	0.015654
Gene Ontology term		
Chaperone-mediated protein folding (GO:0061077)	5	3.40E-04
Positive regulation of oxidative phosphorylation uncoupler activity (GO:2000277)	2	1.11E-04

2.11 Unravelling the Sperm Epigenetic Landscape in Infertile Men with Clinical Varicocele

Principal : **Dipty Singh**

Investigator

Project : Deepshikha Arya, Nafisa

Associates Balasinor, R Gajbhiye, Deepti Tandon, Kushaan Khambata

Collaborator : P Pawar, Lokmanya Tilak Municipal General Hospital

Duration : 2020-2023

Varicocele is one of the leading causes of male infertility. Though varicocele affects spermatogenesis via several distinct mechanisms, elevated oxidative stress has been implicated as a key factor in varicocele induced male infertility. High oxidative stress during spermatogenesis may affect sperm mitochondrial functions and lead to aberrant epigenetic modifications in spermatozoa. This study aims to unravel the sperm genome wide DNA methylation signatures before and after antioxidant treatment and varicocele repair in men with clinical varicocele. This case-control study includes apparently healthy fertile men (n=25) and infertile men with clinical varicocele (n=50). The objectives of this study are: i) evaluation of the genome-wide DNA methylation in spermatozoa of infertile men with clinical varicocele before antioxidant

treatment or varicocele repair; ii) evaluation of DNA methylation status of differentially methylated genes in spermatozoa of infertile men with clinical varicocele after antioxidant treatment or varicocele repair; and iii) evaluation of the histone retention in spermatozoa of infertile men with clinical varicocele before and after antioxidant treatment or varicocele repair. A total of 21 healthy fertile men and 18 infertile men with clinical varicocele (Grade I-III) have been recruited so far. Semen samples were analyzed for basic semen parameters as per WHO (2010) standards. Infertile men with varicocele had significantly lower sperm count, motility, intact morphology and viability compared to control group (Table 5). Lower serum testosterone levels were observed in varicocele group (3.429 ± 1.137 ng/ml Vs 5.708 ± 2.219 ng/ml). Mitochondrial Membrane Potential (MMP) and intracellular reactive oxygen species (iROS) assays significantly lower MMP ($30.38 \pm 12.61\%$) in the varicocele group as compared to control group ($61.20 \pm 8.351\%$) (Fig. 38A). Also, varicocele group had higher iROS (176821 ± 57620 DCF-MFI) than healthy fertile control group (Fig. 38B). High mitochondrial copy number was observed in infertile men with varicocele as compared to healthy fertile men (Fig. 38C).

Table 5: Semen characteristics of healthy fertile controls and varicocele group

Participants Recruited	Mean Age	Sperm Concentration (million/ml)	Sperm Motility (%)	Sperm Morphology (normal forms) (%)	Sperm Viability (%)
Control (n= 21)	32.94 (26 – 40) yrs	111.2 $\pm$ 22.79	75.19 $\pm$ 18.83	7.33 $\pm$ 2.38	53.70 $\pm$ 2.79
Varicocele; Grade I (n=2)		59.50 $\pm$ 64.35	51.50 $\pm$ 44.55	7.41 $\pm$ 0.48	-
Varicocele; Grade II & III (n=16)	31.5 (20 – 45) yrs	27.57 $\pm$ 29.66*	40.28 $\pm$ 29.80*	3.37 $\pm$ 2.60*	33.33 $\pm$ 13.77*

* p<0.05

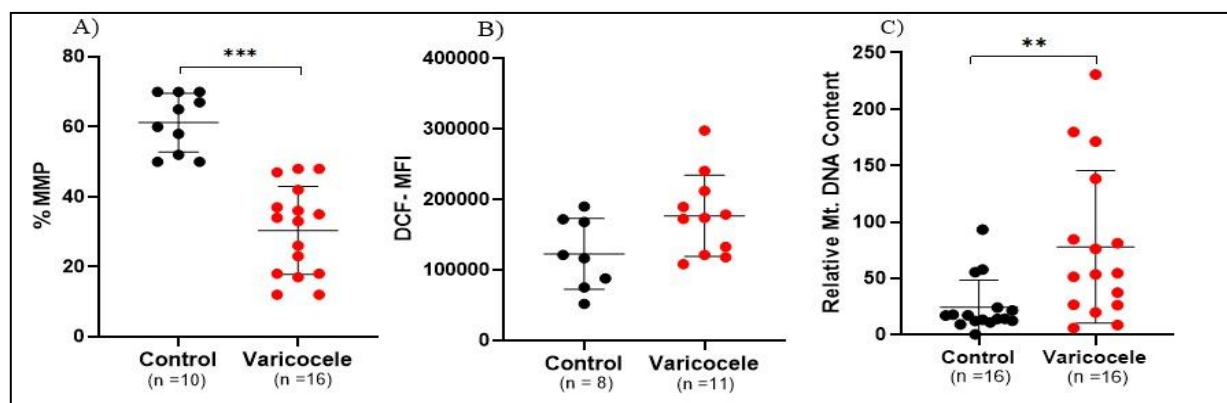


Figure 38: Sperm mitochondrial functions A) MMP, B) ROS & C) Mitochondria copy number. Mean $\pm$ SD. (** $p < 0.01$, *** $p < 0.001$)

Decreased MMP, high mitochondrial copy number and increased iROS levels indicated sperm mitochondrial dysfunction in men with varicocele. Men with varicocele had significantly high sperm DNA fragmentation ($31.88 \pm 18.62\%$), compared to fertile men ($12.59 \pm 7.41\%$) (Fig. 39). Assessment of genome-wide sperm DNA methylation changes and histone retention are underway. The present study would be helpful to understand the genome-wide epigenetic changes in the sperm of infertile men with clinical varicocele before and after antioxidant treatment or varicocele repair.

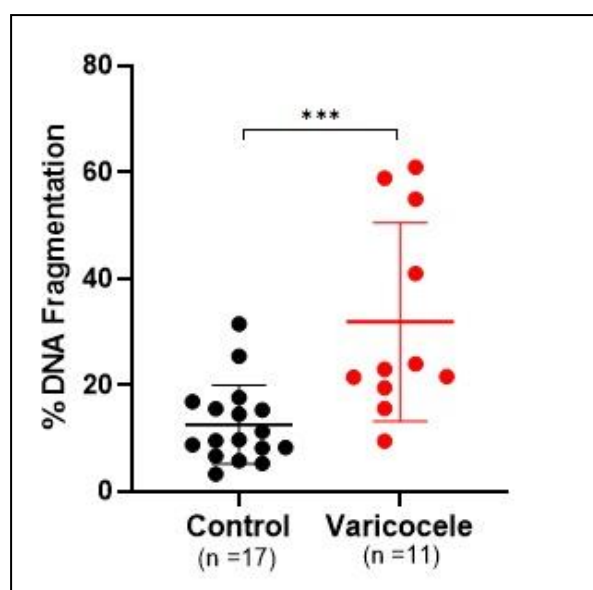


Figure 39: Sperm DNA fragmentation index. Mean $\pm$ SD. (** $p < 0.01$, *** $p < 0.001$)

2.12 Identification and Characterization of Genetic Factors Associated with Multiple Morphological Abnormalities of Sperm Flagella (MMAF)

Principal : DVS Sudhakar
Investigator

Principal Co- : R Gajbhiye
Investigator

Collaborators : R Shah, V Kulkarni, Dipty Singh

Duration : 2022-2025

The project aims to determine the frequency of MMAF phenotype among infertile men with asthenoteratozoospermia (AT) and oligoasthenoteratozoospermia men (OAT) phenotypes in India; identify genetic factors that lead to MMAF phenotype by exome sequencing; characterize the morphological defects in the spermatozoa of men with MMAF phenotype to establish genotype-phenotype correlation. With the help of clinical collaborators, infertile men with MMAF phenotype were identified using retrospective data of infertile men available with NIRRCH andrology clinic. A total of three study participants were recruited till date (from February 2, 2022). Blood and semen samples were collected from the study participants.

DNA samples will be sent to CDFD, Hyderabad after receiving material transfer agreement. Currently we are analysing the sperm flagellar ultra-structure of one MMAF individual using electron microscopy.

2.13 Unravelling Sperm Epigenetic Landscape Regulated by Estrogen Receptors in Adult Male Rats (Partly Funded by SERB Start-up Research Grant)

Principal Investigator : **Kushaan Khambata**

Project Associates : Priyanka Bera

Duration : 2020-2022

Estrogen through its receptors (ER α and ER β) plays an important role in regulation of various aspects of spermatogenesis and male fertility. To study the roles of ERs in male fertility, rat models have been established where treatment with selective ER agonist for ER α (PPT) and ER β (DPN) for 60 days leads to decreased fertility parameters in adult male rats resulting in pre- and post-implantation embryo losses. Since, epigenetic marks in the sperm such as DNA methylation play a crucial role in embryogenesis, the present study aims to investigate the effects of estrogen signaling via ER α and ER β on sperm DNA methylome in rat models.

In the reporting period, DNA from sperm of male rats treated with ER α agonist (PPT) and ER β agonist (DPN) was subjected to whole genome bisulfite sequencing. There were 28074 differentially methylated CpG (DMC) in PPT and 1492 DMC in DPN treatment group identified with the cut-offs of methylation difference 25% and q-value 0.05. Volcano plots are represented in Fig. 40A and B. The chromosome-wise distribution of the differentially methylated CpG (DMC) sites revealed different methylation patterns affected

for PPT and DPN (Fig. 40C and D). The chromosome 1 had the most number of DMCs for PPT (Fig. 40C); whereas, chromosome 18 had most DMCs for DPN (Fig. 40D).

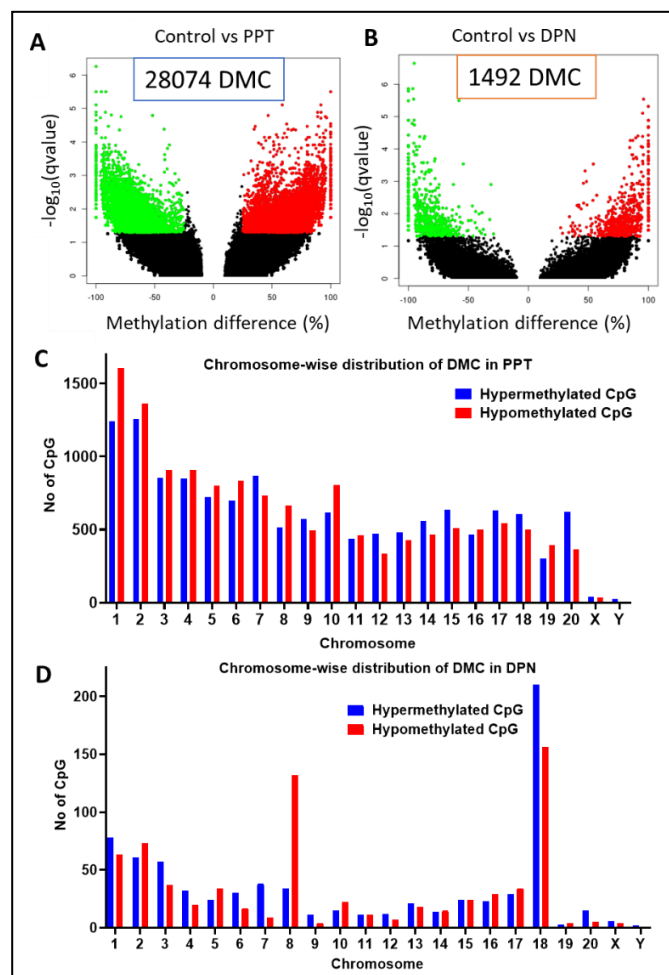


Figure 40: Volcano plots representing the differentially methylated CpG sites (DMC) at cut-off of methylation difference 25% and q-value 0.05 after PPT (A) and DPN (B) treatments. Chromosome-wise distribution of DMC after PPT (C) and DPN (D) treatments.

Genomic feature distribution revealed that majority of these CpG sites lie within the intergenic regions, followed by intronic regions. Small percentage of these sites were also located in the -10kb upstream region, promoter region, exon and 3' end regions of the genes (Fig. 41A and B). There were comparably more DMCs in the CpG shelves and shores region as compared to the annotated CpG island regions. About 8%

of DMCs in PPT and 13% in DPN had transcription factor binding motifs indicating their possible role in transcriptional regulation (Fig. 41C). Majority of the intragenic DMCs were distributed around ± 10 kb from the TSS (Fig. 41D). Among the 28074 DMC identified genome-wide in sperm after PPT treatment, 10865 DMC lie within 4653 differentially methylated genes (DMG), (i.e. from -10 upstream of TSS to transcription stop).

Similarly, for DPN treatment, out of 1492 DMC identified genome-wide, 528 DMC lie within 314 DMG. There were 212 DMG commonly identified for both DPN and PPT treatments. The results so far indicate that the sperm methylome was more extensively affected after ER α agonist treatment possibly due to concurrent changes in chromatin remodelling observed in this treatment.

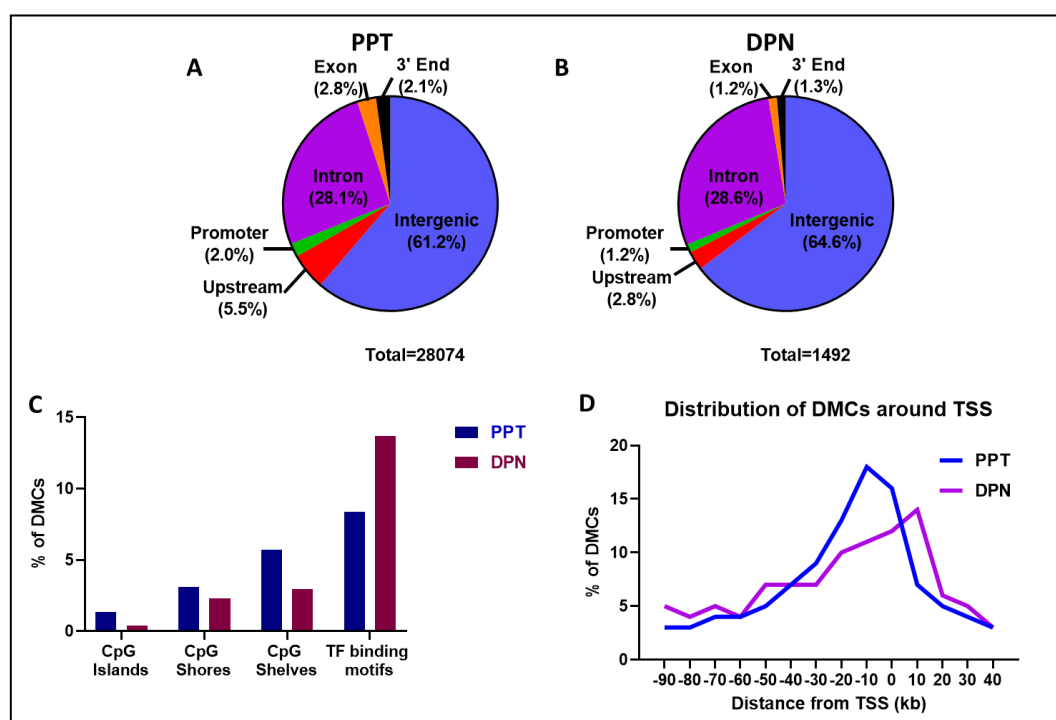


Figure 41: Genomic feature distribution of differentially methylated CpG sites (DMCs) in spermatozoa after PPT (A) and DPN (B) treatment. Distribution of DMCs in CpG islands, shores, shelves and TF binding sites (C). Distribution of DMCs around the TSS (D).

3. RTI/ STIs/HIV/ MICROBICIDES

3.1 Three Dimensions of *Mycoplasma genitalium* Infection - Detection, Cure Rate and Co-infections in Women Attending STI Clinics

Principal Investigator	: Deepti Tandon
Principal Co-Investigators	: Anushree Patil, Jayanti Mania Pramanik, Shilpa Kerkar
Project Associates	: S Chauhan, Shahina Begum, Kiran Munne, Suchitra Surve
Collaborators	: Padmaja Keskar, Pranita M Tipre, Brihanmumbai Municipal Corporation Jayanthi Shastri, Nair Hospital
Duration	: 2019-2022

Mycoplasma genitalium is an emerging STI/RTI associated with wide spectrum of reproductive morbidities like cervicitis, tubal factor infertility, endometritis and pelvic inflammatory diseases. Literature for Asian populations is very sparse regarding its prevalence and response to treatment. Hence the present study was undertaken i) to detect *Mycoplasma genitalium* in women attending STI clinic with clinically diagnosed lower genital tract infections (cervicitis, cervicovaginitis); ii) compare the detection rate of *Mycoplasma genitalium* in endocervical swab, vaginal and urine samples; iii) to assess clinical and microbiological response to treatment among women infected with *Mycoplasma genitalium* following treatment with standard management; and iv) to investigate the presence of co-infection of other common RTI causing organisms with *Mycoplasma genitalium*.

The present study was conducted at two sites, ICMR-NIRRH Family Welfare Clinic, Abhyudya Nagar and STI Clinic, Nagpada, Mumbai. Characteristics of the study populations at these two study sites were different. Abhyudya Nagar clinic caters to women from the community who seek clinical services for routine gynaecological problems whereas women visiting Nagpada clinic are mainly having multiple sexual partners. The STI Clinic Nagpada is a dedicated STI Clinic. Sample size was calculated on basis of anticipated prevalence of *Mycoplasma genitalium* (5.0%) at 5% level of significance and 0.025 marginal error. Total sample size of 340 cases was recruited. Three samples per woman were tested so total 1020 samples were tested for *Mycoplasma genitalium*.

During the reporting year (2021-2022), recruitment of study participants was completed (n=341) from STI Clinic Nagpada. A total of 190 samples have been tested. Mean age and mean BMI of the recruited population was 33.4± 5.7 years and 24.4±4.9kg/m² respectively. Majority (88.3%) were female sex workers by occupation and rest (12.7%) had other occupations. As per Kupuswamy socioeconomic scale of 2018 majority (94.2%, n=322) belonged to upper lower socioeconomic scale. Only 35.1% (n=120) had access to private toilet facilities and majority (64.9%, n=222) used common or public toilets. Smoking of cigarette was reported by 9.6% (n=33), alcohol consumption by 27.5% (n=94) and other addictions of smokeless tobacco by 50.9% (n=174) of the population. Condom as a method of contraception was used by 84.5% (n=289) and 8.5% (n=29) had undergone tubal ligation and rest 7% (n= 23) reported use of other methods such as Intrauterine device and injectable contraceptives. Among the recruited

population, 14.6% (n=50) were seropositive for HIV, 4.4% (n=15) were positive for syphilis and 2.6% (n=9) were seropositive for Hepatitis B surface antigen. Among the 190 samples tested, the prevalence of *Mycoplasma genitalium* was 6.08% (n=14), of *Neisseria gonorrhoeae* was 3.5% (n=8), *Chlamydia trachomatis* 2.2% (n=5), *Trichomonas vaginalis* was 1.3% (n=3), *Ureaplasma urealyticum* was 16.5% (n=38), *Ureaplasma parvum* 20% (n=46) and *Mycoplasma hominis* 39% (n=74). *Mycoplasma genitalium* is an emerging STI and needs attention especially among women having high risk behaviour.

3.2 Longitudinal Cohort Study to Evaluate the Effect of Various Contraception Methods on the Composition and Diversity of the Vaginal Microbiota (Funded by ICMR)

Principal Investigator : **Deepti Tandon**
 Co-Principal Investigator : V Bhor, Anushree Patil, Clara Aranha,
 Project Associates : S Chauhan, Shahina Begum, Kiran Munne
 Collaborators : Vandana Bansal, Nowrosjee Wadia Maternity Hospital
 Duration : 2019-2022

As per an estimate done in 2017, 63% of the married women of reproductive age worldwide use some form of modern or traditional contraception. There has been conflicting evidence in the literature regarding the effect of available contraception methods on the vaginal health of women. This longitudinal cohort study has been planned in which a cohort of 120 women using various contraception methods will be followed over a period of six months. This study will help to map the 'normal or healthy' vaginal microbiome of healthy women living in the urban belt of Maharashtra as well as give insight into the effect of various

contraceptive methods on the vaginal health of women.

Primary objectives of the present study are i) to evaluate vaginal health status of women before and after using various contraception methods by diverse methods (vaginal pH, quantification of leukocytes, Nugents score and Amsel's criteria); ii) to evaluate species-level composition of the vaginal microbial ecosystem in these women over a period of 24 weeks; iii) to determine the vaginal inflammatory profile in these women over a period of time; and iv) to investigate the association between the microbiome profile with clinical symptoms, Nugent score, Amsel's criteria and vaginal inflammation profile. Secondary objective of the study is to stratify the vaginal microbiome profile of the study cohort as per age, BMI, sexual activity, parity and ethnicity.

A cohort of 68 women using various contraceptive methods –barrier, Intrauterine devices, injectable contraception, LNG IUCD and control group are being followed up. Baseline microbiome of 56 women has been profiled by 16SrRNA sequencing. Evaluation of Vaginal Microbiome (VMB) profile showed that the most abundant at phylum and class level were Firmicutes (60.8%) and Bacilli in (55%). The predominant genus was *Lactobacillus* in 51.10% of women and at species level maximum were *Lactobacillus iners* identified in 34.3% of women. In Fig. 42, women with Bacterial Vaginosis (BV) had significantly higher abundance of *Anaerococcus* (p=0.0135), *Peptoniphillus* (p=0.0227) and *Dialister* (p=0.0279). There is evidence of restoration of VMB after 12 months of childbirth with significant decrease in *Bifidobacterium* (p=0.0172). No specific genus showed statistical significance with respect to age, menstrual cycle and sexual and hygienic practices. Longitudinal analysis with respect to the women using various contraceptives is ongoing.

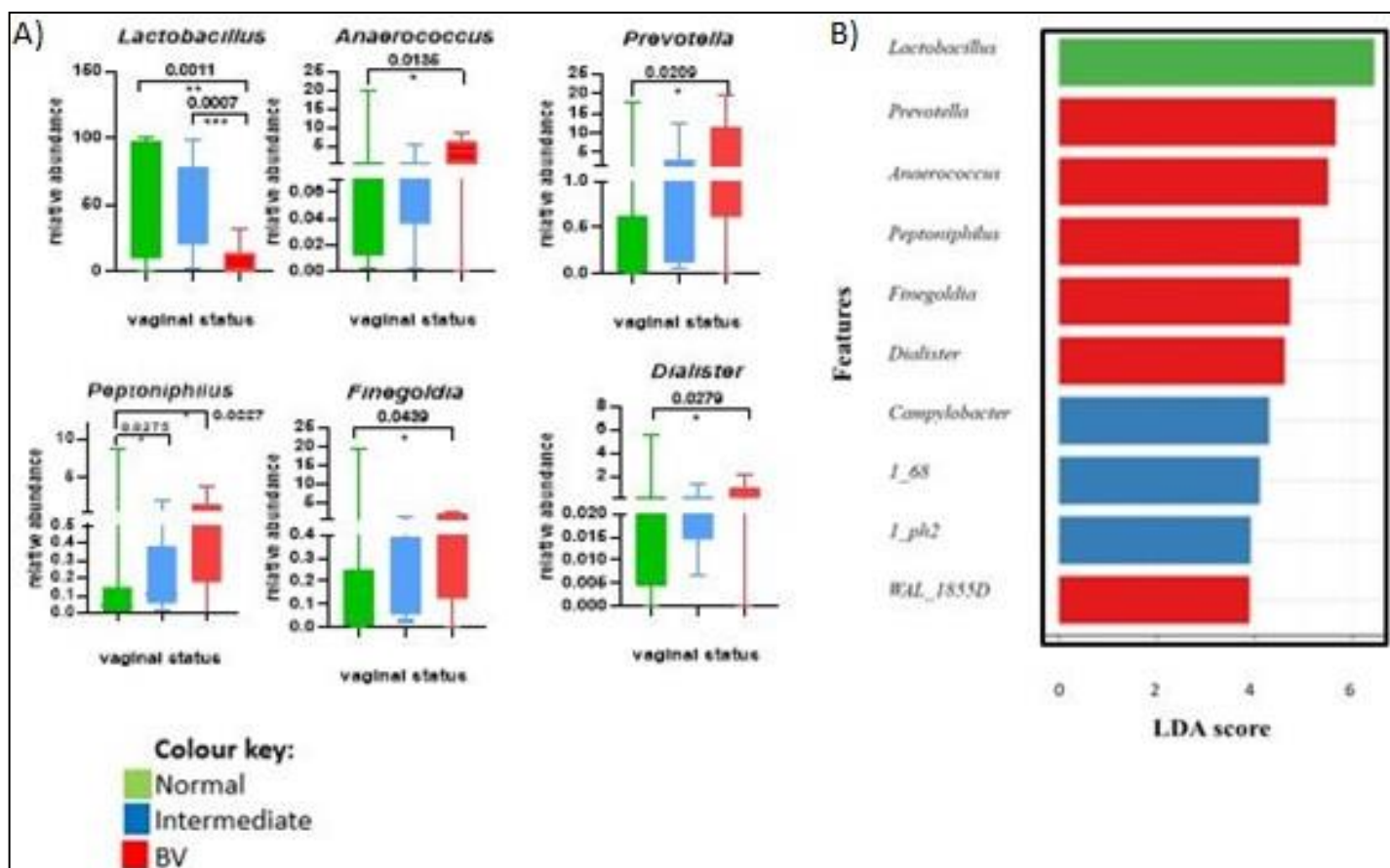


Figure 42: Box and whisker plot representing relative abundance of the predominant bacterial genera classified on the basis of Nugent scoring as Normal (n=30), Intermediate (n=13) and Bacterial Vaginosis (n=9) (A). Significant differences in relative abundance were found between healthy and BV microbiota. Linear discriminant analysis (LDA) effect size (LEfse) plots at genera level show the differences in vaginal microbiota among Normal, Intermediate and Bacterial Vaginosis group using Nugent Scoring (B). Differentially enriched features for women with normal microbiota are indicated with positive LDA scores in green, enriched features in women with Intermediate microbiota are indicated in blue and enriched features in women with BV microbiota are indicated in red. Only features with $p < 0.05$ and an effect size cut-off of 2 are shown.

3.3 Role of *Gardnerella vaginalis* Membrane Vesicles in Pathogenesis of Bacterial Vaginosis

Principal : V Bhor
 Investigator
 Project Associate : Parul Bedi
 Duration : 2021-2024

Gardnerella vaginalis is the predominant anaerobe associated with bacterial vaginosis (BV). Our previous work demonstrated the

cytotoxic potential of membrane vesicles (MVs) produced by *G. vaginalis*, towards vaginal epithelial (VK2) cells. BV is also known to be associated with the pathogenesis of conditions afflicting the upper female reproductive tract (FRT) such as endometritis and pelvic inflammatory disease. This may occur through direct ascension of the bacteria or their products including MVs from the vaginal milieu to the upper FRT.

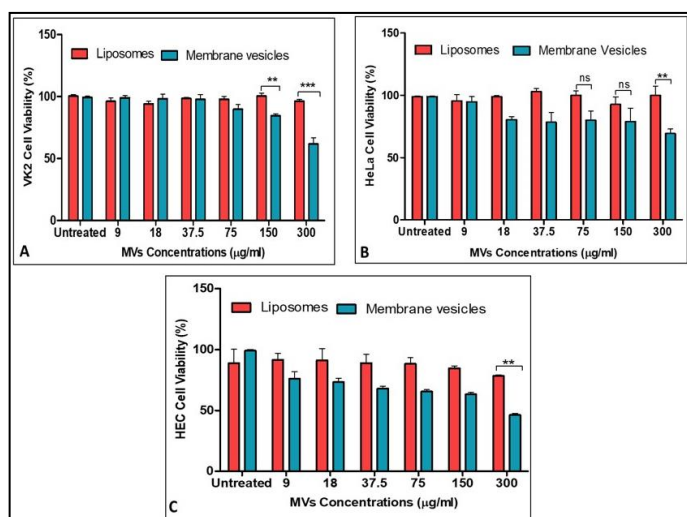


Figure 43: Effect of *G. vaginalis* MVs on FRT cell lines. Reduction in viability of FRT cells (A) VK2 (B) HeLa and (C) HEC-1A upon treatment with varying concentration of *G. vaginalis* MVs (9 to 300 µg/ml) for 24 hours. Cytotoxicity was assessed by MTT assay. BSA entrapped liposomes were used as a negative control. Experiments were repeated thrice and statistical analysis was done using two-way ANOVA with Bonferroni test *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

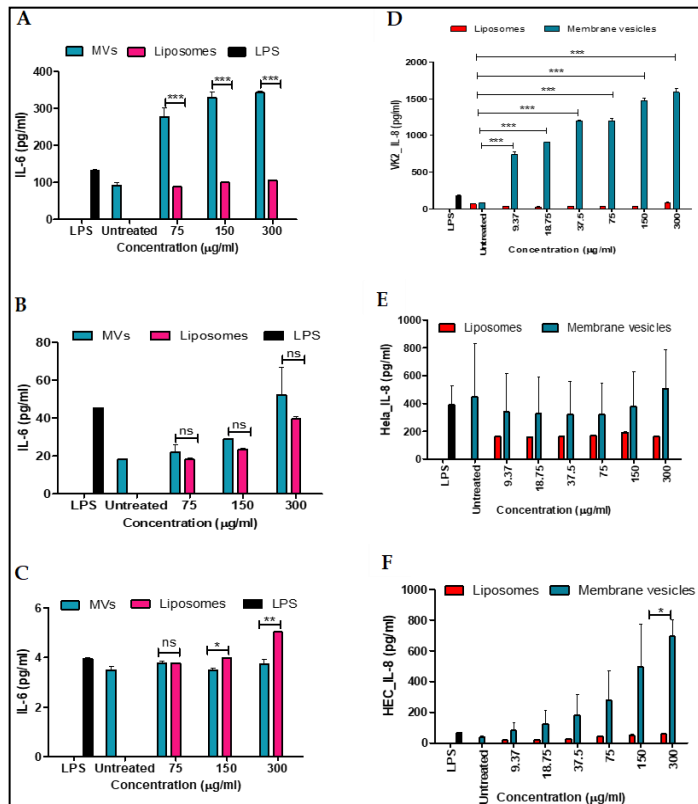


Figure 44: Induction of pro-inflammatory cytokines in FRT cell lines. Levels of pro-inflammatory

cytokines produced by FRT cells following treatment with varying concentrations of *G. vaginalis* MVs (9-300 µg/ml) for 24 hours. IL-6 levels for (A) VK2 (B) HeLa and (C) HEC-1A cells; IL-8 levels for (D) VK2 (E) HeLa and (F) HEC-1A cells. Lipopolysaccharide (300 µg/mL) was used as a positive control and BSA entrapped liposomes were used as a negative control. Experiments were repeated twice and statistical analysis was carried out using two-way ANOVA with Bonferroni test *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

In view of this, we examined the cytotoxic potential of *G. vaginalis* MVs on the endometrial cancer cell line, HEC-1A in comparison with the effect on cell lines from cervical (HeLa) and vaginal epithelium, (VK2). This was done by studying the effect of the treatment of *G. vaginalis* MVs on the viability of the above mentioned cell lines as well as induction of pro-inflammatory cytokines such as IL-6, IL-8 and TNF-alpha. Compared to VK2 cells, which exhibited a 40% reduction in cell viability (Fig. 43A), HEC-1A cells exhibited a greater loss of cell viability (54%) (Fig. 43C) while HeLa cells appeared to be the least affected (29%) (Fig. 43B) upon treatment with MVs (protein).

The specificity of the cytotoxic effect of the *G. vaginalis* MVs was indicated by the absence of loss in viability of the FRT cells upon treatment with BSA entrapped liposomes which were used as negative control. Further, variations in the pro-inflammatory cytokine induction were observed with each of the cell lines. Considerable elevation of IL-6 was observed in case of VK2 cells (Fig. 44A). Conversely, a reduction in the IL-6 levels was observed in HEC-1A cells upon treatment with MVs at a concentration of 150 µg/ml and 300 µg/ml compared to the negative control (Fig. 44C). A dose dependent induction of IL-8 was observed in case of both VK2 (Fig. 44D) and HEC-1A (Fig. 44F). Pro-inflammatory cytokine induction was not observed in case of HeLa cells (Fig. 44B and E). Taken together, these results demonstrate

the variability in the potential of *G. vaginalis* MVs to induce cellular pathogenesis in different cells of the FRT. The susceptibility of the endometrial cell line to *G. vaginalis* MV induced cytotoxicity indicates the possibility of the MVs contributing to cellular pathogenesis in the endometrium, which is also the site of formation of polymicrobial biofilms containing *G. vaginalis*.

3.4 Development of a Multistrain Probiotic Lactobacillus Formulation Effective against Reproductive Tract Infections

3.4.1 Novel Genotypes of Vaginal *Candida Glabrata* Isolates from Premenopausal Asymptomatic Women with Vulvovaginitis (Partly Funded by ICMR)

Principal Investigator : Clara Aranha

Principal Co-Investigator : K Chaaithanya

Duration : 2013-2023

Vulvovaginal candidiasis (VVC) is a mucosal infection, affecting 75% of reproductive-age women at least once in their lifetime and around 30% of them suffer from asymptomatic vulvovaginal candidiasis. *Candida glabrata* is the second most common cause of candidiasis in adults after *Candida albicans* and causes nearly-three-quarters of all systemic candida infections worldwide. The most common causative agent is azole-resistant *C. albicans* and recently *C. glabrata* is emerging as a common pathogen. A total of 19 clinical isolates of vaginal *C. glabrata* strains were investigated to determine the most prevalent sequence type and relatedness among the isolates by using MLST (Multi Locus Sequence Typing). These clinical isolates, which were a subset of our earlier study, had been isolated from vaginal swabs of premenopausal asymptomatic women of reproductive age group from Mumbai. Polymerase chain reaction was carried out to amplify the six housekeeping genes of *C. glabrata* viz. FKS, LEU2, NMT1,

TRP1, UGP1, and URA3. Sanger sequencing was carried out and raw reads were quality checked and assembled into contigs using a reference sequence. The MLST analysis revealed a high genetic diversity among the *C. glabrata* isolates with eight of them assigned to three known sequence type STs (15, 147 & 55) and rest 11 isolates belonging to eight newly reported STs in the PubMLST database (ID: BIGSdb_20220710094751_028573_75016) (Fig. 45). Of these, ST147 was predominant (5/19; 26.3%), followed by ST 55 (2/19; 10.5%) and ST15 (1/19; 5.2%) respectively. This report identified novel *C. glabrata* STs, and this data might provide a basic database for further studies on the epidemiology and evolution of *C. glabrata* species. Further, continuous surveillance on the characterization of *C. glabrata* is needed to understand diversity which may have implications for treatment strategies.

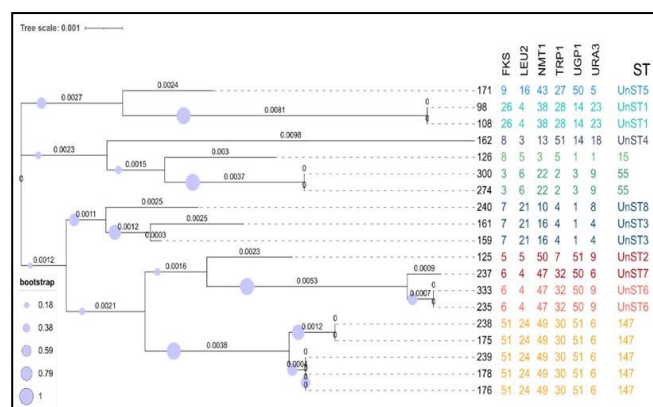


Figure 45: Phylogenetic analysis of the 19 *C. glabrata* isolates. Allelic profiles and Sequence Types were indicated corresponding to each node. Branch lengths were mentioned at the center of each branch. Bootstrap values were indicated as circles in various sizes.

3.4.2 Isolation and Characterization of Exopolysaccharide from Vaginal Lactobacillus Species

Principal Investigator : Clara Aranha

Principal Co-Investigator : V Bhor

Duration : 2013-2023

Lactobacillus species in the healthy vaginal microenvironment prevent urogenital infection by producing lactic acid, H₂O₂, and antimicrobial compounds like bacteriocins, exopolysaccharides (EPS), and biosurfactants. The objective of the present study was to extract and characterize the composition as well as determine the antimicrobial, antiproliferative and antimetastatic properties of cell-free supernatants (CFSs) and EPS from Lactobacilli isolated from the vagina and to elucidate the mechanism of action by which these lactobacilli exert their biological activities. Glycerol stocks of vaginal isolates (n=100) previously recovered from women were screened for EPS secretion by phenol sulfuric acid method. Lactobacillus with efficient EPS production and biofilm formation, identified as *Lactobacillus reuteri* and *Lactobacillus salivarius* were shortlisted for further characterization of exopolysaccharides. EPS purified from Lactobacilli were analyzed for the presence of functional groups by Fourier-transform infrared spectroscopy (FT-IR). The presence of Alkene, alkane, Aromatic amine or Sulfone, Nitro Compound, and Carbon Dioxide were predominant in *L. salivarius* EPS; whereas Phenol was exclusively seen in *L. reuteri*. Both the species showed the presence of 1,2,4-trisubstituted, primary alcohol, alkyl aryl ether/ vinyl ether, alkene monosubstituted, and methyl groups. Treatment with EPS inhibited the growth of urogenital pathogens and affected cell proliferation of cervical cancer cells. *L. salivarius* was more potent than *L. reuteri* in its antimicrobial and anticancer activities. EPS from *L. reuteri* arrested the cells in G2M phase and *L. salivarius* arrested the cells in S and G2 phases of cell cycle. *L. salivarius* EPS showed higher early and late apoptotic cells as measured by Annexin-V flow cytometry

analysis. The cell migration of EPS-treated HeLa cells was observed to be reduced at IC₅₀ dose of these products (Fig. 46). *L. reuteri* and *L. salivarius* produce metabolites that have both antimicrobial and anticancer activities. These species can potentially aid in the treatment of urogenital infections and cervical cancer. Further characterization is in progress.

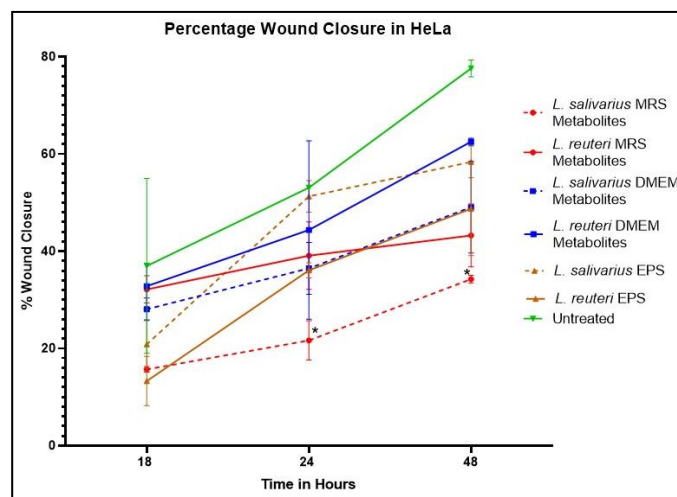


Figure 46: Percentage wound closure in HeLa cells after treatment with IC₅₀ dose of *L. reuteri* and *L. salivarius* metabolites (MRS- 0.0003 mg/ml and DMEM-0.003 mg/ml) and EPS (30mg/ml)

3.4.3 Whole Genome Sequencing of Vaginal Lactobacillus Isolates (Partly Funded by Department of Health Research)

Principal Investigator : Clara Aranha

Principal Co-Investigator : V Bhor

Project Associates : Krupali Pawar, Rinku Pramanick

Duration : 2018-2023

To gain a better insight into the beneficial effects on vaginal health and to validate our *in vitro* findings of the functional properties of the selected lactobacillus species, the genomes of eight shortlisted Lactobacillus isolates were

sequenced and the comparative genome analysis study was conducted on these species and reference genomes. Whole-genome DNA sequencing analysis of *Lactobacillus* strains indicated differences in genome size, GC %, variations, SNP, INDEL, CRISPR-Cas genes, exopolysaccharide biosynthesis genes, presence of plasmids and phages. All strains investigated enclosed intact regions for at least one phage. M46 and M54 showed the presence of three phages. All strains possess CRISPR associated endonuclease Cas3 which could provide immunity against phages. Except for one species, no plasmid signature was identified in

other isolates. We found that each of the vaginal *Lactobacillus* species uniquely possesses or lacks numerous different kinds of protein families. This suggests that each species has experienced lineage-specific gene gain and loss and thus developed a unique set of protein families. Classification of the predicted genes by Clusters of Orthologous Groups (COGs) of genes further showed differences and similarities in function between the different *Lactobacillus* spp (Fig. 47). Further analysis will be undertaken to understand the molecular basis for the probiotic functions of the isolated vaginal *Lactobacillus* spp.

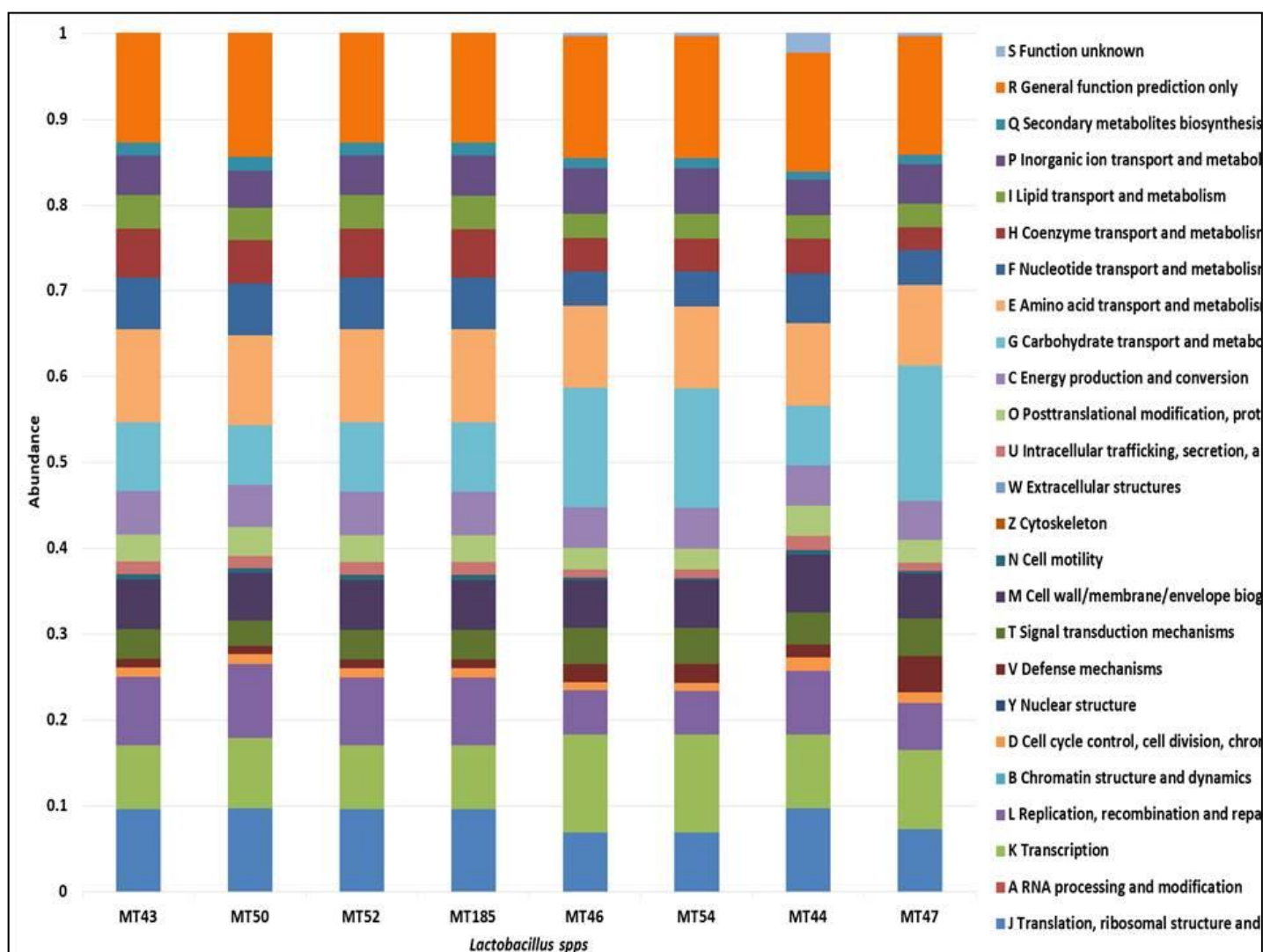


Figure 47: COG function classification in the genomes of the eight *Lactobacillus* spp

3.5 Evaluating the Inflammatory, Microbiome Profile and Co-Infections in Women Diagnosed with Treatment Failure, Relapse or Recurrent Bacterial Vaginosis (Partly Funded by ICMR)

Principal Investigator : **Deepti Tandon**

Principal Co-Investigator : V Bhor, Clara Aranha, Kiran Munne

Project Associate : Anushree Patil, Suchitra Surve, Rachna Dalvi

Collaborator : N Mayadeo

Duration : 2022-2025

Bacterial vaginosis (BV) is a highly prevalent reproductive tract infection with reported prevalence in India ranging from 21% to 44.8% in different communities and clinic settings. Despite giving standard antimicrobials like metronidazole or secnidazole, short-term cure is 30-40% and the recurrence rate is greater than 50%. This collaborative prospective study between NIRRCH and KEM hospital will help us understand the burden of women with BV suffering from recurrent/relapse and treatment failure. It will identify the bacterial species in recurrent/relapse and treatment failure BV. Further correlation with Nugent score, Amsel's criteria and inflammatory markers will give composite information on women suffering from recurrent/relapse/treatment failure and compare it those in remission.

3.6 Identification and Validation of Potential Drug Targets for Infectious Diseases using *in Silico* and Wet Lab Methods (Funded by Science and Engineering Research Board)

Principal Investigator : **Susan Thomas**

Co-Investigator : Taruna Madan

Project : Shuvechha

Associates : Chakraborty, Kshitija Rahate

Duration : 2018-2021

Secreted Aspartyl Proteases (SAPs) are one of the important virulence factors known in *Candida*. However, there are no established drugs against this target. Druggability of the *Candida albicans* aspartyl protease SAP3 was explored using drug discovery protocols to expand the therapeutic options against *Candida* infections. 3D structures of targets of FDA approved drugs were screened for structural homologs of SAP3. Drug-bound structure of protein with least RMSD was shortlisted for further screening. HIV-1 protease was found to share significant structural similarity with *Candida* SAP3. Binding site analysis of the *Candida* protein and the structural homolog was performed to understand the similarity in drug binding pocket. The HIV-1 protease inhibitor was found to bind with comparable binding energies to the active sites of HIV-1 protease (-46.2 kcal/mol) and *C. albicans* SAP3 (-46.8 kcal/mol). Thus, using the molecular fingerprint of this inhibitor, high-throughput virtual screening of peptidomimetic library was performed followed by high precision docking and molecular dynamics (MD) simulation. Based on the stability observed in RMSD and RMSF plots, for the SAP3-peptidomimetics complex, total 4 molecules (P12, P21, P104, and P124) were selected for *in vitro* validation. Stand alone and combination assays of peptidomimetics with fluconazole were performed by broth microdilution technique. Two of the four peptidomimetics were found to inhibit the growth of *Candida*. MIC50 of fluconazole against *C. albicans* was significantly reduced when used in combination with peptidomimetics P21 and P124 (Fig. 48). These

two peptidomimetics were tested for their anti-SAP activity through biochemical assay using azocasein as substrate (Fig. 49). SAP inhibition

activity of P21 and P124 was found to be comparable to pepstatin A, a known aspartyl protease inhibitor, at equivalent concentration.

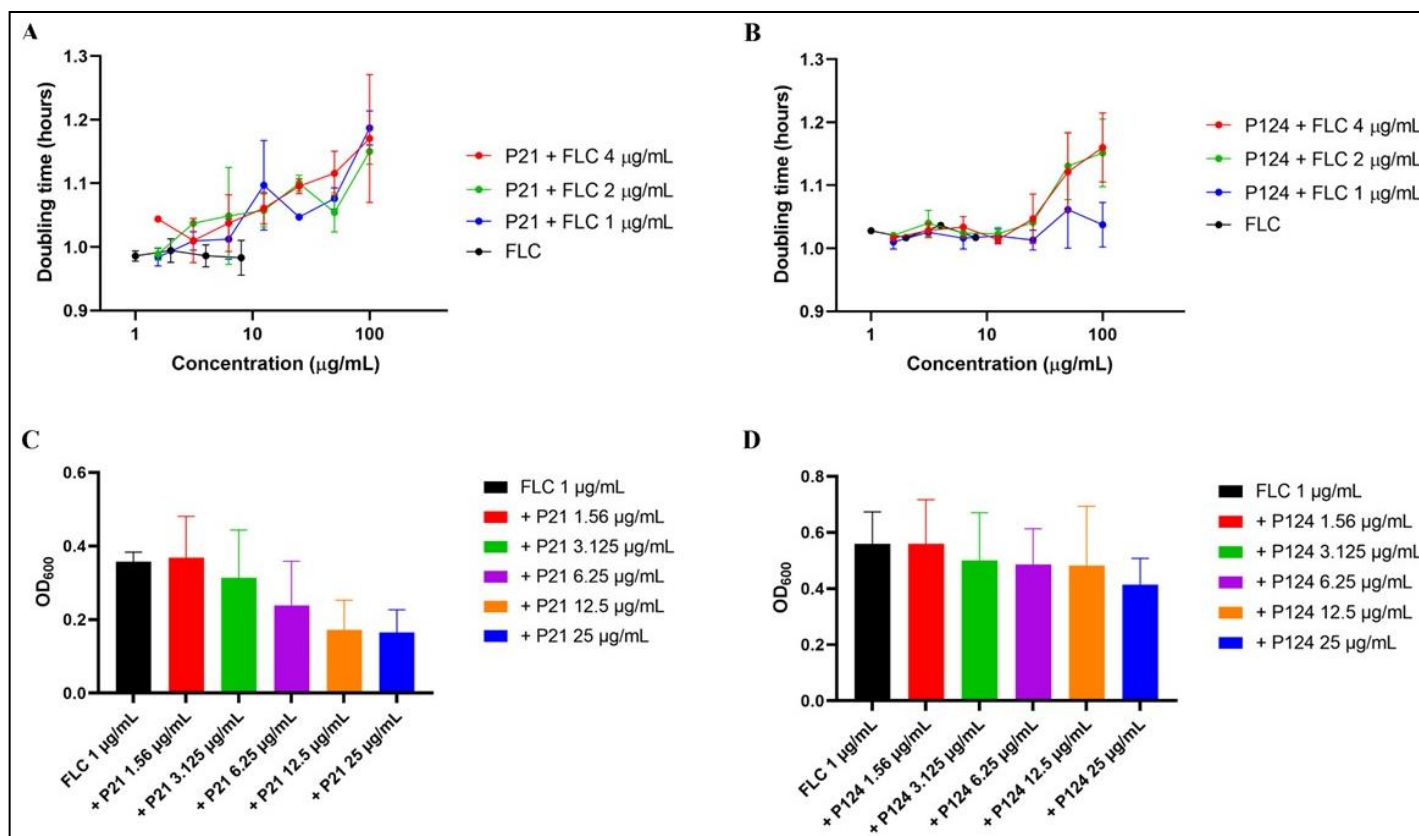


Figure 48: Effect of peptidomimetics on the doubling time (A & B) and growth (C & D) of *C. albicans* in combination with fluconazole. Histogram representation of the inhibitory effect of peptidomimetics are in combination with the lowest concentration of fluconazole (1 microg/mL) expressed in terms of OD₆₀₀.

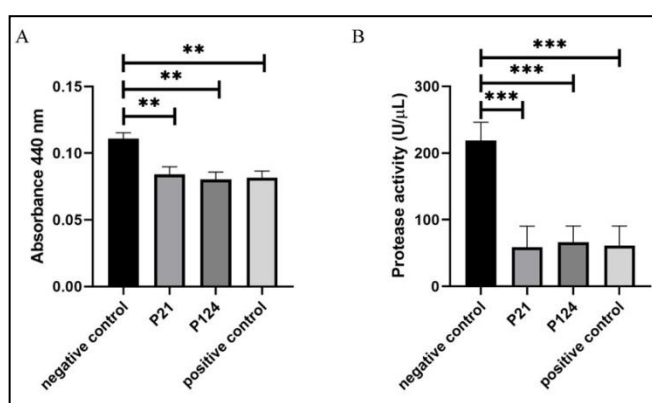


Figure 49: Anti-SAP activity of P21 and P124 demonstrated through (A) Absorbance at 440 nm, and (B) Protease activity, as compared to positive (pepstatin A) and negative controls (without inhibitors). Absorbance at 440 nm shows the amount of substrate degraded during the reaction. Quantity

of enzyme required to induce a change in absorbance of 0.001 at 440 nm is represented as protease activity.

3.7 Infections Associated with Preterm Births

Principal Investigator : **DN Modi**

Project Associate : **A Husein**

Collaborators : **Purnima Satoskar, Shradha Herlekar, Nowrojee Wadia Maternity Hospital**

Duration : **2019-2023**

One of the major causes of preterm birth (PTB) is vaginal infection caused by various bacteria. Opportunistic bacterial colonization in the vagina is harmful to the pregnancy and is associated with preterm birth, stillbirth, or neonatal death. Intrapartum transmission of the bacteria from mother to child during labor can cause neonatal septicemia. Thus it is clinically essential to diagnose vaginal infections in women with PTB. Microbial culture techniques are the gold standard method for detecting vaginal pathogens. However, these are time-consuming and need considerable expertise. Thus, World Health Organization recommends the use of Nucleic Acid Amplification (NAAT) based tests for the detection of pathogens in various disease contexts and even allows clinical decision-making based on the results of these tests. Herein we aim to develop a multiplex PCR assay for the identification of common microorganisms associated with preterm births. A systematic review of 28 Indian studies identified *Enterococcus faecalis*, *Escherichia coli*, Group B Streptococcus, *Klebsiella pneumoniae*, *Ureaplasma urealyticum*, *Gardnerella vaginalis* associated with preterm labor, premature rupture of membrane, and PTB. Thus we designed primers for the above species and developed a multiplex PCR assay using commercially available DNA from these strains. *Lactobacillus iners* was used as a positive control. The sensitivity of the PCR reaction was estimated to be 1-10pg of DNA. After multiple rounds of optimization, these bacteria could be analyzed in 2 sets of multiplex PCR reactions (termed as set 1 and set 2). Set 1 detects *Gardnerella vaginalis*, *Klebsiella pneumoniae*, and *E. coli* while set 2 detects *Neisseria gonorrhoeae*, *Ureaplasma urealyticum*, *Streptococcus aureus*, and *Lactobacillus iners* (Fig. 50). We collected vaginal swab samples of full term (n=10) and preterm birth (n 10) and DNA was extracted. Multiplex PCR was performed using the DNA from swab samples with the Set1 and Set 2 primers.

Additional sample collection and data analysis are in progress.

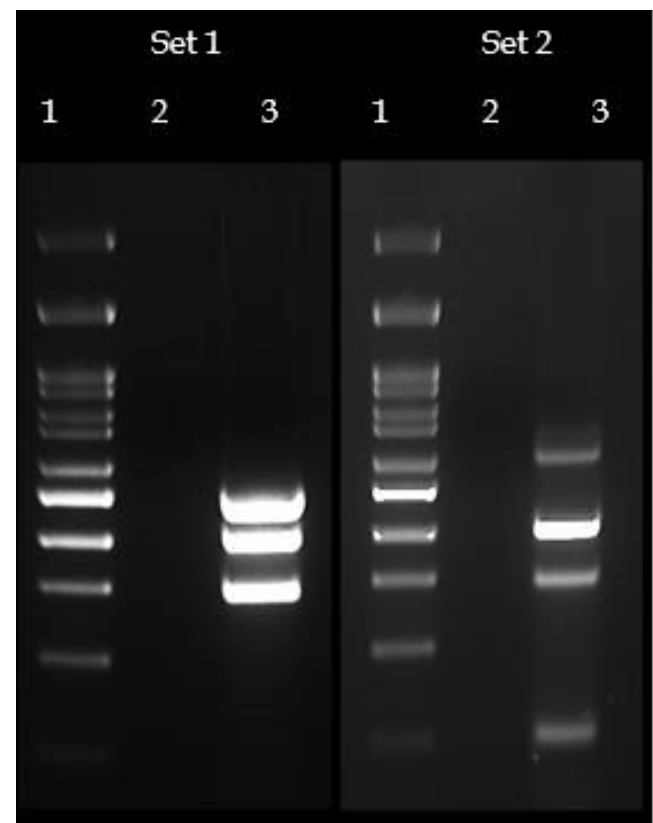


Figure 50: Multiplex PCR from standard strains. (Set 1) Multiplex PCR of *Gardnerella vaginalis*, *Klebsiella pneumoniae*, and *E. coli* (Set 2) Multiplex PCR of *Neisseria gonorrhoeae*, *Ureaplasma urealyticum*, *Streptococcus aureus*, and *Lactobacillus iners*. In both Set 1 and Set 2 Lane 1: DNA ladder, Lane 2: negative control Lane 3: Test.

3.8 Investigation of Integrin alpha4beta7 Expressing Immune Cells in Sexually Transmitted Infections / Reproductive Tract Infections (STIs/RTIs) and HIV

Principal Investigator	: V Bhor
Project Associates	: Nandini Kasarpalkar, Lalita Savardekar
Clinical Collaborators	: Jayanthi Shastri, Sneha Shirodkar, TN Medical College and BYL Nair Hospital, Mumbai
Duration	: 2017-2022

Reproductive Tract Infections (RTIs) and Sexually Transmitted Infections (STIs) such as Bacterial Vaginosis (BV) and Herpes Simplex virus-2 (HSV-2) increase the risk of acquiring HIV infection. The occurrence of these infections could increase the availability of immune cells in the reproductive tract, which could be preferentially targeted by HIV. These include T cells expressing the gut homing receptor integrin $\alpha 4\beta 7$. Retinoic acid (RA), a metabolite of vitamin A i.e. retinol is known to modulate integrin $\alpha 4\beta 7$ expression on immune cells *in vitro*. Therefore, we aimed to investigate the levels of integrin $\alpha 4\beta 7$ expressing CD4 and CD8 T cells and their association with the serum levels of retinol in a group of HIV seronegative women with latent HSV-2 infection (IgG positive). The median serum retinol levels of these women were found to be similar to controls i.e. HIV seronegative women without HSV-2 infection (data not shown). However, retinol levels were observed to be positively

correlated with the frequency of central memory (CM) CD4 T cells expressing integrin $\alpha 4\beta 7$ and CD8 terminally differentiated effector memory T cells (TEMRA) expressing integrin $\alpha 4\beta 7$ in the HIV seronegative women with latent HSV-2 infection (Fig. 51). Further, compared to HIV seronegative controls, frequency of central memory CD4 T cells expressing integrin $\alpha 4\beta 7$ was higher in women with latent HSV-2 infection, while the frequency and count of CD8 TEMRA cells expressing integrin $\alpha 4\beta 7$ was found to be lower in these women. These results suggest that although modulation of the expression of integrin $\alpha 4\beta 7$ in certain CD4 and CD8 T cells subsets is observed during HSV-2 infection, retinoic acid may only be selectively involved in altering the expression of integrin $\alpha 4\beta 7$ on CD4 CM T cells. The role of factors other than retinoic acid in modulating the expression of integrin $\alpha 4\beta 7$ on T cells during RTIs and STIs needs to be further explored.

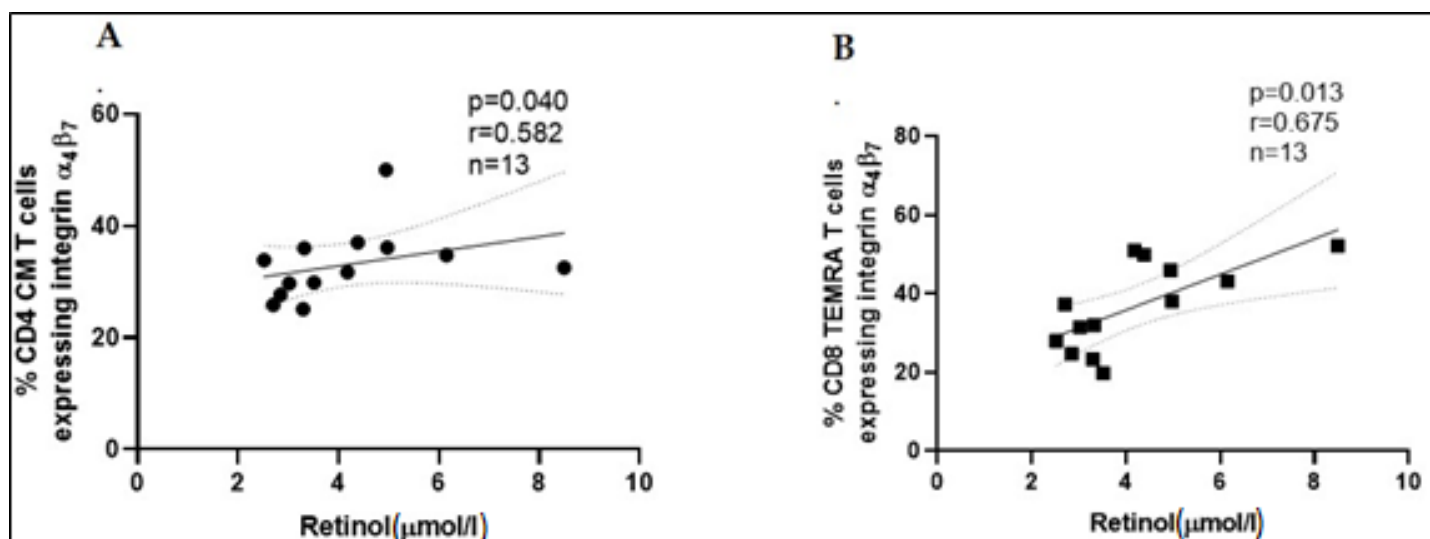


Figure 51: Correlation analysis for serum retinol levels and frequency of T cell subsets in women with latent HSV-2 infection. Serum retinol levels in HIV seronegative women with latent HSV-2 infection (IgG+ve) were positively correlated with frequency of CD4 CM T cells expressing integrin $\alpha 4\beta 7$ (A) and with frequency of CD8 TEMRA cells expressing integrin $\alpha 4\beta 7$ (B). Spearman coefficient was used to analyse the relationship between the variables and $p < 0.05$ was considered to be statistically significant.

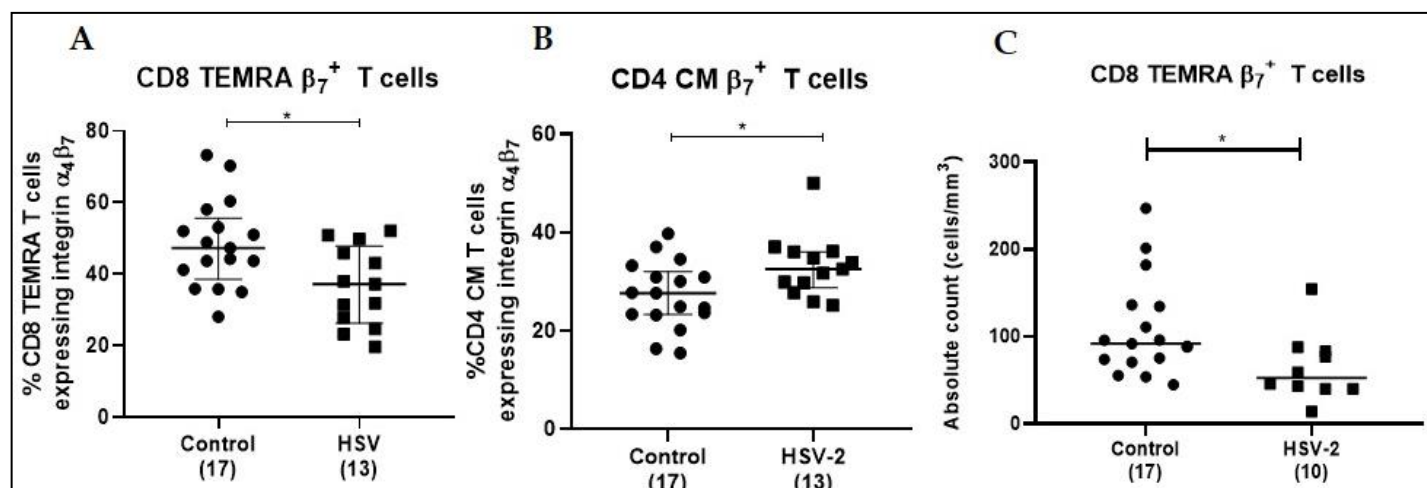


Figure 52: Integrin $\alpha_4\beta_7$ expressing T cell subsets in HIV seronegative women with latent HSV-2 infection. (A) Median frequency distribution of CD8 TEMRA cells and (B) CD4 CM T cells expressing integrin $\alpha_4\beta_7$ in control and latent HSV-2 group (IgG+ve) (C) Absolute count of CD8 TEMRA cells expressing integrin $\alpha_4\beta_7$ in control group and latent HSV-2 group (IgG+ve). Statistical analysis was performed by Mann-Whitney test using Graphpad Prism 8. *depicts $p < 0.05$

3.9 Improving Treatment Literacy and Adherence among People Living with HIV (PLHIV) through Innovative Strategies (Funded by National AIDS Control Organisation)

Principal Investigator : **Kiran Munne**

Principal Co-Investigator : H Sharma, Anti-Retroviral Therapy Center, Bhopal

Project Associates : Namita Parashar, ARTC Jabalpur
A V Kadam, ICMR NARI, Pune
R Sindhu, M Sebastian, National AIDS Control Organisation, Delhi
Harpreet Kaur, Haryana State AIDS Control Society, Haryana

Duration : 2020-2022

The long-term efficacy of ART regimens is dependent on the ability of the patient to have a near-perfect rate of adherence to complex medication schedules which generally include two or more antiretroviral agents. HIV treatment is very effective and safe, and the

benefits outweigh the risks. Also, early HIV treatment prevents immune system damage and preserves health. These messages need to be communicated among PLHIV. Also, there is a need felt for capacity building as well as regular updation of knowledge on newer interventions among healthcare providers in HIV/AIDS sector. Accurate information communicated through a variety of formats using appropriate channels is needed in all the centers where ART is introduced so as to improve treatment literacy and adherence. The objectives of the present study are: i) designing intervention to identify the reasons for knowledge gaps in treatment literacy among PLHIV and providers at Anti-Retroviral Therapy Centers (ARTC) by conducting a formative qualitative research and stakeholder meetings; and ii) implementing and assessing intervention: a) to build the capacity of health care providers through innovative strategies and provide adequate treatment-related knowledge among PLHIV for improving treatment literacy; and b) to implement multi-modal educational interventions for improving treatment literacy and treatment adherence among PLHIV.

During the reporting year, study interventions were developed. All the proposed multimodal interventions for PLHIVs and healthcare providers have been developed and shared with study team and National AIDS Control Organisation (NACO). A total of 280 PLHIV study participants (140 from Bhopal ART centre and 140 from Jabalpur ART centre) have been enrolled. Majority (86%, n=241) were in stage 1 of WHO clinical staging for HIV/AIDS. Around 12.5%, (n= 35) of the recruited participants had HIV TB co-infection. As per the pre-intervention data analysis, adherence percentage of <95% have been seen in around 16% (n=45) of the participants. Pre-intervention questionnaire from PLHIV participants have been scored out of a total score of 15. It was found that around 36.5% (73) study participants have scored $\leq 50\%$. All these participants have been given different interventions each month for 5 months and their pill counts and self-report adherence have been recorded. The remaining follow up and post intervention assessment will be completed and Tenofovir blood levels will be analysed at ICMR-NARI, Pune.

3.10 Immune and Microbiome Correlates of TB Reactivation in PLHIV and a NHP Model *(Partly Funded by Indo-US Joint Program on HIV/AIDS and STI Prevention Research Collaboration Awards, with Financial Support by NIH, USA and ICMR)*

Principal Investigator : **Nupur Mukherjee**

Principal Co-Investigator : **Kiran Munne**

Co-Investigators and Mentors : **Taruna Madan, V Patel**

Project Associates : **AK Singh**

Collaborator : **D Kaushal, Southwest Primate Research Center, San Antonio, USA**

Duration : **2020-2023**

India bears the largest burden of tuberculosis (TB) infected individuals globally where approximately 400 million are estimated to be at risk for reactivation of their latent infections. Also, with 2-3 million PLHIV and HIV-1 prevalence of about 0.3%, the country faces an impending public health crisis that threatens to impede much needed developmental goals. The benefit of IPT (Isoniazid Preventive Therapy) is internationally recognized and recommended (WHO guidelines) for individuals with latent TB infection (LTBI) that acquire HIV. However, this is a daunting goal for the overburdened, resource-limited public health sector in India. Targeted use of IPT in HIV-LTBI individuals presenting a prognostic susceptibility for reactivation may be a more efficient utilization of resources and reduce resistance by drug sparing. The present study seeks to elucidate both innate as well as adaptive immune correlates for their utility as prognostic markers for TB reactivation following HIV infection in a tertiary health care setting based in Mumbai, India. Also, the role and interplay between the gut microbiome and identified immune signatures will be examined in this setting as well as through an exploratory Rhesus macaque study that would seek to complement and validate the clinical data. Possible identification of microbiome-immune based prognostic signatures for TB reactivation in this setting, would, facilitate cost-effective and feasible therapeutic supplementation of ATT (Anti-Tubercular Therapy) and HAART (Highly Active Anti-Retro Viral Therapy) with probiotic and/or immunomodulatory mediators to prevent reactivation. While the risk of reactivation of LTBI in otherwise healthy individuals is 10-15% over a lifetime, this risk

greatly increases (5-15% annually) following HIV-1 co-infection and represents a significant clinical management issue in HIV-1 and MTb co-infected patients. Recruitment of participants is currently ongoing and 102 participants have been recruited till date. IGRA reports have been generated for 87 participants and shared with the clinical collaborators. Immunophenotyping analysis of 101 study participants revealed a possible association of a subtype of innate lymphoid cells (ILCs) with latent TB positive PLHIV.

3.11 Characterization of Integrin $\alpha 4\beta 7$ Expressing Immune Cell Subsets in PLHIV with TB Reactivation and its Association with Microbiome Profile

Principal Investigator : **V Bhor**

Project Associates : P Devadiga, Shilpa Bhowmik, V Patel, Taruna Gupta, Nupur Mukherjee, Kiran Munne, S Birje

Collaborators : Vidya S Nagar, Priya Patil, Grant Medical College and JJ Group of Hospitals
Jayanthi Shastri, Sachee Agrawal, TN Medical College and BYL Nair Hospital.

Duration : 2021-2024

People living with HIV (PLHIV) exhibit increased susceptibility to active tuberculosis (TB) on account of reactivation of a latent tuberculosis infection (LTBI). Among several factors which may be responsible for TB reactivation, gut microbiome dysbiosis and immune dysregulation are likely to play a prominent role. Integrin $\alpha 4\beta 7$, the gut homing receptor, serves as an additional receptor for HIV and has been demonstrated to be

associated with acquisition of HIV as well as disease progression. Any alteration in the expression of integrin $\alpha 4\beta 7$ on immune cells on account of HIV and/or LTBI may result in altered gut homing which in turn may result in impaired immune function. Recent reports indicate a role for gut microbiota in production of retinoic acid which is known to modulate expression of integrin $\alpha 4\beta 7$. In view of this, we attempted to characterize the integrin $\alpha 4\beta 7$ expressing immune cells in HIV infected individuals with LTBI to decipher its potential association with gut microbiome profile as well as to understand the combined action of these two factors in TB reactivation. HIV infected individuals were recruited from both JJ Hospital and Nair Hospital. Following determination of the status of LTBI infection these individuals were grouped into four distinct groups of HIV-infected with and without LTBI infection and HIV-uninfected with and without LTBI infection. Further, depending on the occurrence of active TB infection in these individuals, two additional groups emerged, namely HIV-TB co-infected and HIV-uninfected-TB-infected. LTBI+ve individuals exhibited a significant increase in the frequency and absolute counts of integrin $\beta 7$ expressing CD4+T cells when compared to LTBI-ve individuals among those infected with HIV (Fig. 53a and 1b). Similarly, absolute counts of central memory CD4+ T cells expressing integrin $\beta 7$ increased in LTBI+ve individuals when compared to LTBI-ve individuals in the HIV+ve group, whereas no corresponding change was observed in their frequency (Table 6). Further in the HIV-ve group, frequency of integrin $\beta 7$ expressing CD4+T cells was found to be higher in LTBI-ve individuals when compared to LTBI+ve individuals. Frequency of integrin $\beta 7$ expressing CD8+ T cells, effector memory CD8+ T cells and TEMRA CD8+ T cells increased significantly in LTBI+ve individuals when compared to LTBI-ve in HIV+ve individuals, although no significant change in

absolute count was observed (Fig. 53c, d and Table 6). The observed increase in frequency and counts of integrin $\beta 7$ expressing CD4+ and CD8+ T cells and their subsets in HIV infected LTBI+ve individuals is probably due to increased migration potential of these cells

towards the gut associated lymphoid tissue over lungs. Validation of these findings in the data obtained upon follow up of the HIV infected LTBI+ve individuals is likely to delineate the potential role of the gut homing receptor in TB reactivation.

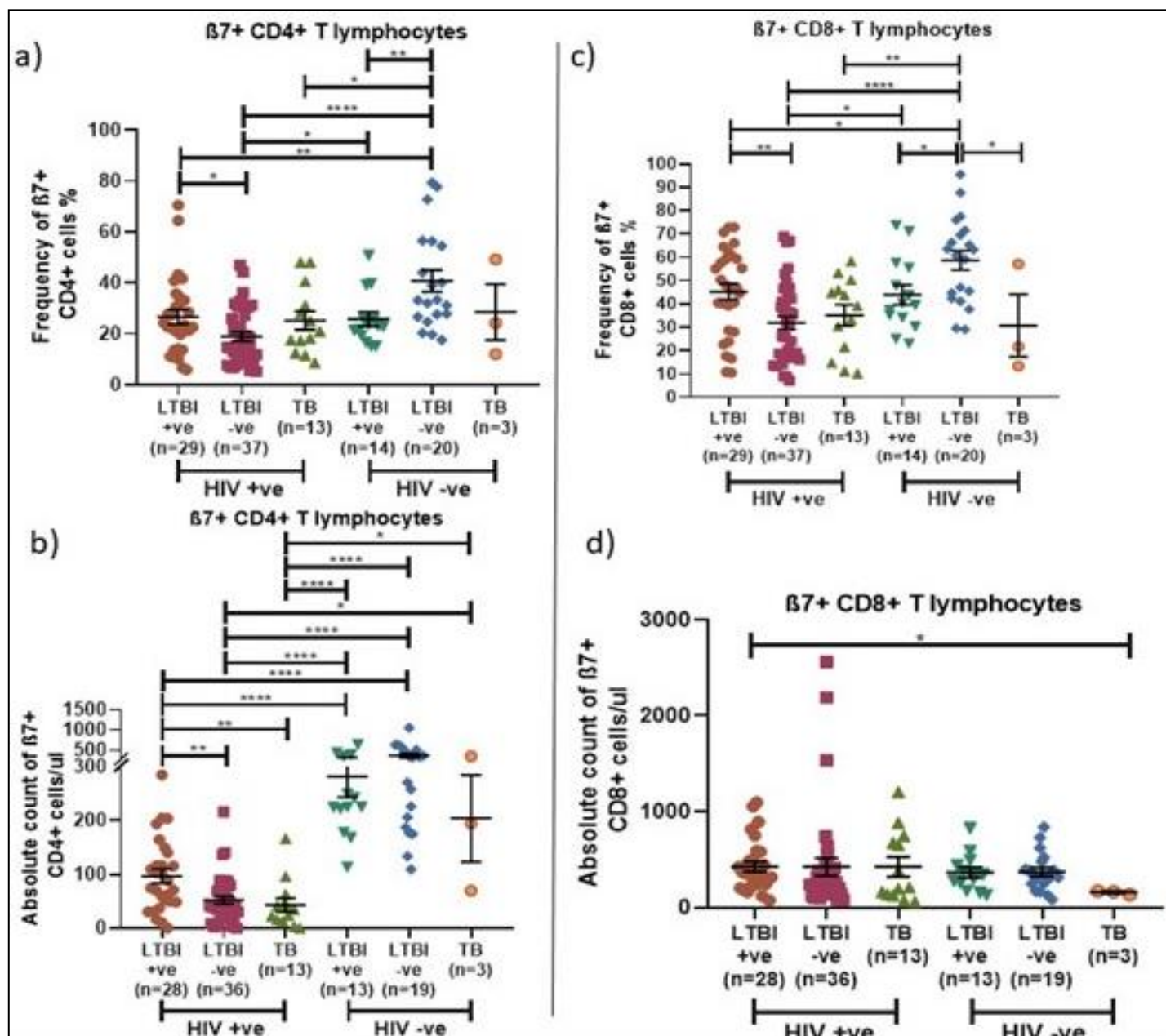


Figure 53: Frequency and counts of integrin $\beta 7$ expressing CD4+T and CD8+ T cells. Statistical significance was calculated by Mann-Whitney U-test; *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$; and ****, $p < 0.001$.

Table 6: Summary of the trends in frequency and absolute counts of integrin $\beta 7$ expressing CD4+ and CD8+ T cell subsets in HIV infected LTBI+ve individuals compared to HIV infected LTBI-ve individuals.

Cells	HIV+ve LTBI+ve compared with HIV+ve LTBI-ve	
	% Frequency	Absolute Count
$\beta 7$ + CD4+ T cells	↑	↑
$\beta 7$ + central memory CD4+ T cells	No significant difference	↑
$\beta 7$ + effector memory CD4+ T cells	No significant difference	↑
$\beta 7$ + CD8+ T cells	↑	No significant difference
$\beta 7$ + effector memory CD8+ T cells	↑	No significant difference

3.12 HIV-1 Specific T cell Responses and Viremic Non-Progression *(Partly Funded by Department of Biotechnology and India Alliance DBT Wellcome Trust)*

Principal Investigator : **V Patel**

Project Associates : AK Singh, Varsha Padwal, Harsha Palav, Shilpa Velhal

Collaborators : Vidya S Nagar, Priya Patil, JJ Group of Hospitals, Mumbai

Duration : 2020-2025

This study reports on HIV-specific T cell responses in HIV-1 infected Viremic Non-Progressors (VNPs), a rare group of people living with HIV that exhibit asymptomatic infection over several years accompanied by stable CD4+ T cell counts in spite of ongoing viral replication. We attempted to identify key virus-specific functional attributes that could underlie the apparently paradoxical virus-host equilibrium observed in VNPs (Table 7). Our results revealed modulation of HIV-specific CD4+ and CD8+ effector T cell responses in VNPs towards a dominant non-cytolytic profile with concomitantly diminished degranulation (CD107a+) ability (Fig. 54).

Table 7: Clinical characteristics of the study participants: a, Data are expressed as the median (range); b, Viral load was estimated at the time of sampling; c, HLA-B*27 and HLA-B*57 allele status of 5 and 1 of the HIV-1 infected participants respectively, was not available due to lack of SSP-PCR amplification; d, Only one participant was positive for both HLA-B*27 and HLA-B*57 allele

	Viremic Controllers [VCs, n= 07]	Viremic Non-Progressors [VNPs, n= 12]	Putative Progressors [PuPs, n= 11]
Age ^a (Years), Range	42 (29-60)	39 (30-49)	35 (21-59)
Gender	Female=03 Male=04	Female=04 Male=08	Female=06 Male=05
CD4+ T cell count ^a (cells/mm ³), Range	900 (501-1469)	680 (501-910)	553 (514-908)
Viral Load ^{a,b} (log10 copies/ml), Range	2.95 (1.73-3.04)	4.73 (4.01-5.35)	4.71 (3.58-5.98)
Duration of infection ^a (Years), Range	10 (8-24)	10 (7-16)	1 (0.5-03)
Antiretroviral therapy (ART) status	Naïve	Naïve	Naïve
HLA-B*27/B*57 status ^c	HLA-B*27 (+ve=0/06) & HLA-B*57 (+ve=1/07)	HLA-B*27 (+ve=1/09) & HLA-B*57 (+ve=0/11)	HLA-B*27 (+ve=02/10) & HLA-B*57 (+ve=1/11) ^d

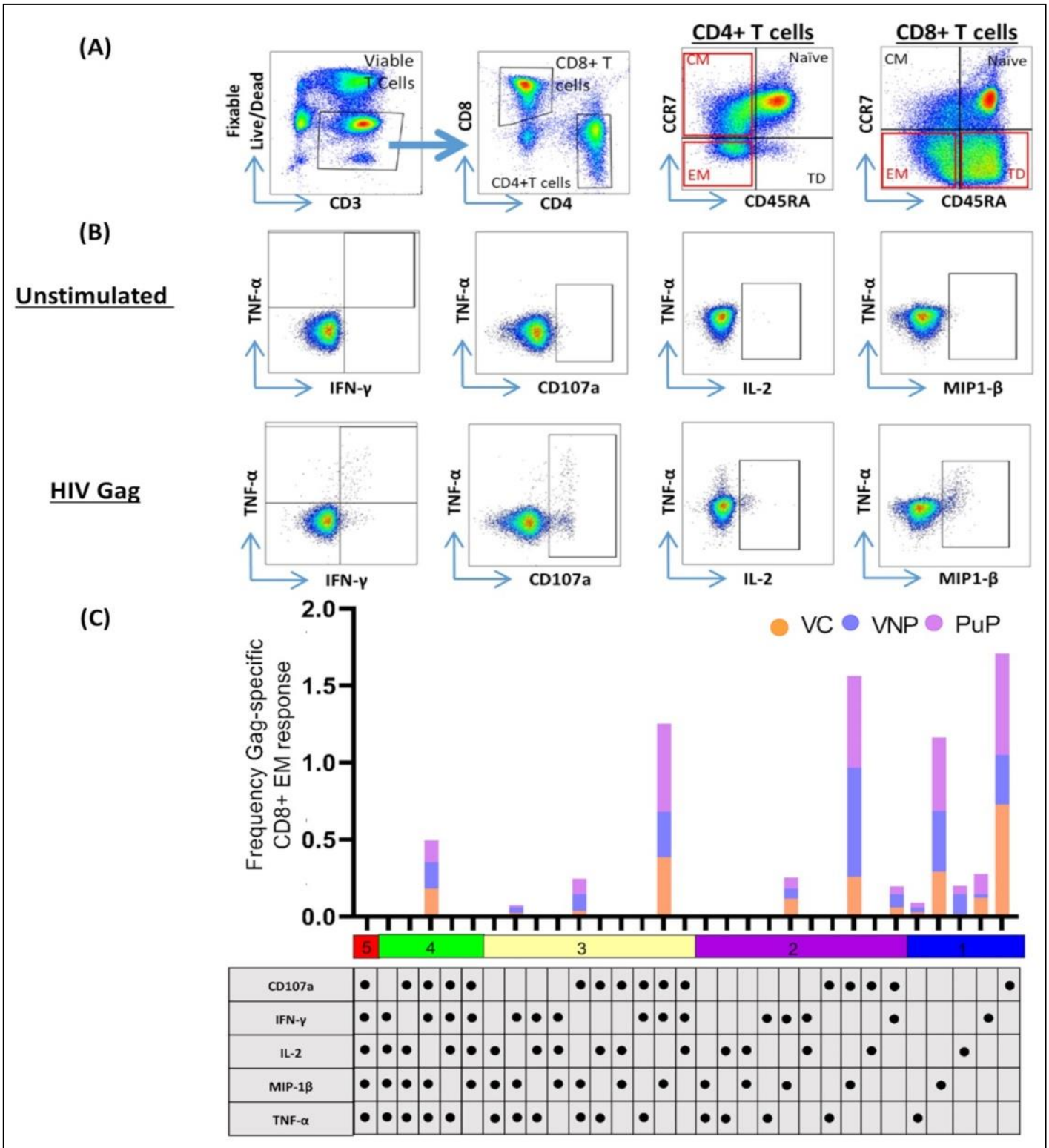


Figure 54: (A) Representative gating strategy for identification of viable CD4+ and CD8+ T cell subsets (in red boxes) for functionality evaluation. (B) Representative plots displaying background response in absence of HIV-antigen (top row) and functional profile in presence of HIV-antigen (Bottom row) individually for each function. (C) Data representing 31 different response patterns (except all negative) obtained through Boolean combination gating of individual function gates. Stacked bars (color coded for VC, VNP and PuP groups) represent mean CD8+EM Gag-specific response for distinct functional profiles (x-axis).

Further, the HIV specific CD8+ effector T cell response was primarily enriched for MIP-1 β producing cells. As expected, concordant with better viral suppression, viremic controllers (VCs) exhibit a robust cytolytic T cell response. Interestingly, putative progressors (PuPs) shared features common to both these responses but did not exhibit a CD4+ central memory IFN- γ producing Gag-specific response that was shared by both non-progressor (VC and VNP) groups, suggesting CD4 helper response is critical for non-progression. Our study also revealed that

cytolytic response in VNPs is primarily limited to polyfunctional cells while both monofunctional and polyfunctional cells significantly contribute to cytolytic responses in VCs. To further understand mechanisms underlying the unique HIV-specific effector T cell response described here in VNPs, we also evaluated and demonstrated a possible role for altered gut homing in these individuals. Our findings inform possible immunotherapeutic interventions to achieve functional cures in the context of ART resistance and serious non-AIDS events (Fig. 55).

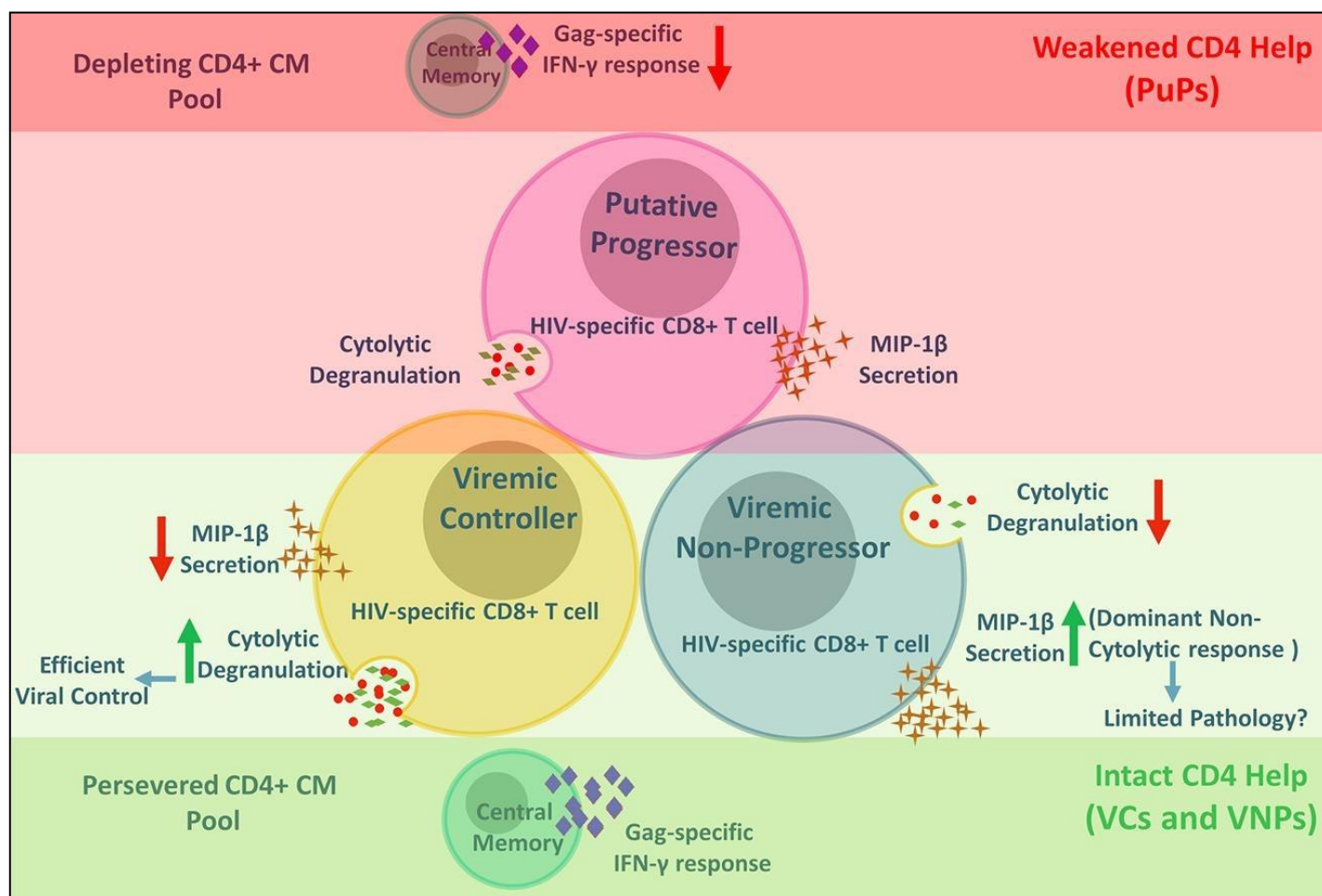


Figure 55: Model for HIV-1 specific T cell immunity Viremic Non-Progressors

4. AGEING

Endocrine, metabolic, genetic and mechanical factors are key determinants in the aetiology of postmenopausal osteoporosis. Besides these factors, inflammation also significantly contributes to its etiopathogenesis. Increased production of pro-inflammatory cytokines is linked with bone physiology and remodeling process.

4.1 Bone Exercise for Life: an Intervention Model on Bone Health and Quality of Life among Menopausal Women (Funded by Department of Health Research)

Principal Investigator : **Lalita Savardekar**
 Project Associates : D Singh, Consultant, General and Renal Medicine
 A Jain, Consultant Radiologist
 S Mohite, Consultant Spine surgeon
 Vandana Bansal, Consultant, Ob/Gyn and Fetal medicine
 Anuradha Vohra, Consultant Yoga Expert
 G Kenkre, Consultant Physiotherapist, Shahina Begum, Neera Mehta, K Valanju
 Duration : 2016-2021

The objectives of the study are i) to assess the awareness towards maintaining bone health among perimenopausal women ii) study the feasibility, acceptability and impact of the 'Bone-x-cise-life' Intervention model' on the bone health and quality of life among the menopausal women affected with osteoporosis. The intervention module was developed based on the information generated from the focussed group discussion (FGD), which included: i)

Yoga sessions 4 times a week in the afternoons; ii) availability of local gardens for brisk walking, use of shoes for walking; iii) regular community activities for increasing rapport among the participants; iv) guidance on calcium and protein rich foods and their consumption; and v) use of IEC, pictorial or simple to understand ways for performing yoga by participants. In summary, walking, exercise, yoga, diet and medicines were included in the intervention module.

Database of the clinic was screened. Four hundred and eighty one women had the requisite T scores (-2.5 to 3.5). The study participants underwent following investigations and consultations. Case record form included the following details: sociodemographic, clinical, gynaecologic and obstetric, diet (24-hour recall and FFQ), physical activity, menopausal symptoms etc. Beck depression inventory, Beck anxiety inventory and Quality of Life questionnaire (QUALEFFO-41) were also pursued. DXA scans to include lumbar, dual femur, forearm BMD estimations, X-ray both knees, ECG and blood tests (haemoglobin, fasting blood glucose, creatinine, albumin, phosphorus, magnesium, thyroid stimulating hormone, 25-hydroxyl vitamin D, serum calcium, intact parathyroid hormone, HbA1c) were carried out. All the study participants underwent evaluation by spine, knee surgeons and general medicine specialist including gynaecological exams. At the time of recruitment, the study participants control (n=27) and yoga group (n=28), were educated on calcium and protein rich diets, brisk walking and bone health measures. A log for walking was maintained for both the groups. The yoga group underwent group classes in 2 batches, 4 times a week under the guidance of yoga consultants and support of peer yoga

volunteers from the community. Women were monitored by the yoga monitoring module. Pictorial yoga booklet helped women to perform the *asanas* in a stepwise manner. The compliance of the women in yoga group for yoga and walking was nearly 100% while that in control group for walking was over 90% suggesting a change in their life style pattern. Due to COVID pandemic, the ongoing recruitment was suspended and discontinued as group yoga classes were not feasible and follow up could not be undertaken. The community awareness so generated by this study brought forward women to get their bone health estimation done, however a modest change in their lifestyle with regard to regular walking was observed.

4.2 Osteoporosis Screening and Intervention Programme using AYUSH Approach: a Community based Programme in Maharashtra State – Public Health Initiatives Study (Funded by Ministry of AYUSH)

Principal Investigator : **KR Kohli, Director, Directorate AYUSH, Maharashtra**

Principal Co-Investigator : **Lalita Savardekar**

Project Associates : **S Khardenavis, R Ghungralkar, RA Podar**
Ayurvedic Medical College, Mumbai

Amruta Deokar, B Datt, Neera Mehta, Rohini Dalvi, Shahina Begum

Duration : 2017-2021

A Public Health Initiatives program was undertaken in collaboration with Directorate of AYUSH, Maharashtra state to address and sensitize menopausal osteoporosis among community, health-care professionals, policy

makers, and the public by screening for the burden of disease using camp approach, and offering short term treatment - *Ghanavati* and *Muktashutibhasma vati/Laksha Guggul* and *Muktashutibhasma vati* to those affected. Twenty three osteoporosis screening and treatment camps were conducted in community and Bone Health Clinic until March 2020 with a total of 2345 post-menopausal women screened. Of these, 937 women were detected with osteoporosis and offered treatment (as above) for 3 months. Over half of the population was above 50 years of age, reflecting that the aged women took benefits from the community-based camps. Among the risk factors for osteoporosis, history of atraumatic fracture, thin built, menopause, and history of tobacco or *misri* use were significantly associated with osteoporosis while sedentary lifestyle and family history of hip fracture did not relate. Over 34.2 % women complained of *Asthishool* and site commonly observed was shin, upper arm or heel with majority of them having mild pain. Knee was predominantly affected among the women (63.1%) followed by lumbar and cervical spine and shoulder joint. Mild and moderate pain in knee, spine and other joints was complained by 58.2% and 24.8% of women respectively. History of *Keshpatan*, *Dantpatan* and *Nakhpatan* was obtained among 39.1%, 27.33% and 13% of women respectively. *Bhrama* was present in 22.1 % women, 46.77 % women had complaint of *Chimchimayana*, 58.77% of women had *Rukshata* and 39.8% women had complaint of leg cramps. Among the women screened in the camps, over one third of them had complaints of constipation and several of those resorted to *mishri* for their bowel habits, however, this leads to increased dryness in the GI tract, thus they become habitual. *Saam Jivha* was observed in over the half the population, thus reflecting that their metabolism was adversely affected. Women had pallor clinically (34.5%) which correlated well with the laboratory findings of moderate and severe

anaemia (37.5%) while 21.1% had mild anaemia. Over one fifth of the population was overweight.

Vata, Pitta, Vata Pradhan or Pitta Pradhan Prakruti had strong association with osteoporosis (Table 8). A total 857 women were treated for osteoporosis with either Tablet *Laksha Guggul* (2 gm per day) along with Tablet *muktashukti bhasma vati* (500 mg per day) for a period of 3 months - osteoporosis with osteoarthritis; Tablet *Ghanavati* (1 gm per day) along with Tablet *muktashukti bhasma vati* (500 mg per day) for a period of 3 months.

Women were also given ancillary care: Tablet *Poonarnava Madur* (1 gm per day) for anemia; Tablet *Triphala guggul* (1 gm per day) for musculoskeletal pains; *Dhanvantar tail* for local application for musculoskeletal pains; Tablet *Pushyanug choorna vati* (1 gm per day) for gynecological diseases like leucorrhoea etc.

To improve the follow up scheduled at 45 days and 90 days of treatment for relief in symptoms and occurrence of any side effects, telephone calls, in addition to home visits, were made. A total of 506 phone calls were made and 81 home visits were made to obtain follow up data on phone (n= 195) and clinic visits (n= 37). Women were also offered free DXA and this helped in

increasing the follow up (approx. 30 to 40 % of women utilized the facility). Commonest reasons for discontinuation of the medicines cited at follow up were - gastro intestinal disturbances like hyperacidity, bloating and abdominal pain etc (n=15), nausea (n=20), loose motions (n=6), indigestion (n=4), travel and relocation (n=10), myths about Ayurvedic medicines (n=8), and others (Table 9). Follow up at 45 days and 90 days revealed that there was a significant decline in the intensity of pain among women with moderate and severe joint pains (*Sandhishool*) and *Asthishool* (bone pains). Over half of the study cohort benefitted from ayurvedic treatment for symptoms of leg cramps after 45 and 90 days of treatment.

The community based camp approach is one of the best modalities to screen women, especially aged women so that they avail the benefits of health screening, diagnosis and treatment. There is a need to increase counselling and awareness among the low income population on the health risks of uncontrolled hypertension and diabetes so that the complications and morbidity can be averted. The increasing prevalence of osteoporosis among these women, necessitate that intervention programs in the regular health care should be advocated.

Table 8: Association of *Prakruti* with osteoporosis

	Normal n=1173(%)	Osteopenia n=177(%)	Osteoporosis n=881(%)	Total
<i>Vata & Vatapradhan</i>	56.5	61	67.1	1362
<i>Pitta & Pittapradhan</i>	22.8	11.3	17.9	445
<i>Kapha & Kaphapradhan</i>	20.6	27.1	15	422

Table 9: Effect of 45 days of Ayurvedic treatment on symptoms of *Sandhishool* in patients of Osteoporosis

<i>Sandhiishool</i> / Pain intensity on Day 0 of Ayurvedic treatment	<i>Sandhiishool</i> / Pain intensity wise number of patients after 45 days of Ayurvedic treatment			
	Patients with no pain n (%)	Patients with mild pain n (%)	Patients with moderate pain n (%)	Patients with severe pain n (%)
Mild pain (126 patients)	15 (11.9%)	111 (88.1%)	0 (0%)	0 (0%)
Moderate pain (105 patients)	11(10.5%)	55 (52.4%)	39 (37.1%)	0 (0%)
Severe pain (4 patients)	0 (0%)	2 (50%)	0 (0%)	2 (50%)

4.3 A Study on Depressive Symptoms in Lower and Middle Socio-Economic Status Urban post-Menopausal Women with Osteoporosis and its Effect on Quality of Life

Principal Investigator : **Neera Mehta**
 Principal Co-Investigator : **Lalita Savardekar**
 Project Associates : **Purnima Vinay Thacker, Consultant Psychologist**
A Jain, Consultant Radiologist
 Duration : 2021-2022

Osteoporosis and depression are leading causes of morbidity and mortality in global population. Studies have shown that depression is one of the unrecognized risk factors of osteoporosis, thus affecting the quality of life. Scant Indian community-based studies exist on the association of osteoporosis and depression among menopausal women, middle & low-income urban population. Hence, the present study was undertaken to study depressive symptoms among the osteoporotic women above 50 years of age. Primary objective of the study is to assess the symptoms of depression among middle and low-income menopausal women with osteoporosis whereas secondary objective is to assess the quality of life among them. This cross-sectional study included a total of 100 osteoporotic menopausal women in the age group of 50-75 years. Depressive symptoms and quality of life was assessed using Patient Health Questionnaire-9 (PHQ-9) and WHO-QOL BREF 26 respectively. Osteoporosis was documented by i-DXA at the Bone Health Clinic. The mean age of women was 62.02±5.93 years. Lower and middle-income menopausal women included were 58% and 42% respectively having attained menopause since 15.83±6.85 years. The PHQ-9

questionnaire revealed mild, moderate and moderately severe depression among 17%, 4% and 1% of women respectively. Physical domain (61 ± 13 SD v/s 86±11 SD), psychological domain (74±16 SD v/s 85±10 SD) and social domain (65±34.52 v/s 84±18.11) was affected among women with depressive symptoms vs no symptoms. Impact on physical domain can be explained as 83% women had various musculoskeletal problems (mechanical low back pain, osteoarthritis knee, lumbar canal stenosis, periarthritis shoulder etc.) which can affect the quality of life among the menopausal women. Regular screening of depressive symptoms is needed in primary care to facilitate early diagnosis and treatment as it impacts the quality of life.

4.4 Study of Drug Utilization Pattern among Elderly Patients (Partly Funded by ICMR)

Principal Investigator : **Lalita Savardekar**
 Project Associates : **Crasto A, Chavan K, Neera Mehta, Shahina Begum**
 Duration : 2020-2021

The objectives of this multicenter study are: i) to evaluate and compare drug utilization pattern in elderly patients in Central Government Health Scheme (CGHS) and non-CGHS settings; ii) to evaluate drug utilization pattern in elderly patients in private and public healthcare in rural and urban settings iii) to assess the prescribing indicators (WHO criteria); and iv) to evaluate potentially inappropriate drug use in elderly. The non CGHS centres included MCGM hospital OPDs viz, VN Desai hospital, MW Desai Hospital, Veer Sawarkar Hospital, MD Agarwal Hospital, MGM hospital and Bone health Clinic OPD to include 1150 men and women aged 60 years and

above. Over 84% of men and women reported of at least one non communicable disease *viz*, hypertension (73.5%), diabetes mellitus (44.8%), Ischemic heart disease (9.7%), stroke (0.56%), chronic obstructive pulmonary diseases (4.8%), musculoskeletal disease (13.1%), hyperthyroid (0.5%), hypothyroid (2.4%) and psychiatric illness (1.0%). Here, the musculoskeletal disease seem to be falsely underreported as majority of the data collection was done from the medicine speciality OPDs. There was a universal

prescription for long-term multivitamin / multimineral combinations. Less than 10% were prescribed antibiotics. Over 95% of the prescribed drugs were from the National List of Essential Medicines, each patient reporting an intake of 1-13 drugs. Majority of the drugs were available free of costs through health care facilities. With no approval from CGHS central office in Delhi for the selected Mumbai dispensaries (despite repeated follow-ups), the study was suspended.

5. MATERNAL HEALTH

5.1 Maternal Near Miss Review and Corrective Measures at District and Women's Hospitals in Maharashtra (Funded by PIP Grant, Government of Maharashtra)

Principal : **Ragini Kulkarni**
Investigator
Co-ordinator : Sanjay Chauhan
Project : Anushree Patil, Shahina Begum, Archana Patil, A Deshpande, District and Women's Hospitals J Aiswarya
Duration : 2017-2022

Government of India released Operational Guidelines for MNM-R in December 2014 which are designed for use by program managers at different levels of public health system and to bring reduction of maternal morbidity and mortality. MNM-R is complementary to Maternal Death Review (MDR). Overall objective of this study is to implement the Maternal Near Miss Review (MNM -R) guidelines developed by Government of India along with MDSR and take corrective measures at selected district and women's hospitals in Maharashtra. The study was conducted at five district/women's hospitals in Maharashtra Nasik, Ratnagiri, Parbhani, Akola and Nanded. Training of the staff at all the hospitals was done when the

project was initiated and reported periodically every year. Data collection for MNM cases was done. All cases which met inclusion criteria given in Operational Guidelines for Near Miss were included and entered in the online software. Data analysis was done after extracting the data from the online software in SPSS. Maternal Near Miss meetings were conducted. After meeting, corrective measures were recommended based on three-delay model by the MNM Committee and corrective measures were taken by these hospitals accordingly. Total number of MNM cases identified during April 2018-March 2022 were 460. Out of these, Hemorrhage (64.6%) was the most common adverse event followed by Hypertension (23%). The study indicates that it is feasible to implement the MNM guidelines at the district hospitals. However, there are many challenges such as dedicated staff is needed for this activity, case sheets are not adequately filled, thus sometimes it is difficult to identify MNM cases, case load of MNM is very less as many cases are referred out and no separate committee exists for MNM. A letter mentioning the above points was communicated through Director, ICMR-NIRRH to Deputy Commissioner, Maternal Health, Ministry of Health and Family Welfare, Government of India. The study findings will be useful for implementation of the MNM-R guidelines of Government of India at district/women's hospitals in the country.

Table 10: MCH Services data and MNM Indicators from the selected district hospitals during 2018-2022

MCH services data and MNM indicators	Akola	Nanded	Nashik	Parbhani	Ratnagiri	Total
No. of deliveries	53717	5701	20952	21294	9186	110850
No. of abortions	2459	55	701	505	418	4138
No. of maternal deaths	16	0	161	14	5	196
No. of MNM cases	125	39	181	59	56	460
No. of live births among MNM	79	37	102	24	37	279
Maternal deaths: MNM	01:07.8	0	01:01.1	01:04.2	01:11.2	01:02.3

Table 11: Distribution of MNM cases as per adverse events (n=460)

*Adverse event as per criteria	Number	Percentage
Pregnancy specific obstetric and medical disorders		
Hemorrhage	297	64.6
Hypertension	106	23
Sepsis	22	4.8
Cardiac failure (cardiomyopathy- antepartum / postpartum)	3	0.7
Postpartum collapse	6	1.3
Preexisting disorders aggravated during pregnancy		
Anaemia	50	10.8
Respiratory dysfunction	15	3.3
Other organ dysfunction #	7	1.5
Diabetic ketoacidosis	1	0.2
# - liver, cardiac and renal dysfunction		
Incidental and accidental causes in pregnancy		
Infections	1	0.2
Anaphylaxis	6	1.3
Total	514*	

* Multiple adverse events in one woman

5.2 Evaluation of Synergistic Impact of Nano-curcumin and Alpha-Linolenic Acid on Pathophysiology of Pre-eclampsia

Principal Investigator : **V Dighe**

Principal Co-Investigator : T Madan

Project Associates : A Tiwari, Shruti Desai, Amruta Gadade, S Jadhav, P Salunke

Duration : 2021-2024

Pre-eclampsia is pregnancy-induced hypertension associated with increased oxidative stress and proteinuria in mothers leading to fetal growth restriction. It is a leading cause of maternal and fetal mortality and morbidity, affecting over 8% of pregnancies worldwide. It involves higher maternal oxidative stress and inflammation with altered fatty acid metabolism. Curcumin and Alpha-linolenic acid (ALA) have anti-inflammatory and antioxidant properties, respectively, and

their effects have been investigated individually on pre-eclampsia. However, the synergistic effect of curcumin and ALA is yet to be investigated. Nano-curcumin is an advancement over natural curcumin showing higher bioavailability, higher aqueous solubility, and increased anti-inflammatory properties. Its effect on suppressing pro-inflammatory cytokines and reducing oxidative stress has been investigated in various conditions like cancer, tuberculosis, rheumatoid arthritis, and malaria. We hypothesize that nano-curcumin and ALA may show a therapeutic effect in the LPS-induced pre-eclampsia model in Wistar albino rats and improve the pregnancy outcome. We have attempted to develop a Lipopolysaccharide (LPS) induced preeclamptic model by giving LPS 20µg/Kg bw on gestation day (GD) 13, followed by increasing the dose to 80µg/Kg bw upto GD18 by intraperitoneal injection. We found increased urinary protein levels and systolic blood pressure in LPS-treated rats compared to the control group. Further

validation of this model will be done with more animals and assess parameters like levels of pro-inflammatory cytokines, sFLT-1, etc. After standardization of the LPS-induced rat preeclampsia model, a Phase-II animal study will be initiated.

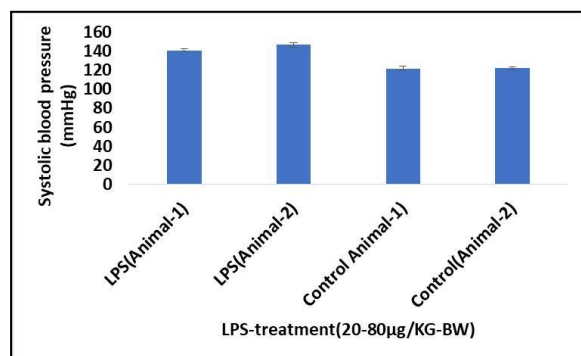


Figure 56: Systolic blood pressures (SBP) of treated and control animals. Bar 1 and 2 are LPS-treated, and 3 and 4 are control animals. LPS-20 μ g/Kg bw on GD 13 was followed by increasing dose till 80 μ g/Kg bw on GD18 by intraperitoneal injection. Control rats were treated with saline.

5.3 Evaluation of Therapeutic Potential of PVF-VEGF in Trophoblast Differentiation and Placental Spiral Artery Remodeling in Murine Models of Preeclampsia (Partly Funded by Department of Biotechnology)

Principal Investigator : **Taruna Madan**

Project Associates : A Bankati Jogdand,
Hajra Gupta

Collaborator : V D Dighe

Duration : 2021-2024

Preeclampsia (PE) is a pregnancy-specific hypertensive disorder characterized by an imbalance of angiogenic-antiangiogenic factors, inflammatory and anti-inflammatory cytokines, and immune cell activation tolerance. Previous studies identified a 27kDa lectin (PVF-VEGF) significantly and specifically induced differentiation of umbilical cord derived mesenchymal stem cells (MSCs) to endothelial

markers (Annual report 2017-18, pp. 80-81). Surfactant protein D is expressed by the trophoblasts and is known to modulate the inflammatory response, a critical etiological factor in PE (Annual Report 2019-20, pp. 78-80). The present study aims to evaluate the therapeutic potential of PVF-VEGF and SP-D in trophoblast differentiation and placental spiral artery remodeling in murine models of Preeclampsia. Perivitelline fluid (PVF) was collected from the Indian horseshoe crab eggs. The rPVF-VEGF (~27 kDa) was transiently expressed in Expi CHO mammalian cells and purified (Fig. 57A). HTR-8/SVneo cells were treated with PVF and rPVF-VEGF in Endothelial Cell Growth Medium (EGM2), with and without growth factors (hFGF-B, VEGF, IGF, and hEGF). In EGM2 Medium, no significant difference was observed in the viability of rPVF-VEGF treated group ($108 \pm 2\%$) as compared to untreated control cells ($100 \pm 4\%$) (Fig. 57B). Several angiogenic markers like VEGF, PLGF, VEGFR-1, vWF, CD31, and anti-angiogenic marker sFlt-1 are altered in trophoblast cells during spiral artery remodeling. A significant upregulation in the expression of angiogenic markers was observed in HTR-8/SVneo cells upon treatment with rPVF-VEGF (Fig. 57C). When compared with the control, the PVF and rPVF-VEGF treated wells showed slower wound closure. In FBS free DMEM media, the percentage wound closure in PVF and rPVF-VEGF treated groups was significantly lowered at 48h and 72h (Fig. 57D). In 2% FBS containing RPMI media, the effect of PVF and rPVF-VEGF on the wound closure was not significant at 72h (Fig. 57E). This lowered wound healing in the PVF and rPVF-VEGF treated groups could be due to the increased pro-angiogenesis in HTR8/SVneo cells. Treatment with LPS significantly upregulated the transcript levels of anti-angiogenic marker - sFlt1 and sFlt1/PLGF ratio (Fig. 58A) and pro-inflammatory TNF alpha and NFkB (Fig. 58B) in HTR-8/SVneo cells.

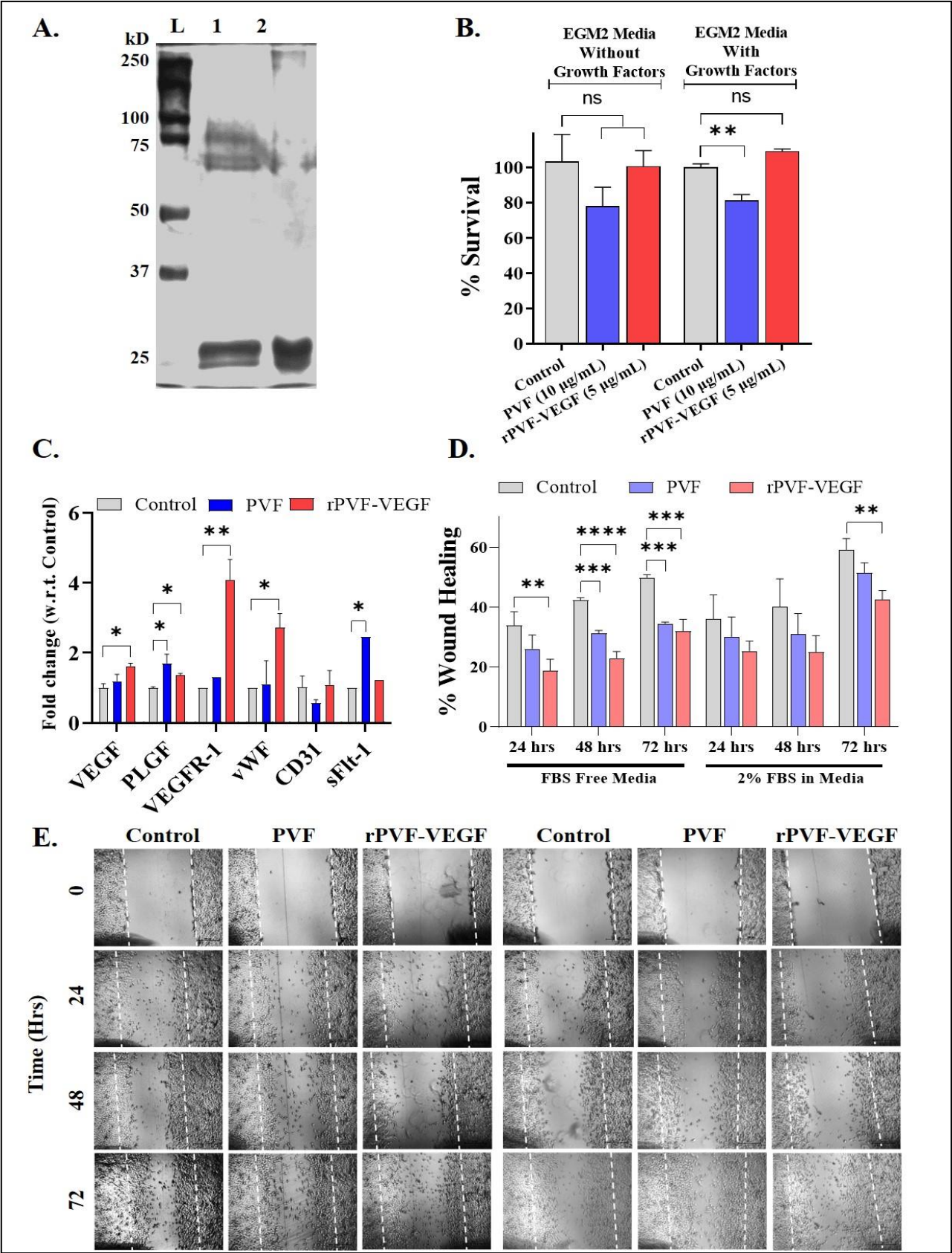


Figure 57: A. SDS-PAGE (10 %) analysis of PVF and rPVF-VEGF with silver staining. Lane 1 showed PVF and Lane 2 showed rPVF-VEGF; B. Cell viability studies in EGM2 media in the presence and absence of cell growth

factors (hFGF-B, VEGF, IGF, and hEGF); C. mRNA expression of VEGF, PLGF, VEGFR-1, vWF, CD3, and sFlt-1 was evaluated in the HTR-8/SVneo cell line after PVF and rPVF-VEGF treatment using real-time RT-PCR assay, 18S rRNA transcript level was used as internal control; D. Scratch assay to evaluate HTR-8/Svneo cell lines migration ability after treatment with PVF and rPVF-VEGF. Percent wound healing at 24 hrs, 48 hrs, and 72 hrs time points was evaluated using Image J software; E. Representative images (4x) of scratch and wound healing in HTR-8/SVneo cell line treated with PVF and rPVF-VEGF at various time points. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

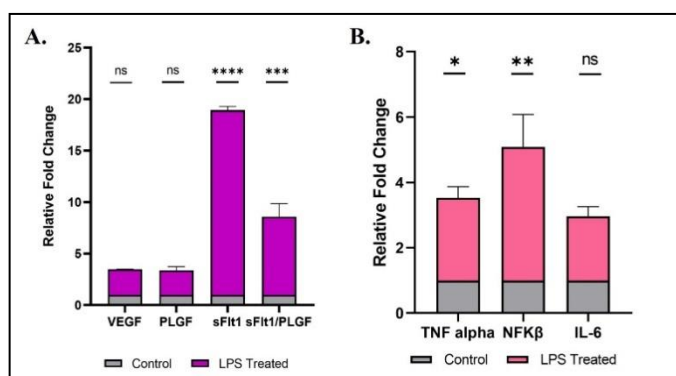


Figure 58: A. LPS induces a PE-like phenotype in HTR8/SVneo cells by establishing an imbalance of angiogenic-antiangiogenic factors; B. LPS produces an exaggerated inflammatory response in HTR8/SVneo cells by upregulation of TNF alpha and NFKB. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

5.4 Aberrant Paternal Imprinting: A Risk Factor for Preeclampsia (Partly Funded by Lady Tata Memorial Trust)

Principal Investigator : Nafisa H Balasinor
Principal Co-Investigator : Anushree Patil
Project Associates : Sweta Nair, Kumari Nishi, Zakiya Ansari
Collaborators : Himangi Warke, Seth GS Medical College & KEM Hospital, Mumbai
Vandana Bansal, Nowrosjee Wadia Maternity Hospital, Mumbai
Duration : 2016-2023

Preeclampsia is a gestational disorder involving high blood pressure and proteinuria or elevated

liver enzymes. In the developing countries, it occurs in about 2-18% of all pregnancies. The risk factors include advanced maternal age, primi-gravidity, obesity, diabetes, multiple gestation, pre-pregnancy hypertension and hypertension in previous pregnancy. The placenta has been known to be the main cause for the genesis of preeclampsia. Experiments in mice involving the development of normal (having one maternal and one paternal genome), androgenetic (having two paternal genomes) and gynogenetic (having two maternal genomes) revealed that the paternal genome is important for the development of the placenta, unravelling the phenomenon of genomic imprinting. Imprinted genes which are expressed in a parent-of-origin specific manner, play a crucial role in placental growth and development. The expression of imprinted genes is regulated by methylation at CpG sites of differentially methylated regions (DMRs). Aberrant expression of several imprinted genes in the placenta has been associated with preeclampsia. However, the epigenetic contribution of the paternal genome to preeclampsia has not been studied till now. Hence, the present study was undertaken to study methylation levels of imprinted gene DMRs in the spermatozoa and placenta, quantify the expression levels of imprinted genes and C19MC miRNAs in the placenta and investigate the global DNA methylation in the spermatozoa. Couples in which the female partners were suffering from early-onset preeclampsia (preeclampsia onset ≤ 34 weeks of gestation) were recruited in the case group while, couples in which the female partners

were normotensive and healthy were recruited in the control group. Till date, 25 and 14 couples have been recruited in the control and case group, respectively. The semen parameters were in the normal range in both the groups according to WHO 2010 standards. The gestational age at delivery, birth weight and placental weights were significantly lower in the case group. Four cases of Intra-uterine Fetal Death and three cases of Intra-uterine Growth Restriction were noted in the case group. Methylation analysis of specific gene loci was done by pyrosequencing.

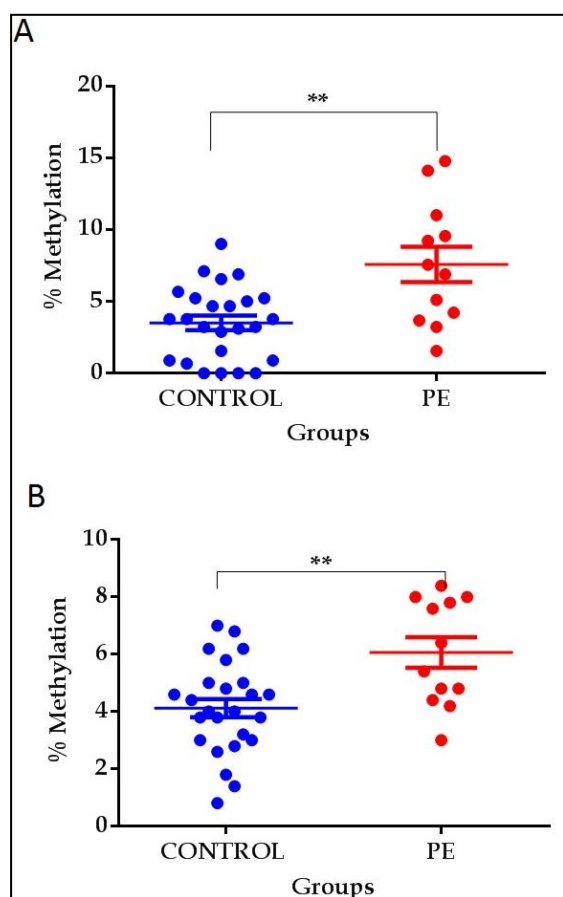


Figure 59: Average methylation in spermatozoa in controls (n=25) and cases (n=12) at A) KvDMR and, B) PEG10 DMRs. **p<0.01, data are presented as mean±SEM

Paternally unmethylated DMRs, KvDMR and PEG10 DMR showed hypermethylation (Fig. 59) while hypomethylation was observed at paternally methylated DLK1-GTL2 IG-DMR

(Fig. 60) in the spermatozoa of cases. The average methylation was not significantly different for H19-IGF2 and MEST DMRs. The results of gene expression study were presented in previous year's annual report (Annual report 2020-2021 pg 83-84). Small RNA sequencing was done to study the expression of C19MC's miRNAs in the placental villi. A total of 306 miRNAs from chromosomes 19 and 14 were found to be differentially expressed in the case group villi. At p value threshold ≤ 0.05 , 21 differentially expressed miRNAs (DEMs) were observed, out of which, four (hsa-miR-518a-5p, hsa-mir-519d, hsa-miR-520a-5p and hsa-miR-527) were from C19MC. Validation through qPCR and identification of their mRNA targets is underway. The study will help in understanding whether sperm DNA methylation is associated with preeclampsia.

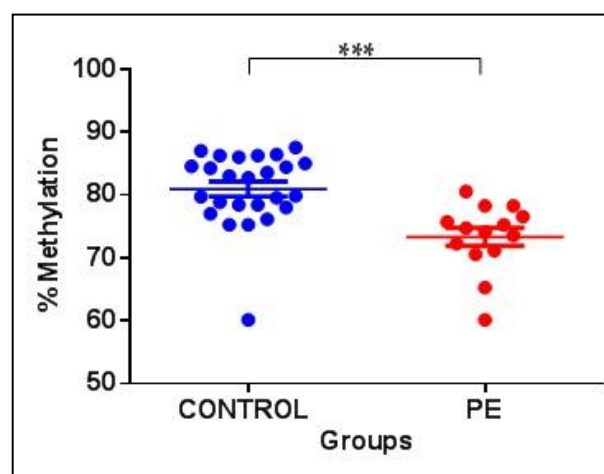


Figure 60: Average methylation in spermatozoa in controls (n=25) and cases (n=12) at DLK1-GTL2 IG-DMR. ***p<0.001, data are presented as mean ±SEM.

5.5 Omics of Placental Exosomes in Early Onset Preeclampsia: An Approach towards Identifying Predictive Biomarkers (Phase-II) (Funded by Department of Biotechnology)

Principal Investigator : Nafisa H Balasinor
Principal Co-Investigator : Taruna Madan, DK Das

Project : Uma Shinde, Aishwarya Associates Rao
 Collaborators : N Mayadeo, Seth GS Medical College & KEM Hospital, Mumbai
 Vandana Bansal, Geeta Balsarkar, Nowrosjee Wadia Maternity Hospital, Mumbai
 Duration : 2020-2023

Preeclampsia (PE) known for defective placental development, adversely affects approximately 1.5% Indian pregnant women. Early onset PE (EOPE), detected before 34 weeks of gestation, is associated with higher maternal and fetal morbidity and mortality. Early prediction of EOPE and preventive interventions may reduce complications in high risk pregnant women. Placental exosomes, small vesicles containing proteins and nucleic acids of trophoblast origin, appear in the serum by 6 weeks of gestation. The cargo of placental exosomes is reported to significantly influence

placental development by regulating inflammation and angiogenesis at the fetomaternal interface. Placental exosomal concentration is altered in preeclampsia. In view of non-availability of placental tissue for evaluation during pregnancy, circulating placental-exosomal derived factors could be efficient and specific predictors of EOPE. Comparative methylomic, proteomics and transcriptomics analysis of circulating placental exosomes would lead to identification of exosomal factors with potential for early prediction of EOPE. Previously (Annual report 2020-21, pp 85-86), double positive PLAP+/HLA-G+ exosomes of both syncytiotrophoblast origin (PLAP) and extravillous trophoblast (EVT) origin (HLA-G) were successfully isolated. A significant increase in pro-inflammatory and anti-angiogenic markers was observed in control PBMCs treated with placental exosomes from EOPE cases than the normotensive pregnant group.

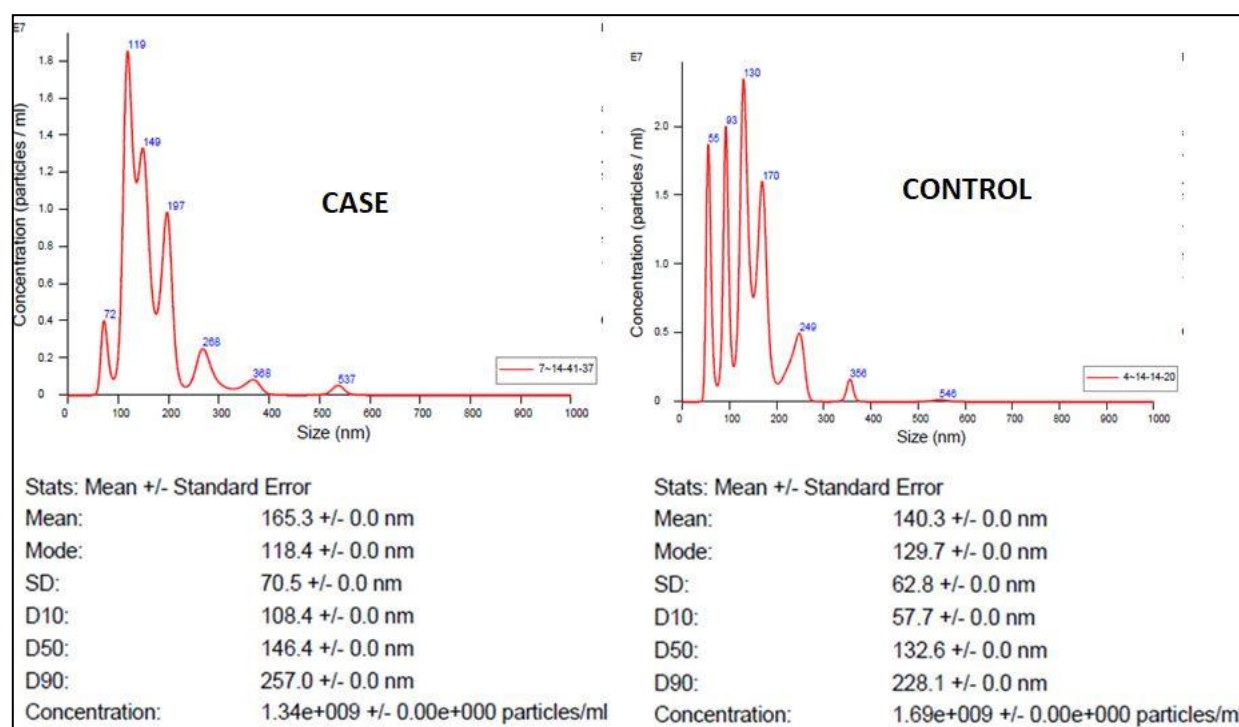


Figure 61: Nanoparticle tracking analysis of circulating placental exosomes using NANOSIGHT - LM 20 equipment.

In the reporting year, samples from a total of 891 (target n=1700) participants have been collected and after follow-up, 39 EOPE cases have been identified. Isolated circulating placental exosomes have been characterized by TEM and NTA (Fig. 61) and are found to be in the desired size range (50-150 nm). Preliminary findings of miRNA transcriptomics revealed 15 dysregulated miRNAs (6 upregulated and 9 downregulated) (Table 12) in preeclamptic circulating placental exosomes compared to controls. A total of 90 miRNAs were found to be present only in EOPE exosomes and 215 in controls. Most of these miRNAs were associated with adipocytokine signaling pathway and peroxisome function and biogenesis (as per the miRNA Enrichment Analysis and Annotation tool) (Fig. 62). Dysregulated metabolic pathways are known to be integral to the development of hypertension including preeclampsia. Dysregulation of miRNA associated with Allograft rejection suggests involvement of immune/inflammatory pathways which are known to be affected in preeclampsia.

Table 12: List of significant upregulated and downregulated miRNAs identified in circulating placental exosomes of women with EOPE compared to controls.

miRNA ID	Fold Change (Log2)	P Value	Reported in Placenta/PE
hsa-miR-107	6.94	<0.01	Yes
hsa- miR- 320b	3.60	0.04	No
hsa- miR- 582-5p	3.06	0.01	Yes
hsa- miR- 520c-3p	2.70	0.05	Yes
hsa- miR- 203a-3p	2.38	<0.01	Yes
hsa- miR- 205-5p	2.37	0.02	Yes
hsa- miR- 181c-5p	3.43	0.13	Yes
hsa- miR- 200c-3p	-2.28	0.02	Yes
hsa- miR- 200a-3p	-2.37	0.02	Yes
hsa- let- 7g-5p	-2.49	0.01	Yes
hsa- miR- 520f-3p	-2.75	0.06	No
hsa- miR- 141-3p	-3.49	<0.01	Yes
hsa- miR- 142-5p	-3.72	<0.01	No
hsa- miR- 577	-3.75	<0.01	No
hsa- miR- 142-3p	-5.89	<0.01	Yes
hsa- miR- 451a	-6.99	<0.01	Yes

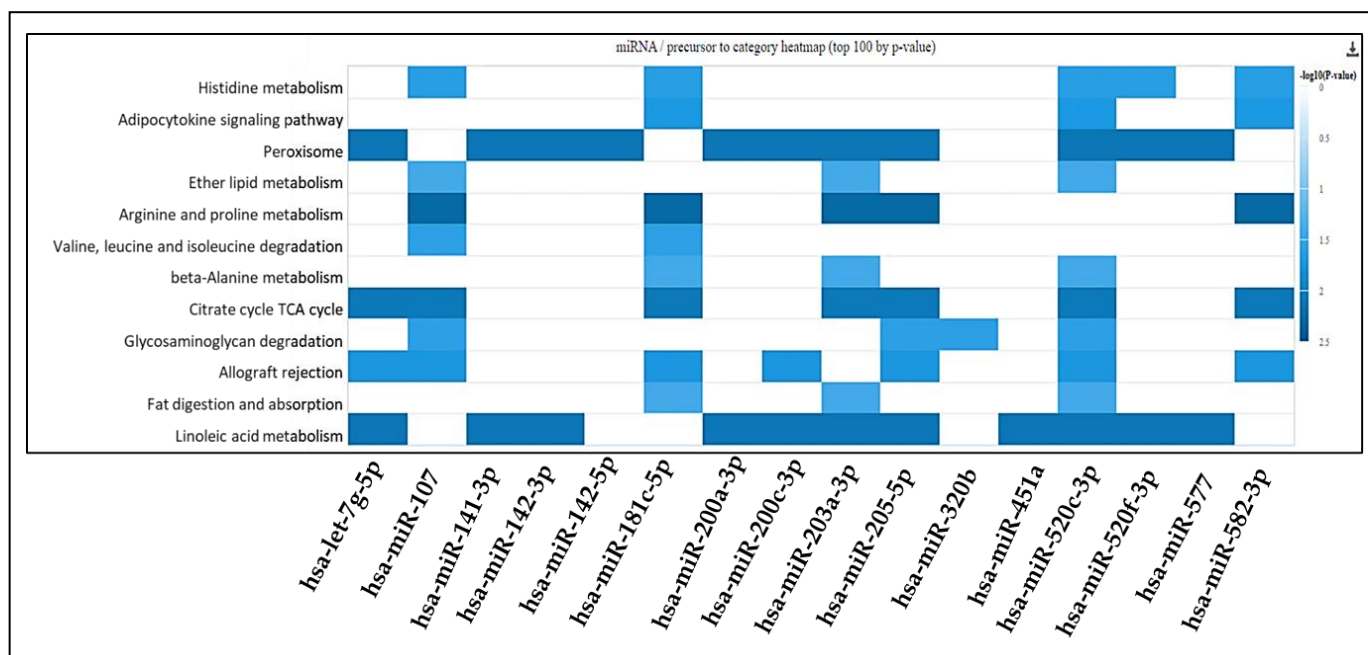


Figure 62: Heat map of GSEA of 16 dysregulated circulating placental exosomal miRNAs in preeclampsia at full term pregnancy stage.

5.6 Genetic and Epigenetic Changes in Imprinted Genes in Male Partners of Women Experiencing Recurrent Spontaneous Abortions (Funded by Science and Engineering Research Board)

Principal Investigator : **Nafisa H Balasinor**
 Principal Co-Investigator : Kushaan Khambata
 Project Associates : Anushree Patil, Dipty Singh, Sanketa Raut, Sweta Mohan
 Collaborators : Himangi Warke, Seth GS Medical College & KEM Hospital, Mumbai
 Vandana Bansal, Nowrosjee Wadia Maternity Hospital, Mumbai
 Duration : 2014-2022

Recurrent Spontaneous Abortion (RSA) / Recurrent Pregnancy Loss (RPL) presents a frustrating reproductive disorder, where a couple undergoes two or more consequent pregnancy losses before the 20th week of

gestation. It affects nearly 7% of Indian women and 1-2% of the women globally. Although several female causes (like genetic, anatomical, endocrine, immunological, infectious and haematological) are identified, paternal factors have not been paid much attention for their association with RSA as nearly 50% of cases remain idiopathic. In the previous year, the DNA methylation status of several important genes implicated in embryo development in the spermatozoa from male partners of couples undergoing idiopathic RPL were investigated (Annual report 2018-2019, pp. 61-63; Annual report 2020-2021, pp. 88-91). In the reporting year, the diagnostic potential of a combination of five imprinted loci was investigated by multiple logistic regression. The average methylation levels of differentially methylated sites for the genes IGF2-H19 DMR, IG-DMR, ZAC, KvDMR, and PEG3 for control (n=73) and RPL (n=80) samples analysed in our previous study were subjected to multiple logistic regression to obtain a predicted probability score for each sample. This probability score was then subjected ROC analysis. The AUC for the ROC curve was 0.88 (Fig. 63A).

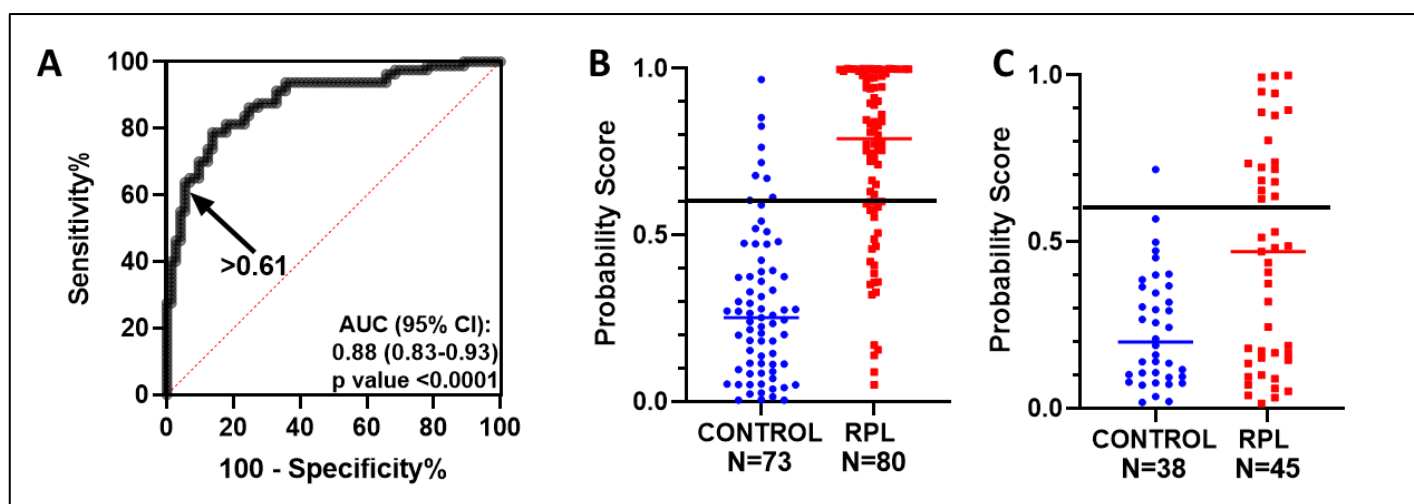


Figure 63: Multiple regression and ROC analysis: (A) ROC curve for the probability score of average methylation levels of differentially methylated CpGs in IGF2-H19 DMR, IG-DMR, ZAC, KvDMR, and PEG3 for the samples analysed in the previous study. Dot plot with median for the probability score for the control (n=73) and RPL samples (n=80) in the previous study (B) and for the probability score for the control (n=38) and RPL (n=45) samples in the current validation study (C). The horizontal line indicates the threshold value of 0.61.

A threshold of 0.61 was selected, at which the specificity was 90.41% and sensitivity was 70%. Fig. 63B represents probability scores for control and RPL groups above and below the threshold. In order to validate the probability score threshold for the combination of five imprinted genes to distinguish the epigenetically abnormal spermatozoa, pyrosequencing for the selected genes was performed in additional 38 control and 45 RPL spermatozoa samples and predicted probability score was calculated. In the control group, 97% samples were correctly classified having probability score below 0.61. Whereas, 40% of the RPL cases were above this threshold value (Fig. 63C). In the validation cohort, there were samples in the RPL group with probability values similar to control group, indicating that sperm DNA methylation may not be a contributing factor in those cases. As RPL is a multifactorial condition these cases would still remain idiopathic. To summarise, the results of this validation study indicate that this threshold can correctly classify the control samples, while identifying a subset of RPL cases with epigenetic anomalies in the sperm.

5.7 Involvement of Paternal Imprinting at MicroRNA Clusters of Chromosomes 14 and 19 in Recurrent Spontaneous Abortions

Principal Investigator : **Nafisa H Balasinor**
 Principal Co-Investigator : Anushree Patil
 Project Associates : Mamata Datar, Nishi Kumari
 Collaborators : Vandana Bansal, Nowrosjee Wadia Maternity Hospital, Mumbai
 Kavita Salve, Naigaon Maternity Home, Mumbai
 Duration : 2018-2023

Studies in our laboratory have demonstrated a significant association of aberrant imprinting in the spermatozoa with Recurrent Spontaneous Abortions (RSA). Epigenetic phenomena such as genomic imprinting and miRNAs regulation play important roles in embryonic and placental development. Two large imprinted miRNA clusters - Chromosome 14 miRNA cluster (C14MC) and Chromosome 19 miRNA cluster (C19MC) are known to be expressed in placenta during pregnancy. Altered expression of these clusters can be a risk factor for RSA. The study aims to analyze the imprinting status of these clusters in spermatozoa and their expression in villi in the context of RSA. The imprinting of the C14MC cluster is regulated by IG-DMR and it is paternally methylated. A total of 10 CpG sites of the IG-DMR were analyzed. Percentage methylation in spermatozoa ranged from 22% to 89% in the control group and 27% to 95% in the RSA group. Average methylation was 63.42% and 68.06% in Control and RSA groups respectively. Percentage methylation in chorionic villi ranged from 26% to 91% in the control group and 27% to 88% in the RSA group. Average methylation was 68.06% and 62.66% in Control and RSA groups respectively. No significant change in methylation at C14MC was observed in RSA.

For methylation analysis of C19MC DMR, a total of 27 CpG sites were analyzed. This DMR is maternally methylated and paternally expressed. Percentage methylation in spermatozoa ranged from 4% to 44% in control group and 7%-71% in RSA group. Average methylation was 13.81% and 25.81% in Control and RSA groups respectively. Percentage methylation in chorionic villi ranged from 42% to 79% and group 24%-83% for RSA group. Average methylation was 57.78% and 55.01% in Control and RSA groups respectively. miRNA sequencing of chorionic villi was carried out.

Total 6 miRNAs from C14MC and C19MC microRNA coding clusters were significantly differentially expressed in the RSA group. From C14MC, miR-342-5p and miR-151b were found to be downregulated, miR-654-3p, miR-665 were upregulated and two miRNAs from C19MC namely has-miR-524-3p and has-miR-525-3p were significantly downregulated in RSA group compared to control group. Real-time PCR revealed that miR-151b, miR-342-5p, miR-524-3p and miR-525-3p were significantly downregulated; miR-654-3p was upregulated and miR-665 was unaffected (Fig. 64). The list of predicted mRNA targets of these differential miRNAs was obtained by TargetScan. mRNA sequencing data was compared with predicted targets to get the actual mRNA targets that are expressed in chorionic villi. Target mRNAs were further analyzed using DAVID tool. Biological processes such as cell migration, cell adhesion, and angiogenesis were enriched which are crucial for early placenta development.

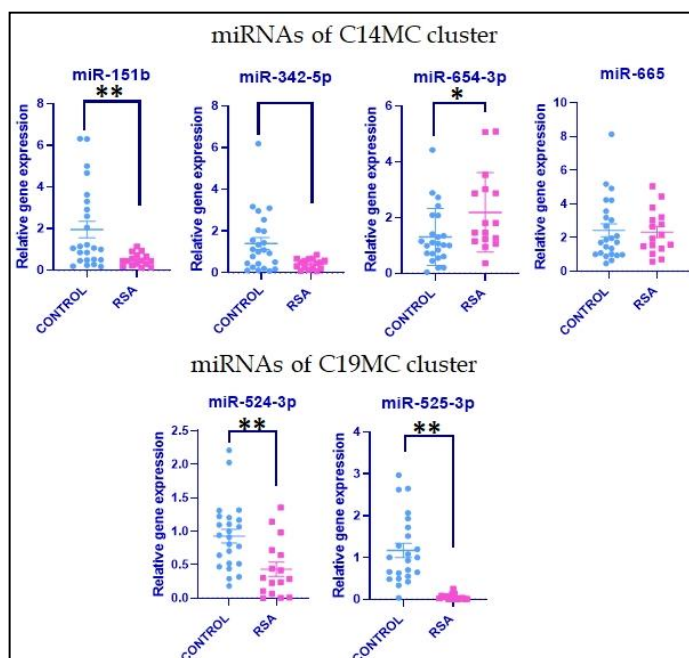


Figure 64: Expression of C14MC and C19MC miRNAs in the chorionic villi from control (n=25) and RSA (n=16) group, Values are mean $\pm$ SEM; *p<0.05, **p<0.001.

5.8 Idiopathic Recurrent Pregnancy Loss: Possible Association with Paternal Exposure to Endocrine Disruptors and Epigenetic Modifications in Sperm

Principal Investigator : **Dipty Singh**
 Project Associates : Delna Irani, Nafisa Balasinar, Anushree Patil, Deepti Tandon
 Collaborators : Geetha Balsarkar, Nowrojee Wadia Maternity Hospital, Mumbai
 Padmaja Samant, Seth GS Medical College & KEM Hospital, Mumbai
 Duration : 2018-2023

Recent studies have reported plausible effects of Endocrine Disrupting Chemicals (EDCs) on sperm epigenome; however, consequences of sperm epigenome modifications due to EDGs on embryonic and placental development remain elusive. This study aims to explore possible association of paternal exposure to endocrine disruptors with sperm epigenetic modifications in male partners of iRPL couples.

In this case-control study, group I included apparently healthy fertile couples (n=100) and group II included couples experiencing first trimester iRPL (n=100). A total of 44 couples in control group and 53 in iRPL group have been recruited till date. During the reporting year, genome-wide DNA methylation studies of spermatozoa from male partners of healthy fertile and iRPL couples were carried out. Five sperm genomic DNA samples from each group were outsourced (Sandor Life Sciences, Hyderabad) for Whole-Genome Bisulfite Sequencing (WGBS). A total of 9497 Differentially Methylated CpGs (DMCs) and 2087 Differentially Methylated Genes (DMGs)

were identified. DMGs common to embryo development (GO:0009790) and placenta development (GO:0001890) were obtained (Fig. 65A, 65B). These common DMGs were further screened using UCSC browser for regulatory features and selected for validation. 10 DMCs were selected which were located within PPARG, KCNQ1, HOXC4, MAPK14, MAP3K4, DNMT1, SETD2, IGF2R, MEG3, PRELID1 genes. Validation of the pooled samples was done and the methylation difference of selected DMCs obtained by WGBS was reflected in the same pooled samples by pyrosequencing. During the reporting year, a total of 39 control and 47 iRPL sperm bisulfite converted DNA samples were individually subjected to pyrosequencing validation for all the above mentioned DMCs. Last year we had reported pyrosequencing validation of 2 DMCs (PPARG, KCNQ1) with a smaller sample size (n=20).

It was observed that a sub-population of around 10.63% of the total iRPL population showed hypomethylation of the PPARG DMC in the range of 0%-15% ($p=0.123$) (Fig. 66A). In case of KCNQ1 DMC, a sub-population of 23.4% of the total iRPL population exhibited hypomethylation in the range of 0%-12% with significant difference ($p=0.006$) as compared to control (Fig. 66B). A sub-population of 8.51% of the total iRPL population exhibited slight hypomethylation of the MAP3K4 DMC in the range 66%-82% ($p=0.248$) (Fig. 66C). SETD2 DMC methylation analysis noted a sub-population of 12.76% of the total iRPL population showing hypomethylation in the ranges 24%-34% ($p=0.074$) (Fig. 66D). The other 6 DMCs showed no sub-population distinct to the iRPL study group. Assessment of spermatozoan histone modifications and serum EDCs is underway. This study will be helpful in understanding possible association between RPL and/or sperm epigenetic changes.

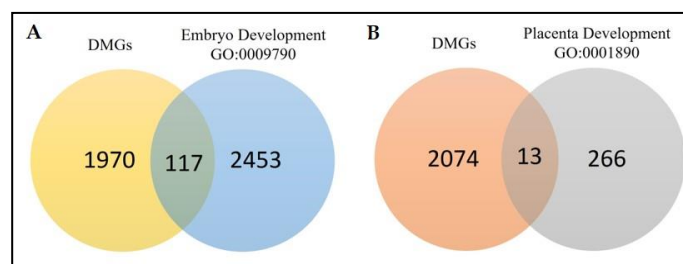


Figure 65: Differentially methylated genes (DMGs) common to human (A) Embryo development (GO:0009790) and (B) Placenta development (GO:0001890) pathways.

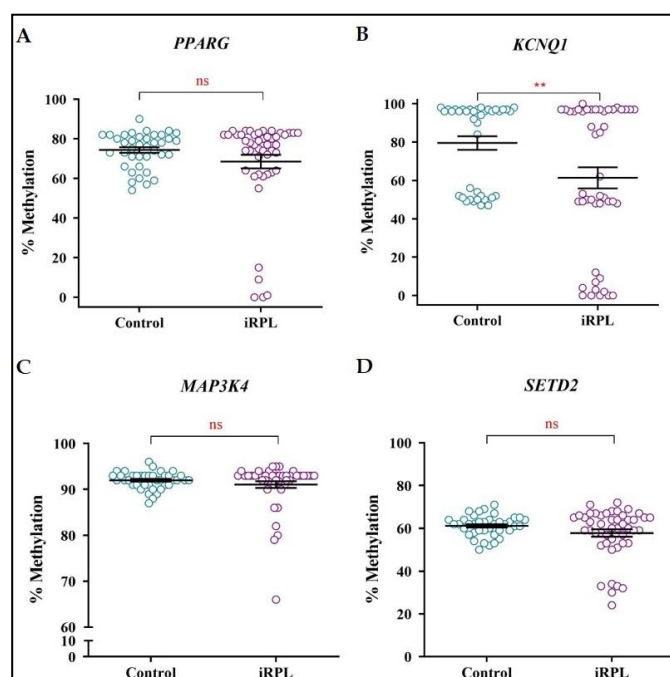


Figure 66: Methylation status of selected DMCs of DMGs in Control (n = 39) vs. iRPL (n = 47). (A) PPARG, (B) KCNQ1, (C) MAP3K4 and (D) SETD2. (** $p \leq 0.01$, ns - not significant). Data are represented as mean $\pm$ SE.

5.9 Enhancing Male Participation in Interventions to Prevent Unintended Pregnancy (Funded by National Institutes of Health, USA)

Principal Investigator : **Shahina Begum**
 Collaborators : Anita Raj, University of California San Diego, USA
 N Saggruti, Population Council, India
 Duration : 2018-2022

The objective of the study was to provide counselling to husbands and wives to achieve reproductive health and marital equity (CHARM2) and assess the impact of this intervention on modern contraceptive use among 1200 young married couples aged 18-29 years (600 each in CHARM2 and control groups) during baseline, 9 (FU9) and 18 (FU18) months follow up surveys. The couple retention rate was approx. 90% in each follow up survey. The full intervention (CHARM2) was received by 87.5% of couples. The analysis showed (Fig. 67) increase in contraceptive use in 9 & 18 months follow up survey (FU9) among non-pregnant women.

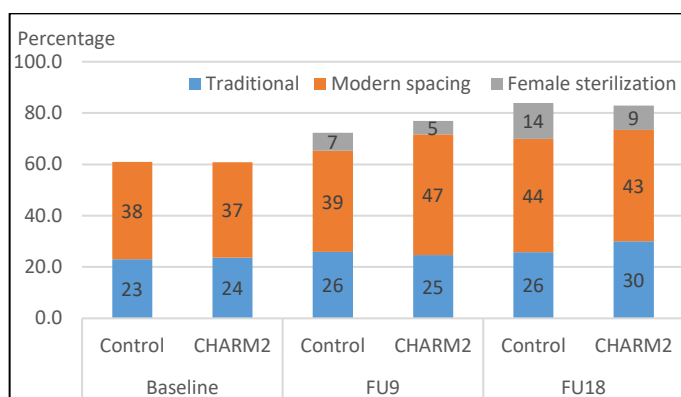


Figure 67: Type of contraceptive use among non-pregnant women over the study period

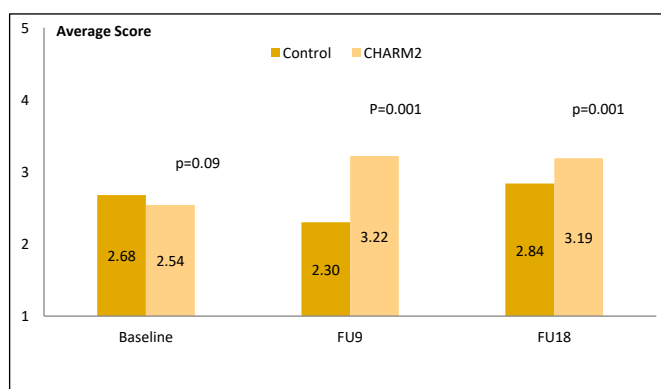


Figure 68: Quality of family planning services over the study period

Substantial increase in female sterilization was observed in control arm compared to CHARM2 arm. There was 6 percentage point increase in traditional method from baseline to 18 month

follow up survey. A significant increase in quality of family planning services was observed during the follow up surveys (Fig. 68). The study is completed.

5.10 Strengthening Maternal and Perinatal Death Surveillance and Response (MPDSR) Action in Tribal Blocks of Palghar District in Maharashtra (Funded by ICMR)

Principal Investigator	: Ragini Kulkarni
Principal Co-Investigator	: D Suryawanshi, District Health Officer, Palghar
Project Associates	: S L Chauhan, M Chavan, Shahina Begum, Anushree Patil, Neha Salvi
Supporting Staff	: I S Mashal, Srishti Verma, Vidisha Chouri, Deepali Ambre, Kanchan Marde, Sonali Thakur
Duration	: February 2021 - November 2021

Maternal Death Surveillance and Response (MDSR) has been included in the Guidelines of MDR by Government of India. However, the current scenario in India indicates that while the review process is done in the MDR meetings, the 'response' component still needs to be strengthened. Identifying corrective actions on the basis of the three-delay model among the maternal deaths reviewed could reduce the future deaths. Hence in the present study, the maternal deaths in the selected area will be reviewed and corrective actions will also be taken. On similar lines WHO has proposed a concept of PNDSR for capturing the number and causes of all stillbirths and early neonatal deaths. Currently, combined Maternal and Child Death Review meetings are conducted monthly at district level in which early neonatal deaths (END) are also reviewed. However, stillbirths are not reviewed during these

meetings. The present project will facilitate review of stillbirths during these meetings along with the review of maternal deaths. The overall objective of this study is to strengthen the maternal and perinatal death surveillance and to enable district and block health system to identify potential reasons and take appropriate actions to prevent maternal and perinatal deaths in the selected two blocks (Dahanu and Jawhar) of the Palghar district in the state of Maharashtra. The formative phase of the project was conducted for six months. The objective of this phase was to know the awareness and perceptions about maternal and new-born care and treatment seeking behaviour among reproductive age group women and community workers in the study area; to identify gaps in the filled maternal and perinatal death forms and the conduction of death review meetings. Twenty-four FGDs were conducted among three groups: Reproductive age group women (18-45 years), Accredited Social Health Activists (ASHAs) and Auxiliary Nurse Midwives in Jawhar and Dahanu blocks of Palghar district. Filled maternal and perinatal death forms were reviewed and the research team participated in the maternal death review meeting. The responses of women related to antenatal, intranatal and postnatal care are quite encouraging due to the implementation of RMNCH programmes. Majority of the women were aware of early registration of pregnancy, danger signs of pregnancy and most of them preferred institutional deliveries. Women had certain misconceptions about diet in postnatal period and consumption of Iron and folic acid tablets. Infrastructure and facilities and availability of skilled manpower were lacking in some of the areas. Difficulty in transport for women in labour to reach the health facilities were reported by women and the health workers. Based on the findings of the formative phase, interventions will be planned and conducted in the implementation phase.

5.11 Implementation Research to Explore Operational Feasibility, Acceptability and Cost-Effectiveness of using IV Ferric Carboxy Maltose (FCM) in Management of Iron Deficiency Anemia (IDA) among Pregnant Women through Sub District Health System in Maharashtra (Partly Funded by Department of Health Research)

Principal Investigator : **Beena Joshi**
Principal Co- Investigator : **Deepti Tandon**
Project Associates : **Ragini Kulkarni, Srimathi G, B Mahale**
Duration : **2022-2023**

Intensified National Iron Plus Initiative (-I-NIPI) of Government of India recommends using intravenous Ferric Carboxymaltose (IV-FCM) as an alternative to Intravenous iron sucrose in treatment of moderate to severe iron deficiency anemia in pregnancy. However most states have yet to implement it in the program. Thus the objectives of the current study are to explore operational feasibility of using IV-FCM for treatment of moderate to severe iron deficiency anemia during pregnancy; estimate improvements in hemoglobin and ferritin indices post-treatment and the need for blood transfusions during pregnancy; evaluate the perspectives from both provider and pregnant women receiving parenteral iron on ease of use and side effects; estimate post treatment pregnancy and neonatal outcomes; estimate health system cost; out of pocket expenditure and quality of life of women treated for anemia in pregnancy; assess cost-effectiveness of using IV-FCM in treatment of Iron deficiency anemia in pregnancy vs the standard of care (IV Iron Sucrose). The project is currently ongoing in two talukas - Dahanu and Jawhar in Palghar district. This open-label, comparative feasibility study includes consenting pregnant women of

age 18-40 years between 22-32 weeks of gestational age, diagnosed with iron deficiency anemia with hemoglobin levels between 5-9.9gm/dl. They are randomly allocated (1:1) to receive either intravenous iron sucrose which is the standard of care in the public health setting vs IV Ferric carboxymaltose for treatment of moderate to severe anemia in pregnancy. Investigators and research team have been sensitised about the study and screening for the

participants has been initiated. Total 130 pregnant women were screened during the reporting period. The screened participants are investigated for hemoglobin, red cell indices, serum ferritin, TIBC, Vit B12 and Hb electrophoresis. The participants who fit the inclusion criteria on the basis of hematological parameters are included in the study. The study is ongoing.

Table 13: Details of enrolment and dose administration

Details	Dahanu	Jawhar
No. of patients screened	29	101
No. of patients who opted out	2	7
Total no. of patients who received IV iron preparation	5	44
No. of patients who received IV Iron sucrose	2	21
No. of patients who received IV FCM	3	23
No. of patients who delivered before 4 week follow up	1	8
No. of patients followed up after 4 weeks of IV administration	4	24
Post IV iron infusion adverse events	0	1

5.12 Study of Adverse Event Profiles of Copper Containing Intrauterine Contraceptive Devices TCu380A and TCu200B

Principal Investigator : **Lalita Savardekar**
 Project : Varsha Vaze, Anuradha
 Associates : Muzumdar
 Duration : 2020 – 2021

This retrospective cohort study is undertaken to compare adverse event rates associated with the use of TCu380A and TCu200B intrauterine contraceptive devices, determine continuation rate of TCu380A and TCu200B during 5 years of use and study the factors associated with discontinuation of use of TCu380A and TCu200B during 5 years of use. Analysis of data records of 695 Copper T insertions among 437 women with either Copper T 200B (42.6%) or Copper T 380A (57.4%) revealed significant adverse events at removal of the device for

TCu380A vs TCu 200. However serious adverse event of accidental pregnancy was higher among TCu 200 users. The study analysis is ongoing.

5.13 Evaluating the Role and Proteolytic Processing of Trop1 and Trop2 in Normal Placentation and Placental Pathologies (Partly Funded by Department of Biotechnology)

Principal Investigator : **Bhakti R Pathak**
 Project : Apoorva S Pawar, Antara
 Associates : Banerjee, Ananya Breed, Meghali Borkortoky
 Collaborators : Pooja Bandekar, Nowrosjee Wadia Maternity Hospital, Mumbai
 Duration : 2021-2026

Human TACSTD family members- Trop1 and Trop2 [Trophoblast protein 1; more commonly known as (EpCAM) and Trophoblast protein 2] were first identified as proteins expressed by the trophoblasts and were later found to be overexpressed by many cancers of epithelial origin. They are well-known for their role in tumor cell proliferation, invasion, and maintenance of cancer stem cells. However, their specific role in placental function remains largely unelucidated. Both the proteins are reported to undergo proteolytic processing which may regulate their activities. Matriptase is one such regulating protease identified by our laboratory to cleave human Trop2. Its overexpression in the trophoblasts is known to have detrimental effects on mouse placentation. The current study aims at studying the expression, proteolytic processing, and role of Trop 1 and Trop2 (targets of matriptase) in placenta across gestation in normal and pathological placentation in rat model. In the reporting year, expression of EpCAM, Trop2, matriptase (the enzyme responsible for cleavage of EpCAM & Trop2), HAI-1

(Hepatocyte growth Factor Activator Inhibitor-1, a known inhibitor of matriptase), and claudin-7 (binding partner of EpCAM and Trop2) was studied in the rat placenta across gestation. The gestational time points covered the period of rat placenta maturation. Early time points GD12.5 and GD15.5 represent the time of rapid placental growth whereas GD19.5 is a late gestational time. The transcripts for targets showed differential expression levels in the placenta across gestation. *EpCAM*, matriptase, and *HAI-1* showed a steady increase in the expression from GD12.5 to GD19.5 (Fig. 69). Trop2 transcript levels increased after GD15.5. Notably, the expression of claudin-7 increases across gestation in an exponential manner. On the other hand, the expression of vimentin (a mesenchymal marker) remained constant throughout the gestation. This indicates that as the rat gestation progresses, the placental expression of these genes also increases suggesting their possible roles in the maturation of placenta. The expression of these targets at the protein level is also being investigated.

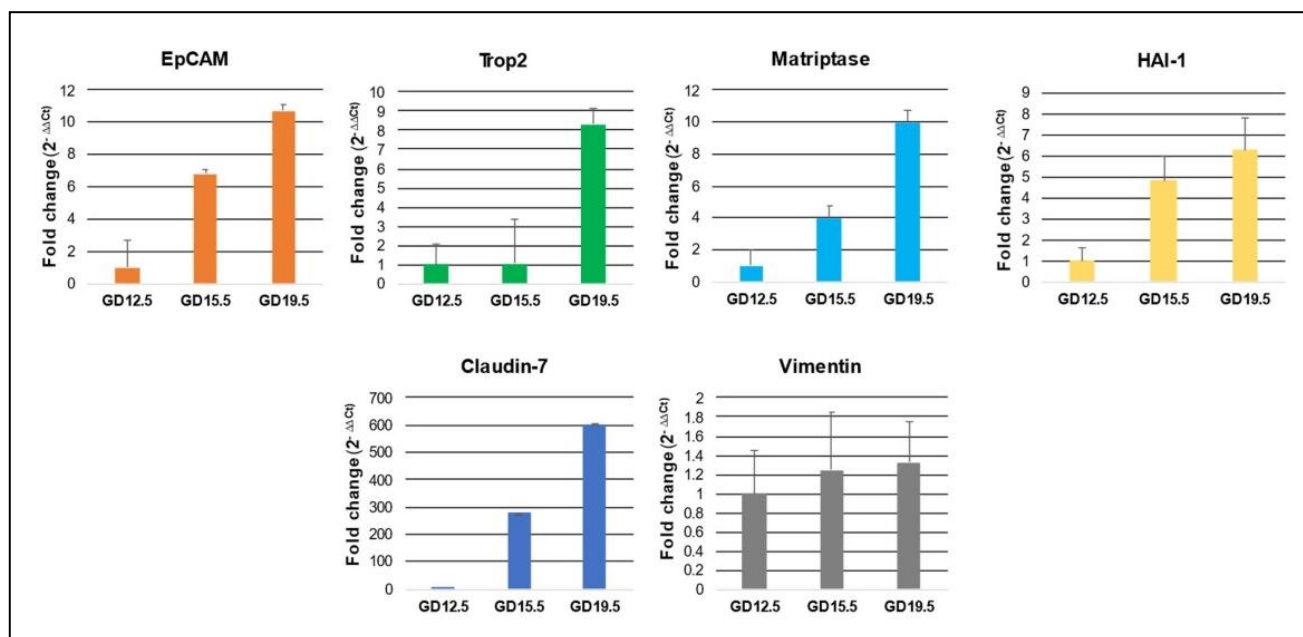


Figure 69: Quantitation of expression of indicated genes in the rat placentas across gestation by real-time qRT-PCR (n=2). Beta-actin was used as the reference gene and the relative expression was calculated using the $2^{-\Delta\Delta C_t}$. The results shown are mean $\pm$ SEM.

5.14 Development of a Microfluidic-Based Tool for Assessing Placental Functions and Evaluating Its Potential Application in Pregnancy-Related Disorders (Funded by Imprint II C DST-SERB)

Principal Investigator : **DN Modi**

Project Associates : A Bhide, Mamta Sawant

Collaborators : A Majumder, Debjani Paul, IIT-Bombay

Duration : 2020-2025

India has a high burden of pregnancy-related complications and many of them are due to

placental dysfunctions. Due to the lack of appropriate model systems, the research on placental (patho-)physiology is impeded. Currently, the model systems used are mouse placenta, and *in vitro* 2D assays using cell lines. However the mouse placenta is structurally and functionally different from the human placenta, the 2D *in vitro* assays fail to recapitulate the architecture of the human placenta. Therefore, there is a need for good model systems for the human placenta to carry out basic and applied research. This project aims to develop a placenta-on-chip that simulates the human fetomaternal interface. To recapitulate different aspects of placental biology and use them in various applications, prototypes of five different types of devices were micro-fabricated and optimized (Fig. 70).

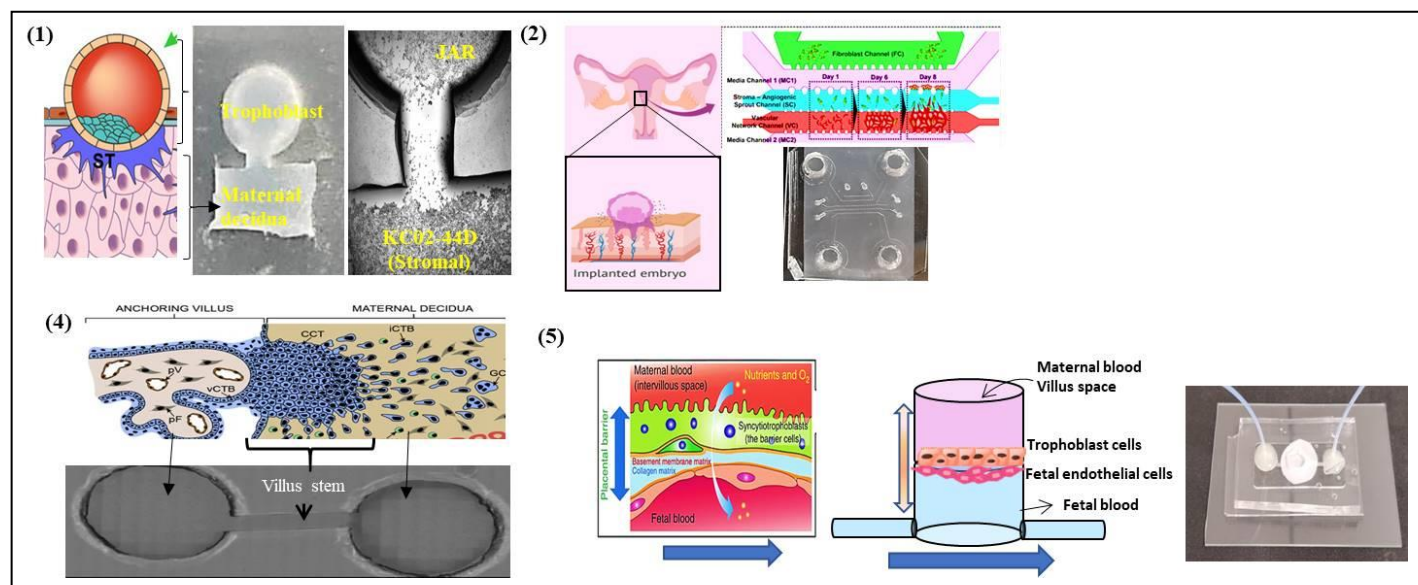


Figure 70: Micro-physiological systems to assess placental function. (1) and (2) Device to study embryo implantation phase; (3) Device for 3D organization of embryos and placental villi; (4) Device to study Anchoring villus; (5) Device to assess placental barrier function with flow kinetics in floating villus.

The first device was to simulate the fetomaternal interface at a very early time in pregnancy mimicking the process of embryo implantation. This is a 2D design in which trophoblast cells and endometrial epithelial cells were successfully co-cultured in two connected chambers. The second design was to

mimic the anchoring/stem villus to study extravillous trophoblast migration in response to decidual stimulus. Studying the migration of villus trophoblast is an important aspect of placental biology and this device was designed keeping in mind the need to study the migratory ability of two or more cell types

simultaneously. In this device, the coating conditions and cell numbers required to maintain and culture the cells for the long term (> 7 days) were optimized. We have successfully co-cultured one placental and one endometrial cell type on this device and their migratory ability by live and static imaging has been studied. COMSOL simulation has been done to optimize the parameters of the 2D device. The other devices with an extension of this design cover additional aspects of placental biology mainly to study cell-cell interactions for migration/invasion with more than two cell types. An important milestone achieved was a prototype for rapid generation of placental spheroids in a reproducible and high throughput manner. This is a 384 well-format PDMS-made micro-fabricated device optimized for the generation of uniformly sized placental spheroids in 24h in an ultra-high throughput manner. The utility of this device in drug testing has been demonstrated. Other prototypes developed include a design for 3D device mimicking the barrier function of the placenta on-chip. This device will help in understanding transplacental drug transport under flow conditions. The ongoing work is optimizing cell culture and growth conditions in the 3D device, adapting the 2D and 3D devices for studying cell migration and invasion under flow conditions, and COMSOL simulations of these devices.

5.15 Understanding the Role of Cannabinoid Receptors in Placental Calcium Transport

Principal Investigator : **Kumari Nishi**
 Project Associates : Nafisa Balasinor,
 Dipty Singh, Zakiya Ansari, S Mandavkar,
 Vaishali Nakhawa
 Duration : 2020-2023

The endocannabinoid system (ECS) is an emerging pathway known for its modulatory role in various physiological processes. The system functions both centrally and peripherally with the help of endogenous lipid molecules known as endocannabinoids (eCBs). eCBs are fatty acid analogs that are metabolites of membrane lipids. These endogenous molecules activate the type 1 and type 2 cannabinoid receptors (Cnr1 and Cnr2) that differ in their distribution. The best-characterized eCBs are N-arachidonylethanolamine (also known as anandamide, AEA) and 2-arachidonoylglycerol (2-AG), both derived from arachidonic acid. The components of ECS i.e. cannabinoid receptors and enzymatic machinery are present in the human first trimester chorionic villi. The present study was undertaken to understand the role of endocannabinoid system in placenta in the C57BL/6 mice. The explant culture of placentae collected on gestation day 18.5 were treated with the cannabinoid receptor 1 (Cnr1) agonist N-Arachidonyldopamine (NADA) and antagonist (AM251). The transcript levels of placental calcium transporter genes i.e., Trpv1, Trpv5, vitamin D receptor (Vdr) and plasma membrane calcium-transporting ATPase1 (Atp2b1) were determined.

Vdr and Atp2b1 transcript levels were significantly upregulated in the placentae treated with CB1 agonist as compared to CB1 antagonist. The immunofluorescence studies also indicated an increased expression of PMCA1 and SLC8A1 in the placentae treated with CB1 agonist as compared to those treated with CB1 antagonist suggesting NADA mediated activation of these calcium transporter proteins through CB1 (Annual report 2020-21, pp. 87-88). The Alizarin Red S staining indicated increased calcium staining in the placental tissues treated with CB1 agonist as compared to antagonist (Fig. 71 A-C). The staining intensity was higher in the chorionic

plate and decidua region in CB1 agonist treated group. The trophoblasts and endothelial cells were stained higher for Alizarin Red S in the agonist group (Fig. 71 A-C).

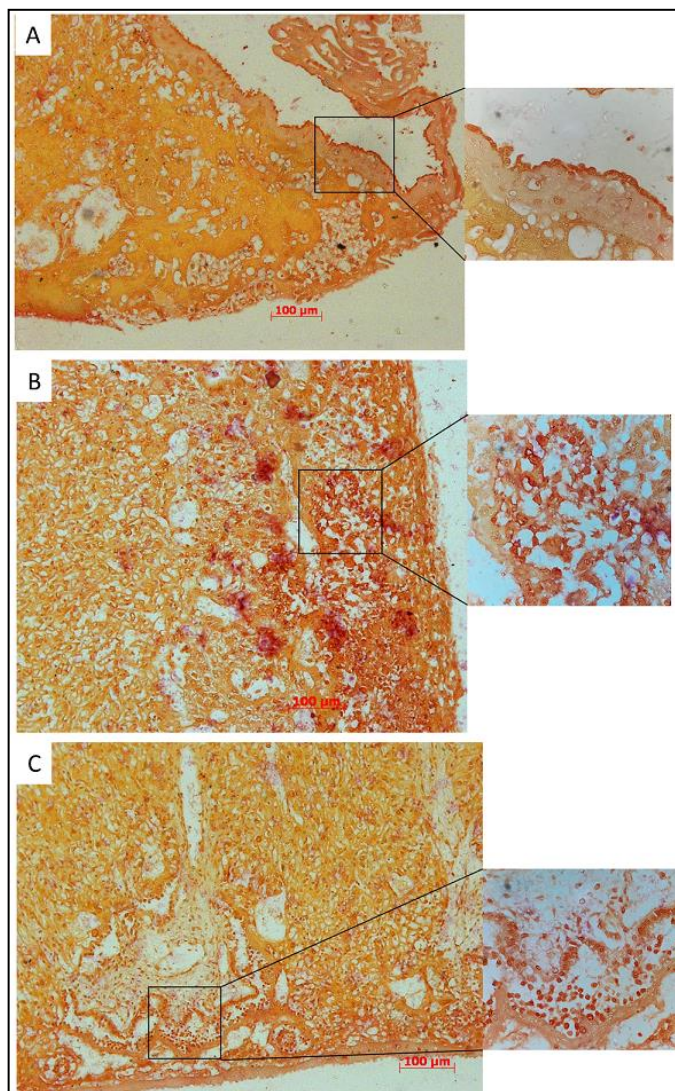


Figure 71: Alizarin Red S staining for calcium in mice placenta (gd18) from control [A]; Cnr1 agonist

(NADA)+CaCl₂ (1.0 mM) [B], and Cnr1 antagonist (AM251) + CaCl₂ (1.0 mM) [C](100X magnification) treated group. Red colour indicates calcium deposits.

The staining intensity in the placentae treated with agonist was significantly higher ($p < 0.005$) as compared to control and antagonist treated placentae (Fig. 72). The findings from the study suggest that the ECS may regulate expression of placental calcium transporters and calcium uptake. This provides us an opportunity to design future research to further apprehend how ECS can regulate placental and fetal development.

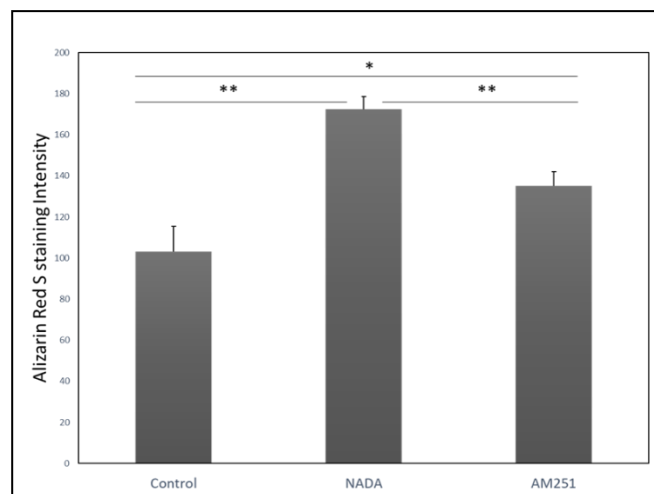


Figure 72: Intensity of Alizarin Red S staining was quantified using ImageJ software. The intensity in the agonist (NADA) treated placentae was found to be higher as compared to antagonist (AM251) and control placentae. Ten sections per treatment were analysed and mean calculated. ** $p < 0.005$; * $p < 0.05$.

6. CHILD HEALTH RESEARCH

6.1 Comprehensive Genetic Evaluation of Fetus in Antenatally Detected Abnormal Pregnancies with Fetal Malformations: Outcomes, Benefits and Limitations - A Pilot Study (*Funded by DHR-GIA Scheme*)

Principal Investigator : **S Pande**

Principal Co-Investigator : **DK Das**

Project Associates : **Shaini Joseph, V Bhanothu, Seema Kadam, Neha Minde, A Khan, H Gawde**

Collaborator : **Vandana Bansal, Nowrojee Wadia Hospital**

Duration : **2021-2024**

This study aims to identify genetic mutations associated with fetal malformations in an Indian cohort, evaluate the efficacy of the available genetic platforms and use the data generated for effective genetic diagnosis and possible management options. Ten products of conception (POC) samples were collected and were subjected to comprehensive genetic evaluation using a reflex approach. Two samples were used for validation and rest were enrolled for the study. The muscle and skin tissues of the fetus, and placenta were collected. Blood samples from the mothers were collected to rule out maternal contamination. Karyotyping and FISH for nine samples have been completed. Whole exome sequencing of one of the seven samples identified a variant of uncertain significance. The couple was counseled and advised to get tested. No chromosomal abnormalities were detected in the other six samples by Karyotype and FISH testing. The identification of frequency and pattern of known and novel mutations,

microdeletion/duplications and chromosomal abnormalities and the data generated in Indian cohort will be helpful in reduction of reproductive wastage and improving pregnancy outcome. This information will be helpful to offer prenatal diagnosis (if any) and extensive counseling to reduce society burden.

6.2 Assessment of Neonatal Screening Approaches for Sickle Cell Disease and the Effect of Early Intervention in Management of the Disease in Tribal Populations: Research cum Intervention Study (*Partly Funded by ICMR*)

Site Principal Investigator : **S Chauhan**

Site Principal Co-Investigator : **Suchitra Surve**

Site Co-Investigators : **Ragini Kulkarni, A Thorat, Palghar, Neha Salvi, A Maile, MRHRU-Dahanu, D Suryavanshi, District Health Officer, A Chavan, Neeta Gangurde, Sub District Hospital, Dahanu**

Project Associates : **Khushboo Chowdhari, Yugali Kore, Aarati Patil, Jidnyasa Kore, Shweta Dube, A Gawad, S Solanki**

Duration : **2019 - 2024**

Sickle cell disease is an important public health problem in India with highest prevalence amongst the tribal ethnic groups with an increased risk for severe morbidity and mortality during the first 3 years of life. The new-born screening is crucial to identify infants with sickle cell disease to initiate prophylactic

and comprehensive medical care. At present, there is no national neonatal screening programme in India. Therefore, a multi centric study is undertaken to establish a newborn screening program for Sickle cell disease in tribal populations of seven different states of India for early detection, to assess the magnitude of the problem, to identify the barriers for undertaking such programme; and to measure the benefit of early comprehensive care of affected babies. This study is being implemented through 7 centers across the country (Maharashtra - 2 centres, Gujrat, Tamil Nadu, Odisha, Madhya Pradesh and Rajasthan). The study also aims to evaluate the genotypic and phenotypic correlation to understand the role of genetic modifiers in disease severity. The study is being implemented at Dahanu block of Palghar District [Subdistrict Hospital (SDH), Dahanu and Kasa] through Model Research Health Unit, Dahanu.

Table 14: Total newborns screened and findings (Apr-2020 to March 2021)

Screening	Number
Total number of deliveries	3438
Total newborn screening by HPLC**	2087
Normal	1748
HbS heterozygous	172
HbS homozygous	10
Other Hemoglobinopathies	53

** Other Hemoglobinopathies-53

53[(22- S window-raised A2, 14- C Window, 02- Raised A2, 04- AS Trait with Unknown Window, 10-Unknown Window, 01-AS Trait with Raised A2)] +

Undetermined result / Results with Unknown significance: 104 [21- A0> F, 74- High P3, 06 - High P3 with A0>F, 03 -Technical Error)]

During the reporting period, out of a total of 3438 deliveries at SDH, 2087 newborns were screened for Sickle Cell Disease. Table 14

depicts the pattern of haemoglobinopathies amongst them. Fifty-three babies [(22 - S window-raised A2; 14 - C window; 02 - raised A2; 04 - AS trait with unknown window; 10 - unknown window; 01 - AS trait with raised A2)] had other haemoglobinopathies of undetermined significance and 104 babies had undetermined result [21- A0> F, 74- High P3, 06 - High P3 with A0>F, 03 -Technical Error)]. So far, total 3785 newborns have been screened for sickle cell disease. A total of 14 babies were sickle cell homozygous (sickle cell disease), 3 babies were compound heterozygous for HbS- β thalassemia and 301 babies were Sickle cell Heterozygous (sickle cell trait). The babies are being followed up at 6 weeks for confirmation of diagnosis as per the protocol. Screening of family members including parents and siblings is being done for babies having trait or disease followed by genetic counselling. Sickle cell homozygous babies are being given pneumococcal vaccinations and prophylactic antibiotics from 3 months as per standard of care and will be followed up to 3 years of life. Till now 5 babies have been started on Hydroxyurea and prophylactic antibiotics.

6.3 Population based Birth Defect (BD) Surveillance in Linkage with Rashtriya Bal Swasthya Karyakram (RBSK) Programme in Rural Blocks of Palghar District in Maharashtra (Funded by Department of Health Research)

Principal Investigator	: SL Chauhan
Principal Co-Investigator	: Suchitra Surve, Neha Salvi
Co-Investigator	: Ragini Kulkarni
Site Co-Investigators	: A Thorat, D Suryawanshi, M Chavan, P Pagi
Project Associates	: Priyanka Gawai, U Pachalkar
Duration	: 2020 – 2023

India has the largest number of newborns with birth defects with a prevalence of 61 to 69.9/1000 live births in India. Despite the high number of birth defects in India, there is a dearth of database on birth defects. Presently some groups are conducting birth defect surveillance; predominantly hospital based passive surveillance where participation is voluntary. Therefore, population-based birth defect surveillance was initiated in November 2020 at MRHRU Dahanu in seven rural blocks of Palghar district, Maharashtra. This study links Rashtriya Bal Swasthya Karyakram Program

(RBSK) to birth defect surveillance with a view to utilize existing resources and in turn strengthen the Birth defect component of RBSK. The screening for all the newborns for birth defects will be following the implementation mechanism of RBSK i.e public health facility based screening using existing manpower (MOs, ANMs, ASHAs) and community based newborn screening by ASHAs during HBNC visits. The project staff will visit all the facilities every one or two months and screen records for birth defects.

Table 15: Blockwise distribution of common major birth defects from April 2021 till March 2022

Type of birth defects	Dahanu	Jawhar	Palghar	Wada	Vikramgad	Talasari	Mokhada	Total
Cleft Lip and Palate	8	4	7	4	1	6	1	31
Other (hypospadias, ear, eye & limb related abnormalities etc)	16	14	6	12	3	8	2	61
Club Foot	6	5	4	5	8	0	0	28
CHD	6	3	5	4	4	1	3	26
Imperforate anus	0	5	1	0	1	0	2	9
Hydrocephalus	3	1	1	1	1	1	1	9
Collodion Baby	2	3	0	0	1	0	1	7
Down Syndrome	0	3	1	1	1	2	0	8
Spina Bifida (NTD)	2	0	2	0	1	0	0	5
Total	43	38	27	27	21	18	10	184

During the reporting year, refresher training for ASHAs to identify visible birth defects was completed. A total of 979 ASHAs were trained in 19 PHCs using IEC material. To measure the change in the knowledge of the ASHAs in identification of visible birth defects a pre-and post-tests were conducted. A number of challenges in terms of registers and reporting of birth defects were identified. The same was discussed during the internal review meeting and letter addressing the challenges were sent to CS and DHO. During the second round of data collection, ANMs who were present on the day of data collection were trained on identification and orientation of referrals and reporting of birth defects. At community level, data collection of ASHAs is mediated through Block Facilitators (BFs). This is done primarily through cell phone and with the help of

Whatsapp groups. The Whatsapp group was created as per the suggestion by RBSK nodal officers to facilitate linkage between RBSK and ASHAs. This group also serves to gather information on birth defects from BF's with their permission.

6.4 Community Based Screening and Management of Latent TB among Under-Five Children from Urban Slums in Mumbai (Funded by Department of Health Research)

Principal Investigator : **Suchitra Surve**
Principal Co-Investigator : **Ira Shah, Bai Jerbai**
Wadia Hospital for Children, Mumbai

Co-Investigators	: V Bhor, Shahina Begum, Kiran Munne
Project Associates	: Varsha Trayambake, Rachana Dalvi, Sharmila Kamat, P Venkateshwaran, M Periyappa, Madhuri Shikhare, R Kamble
Collaborators	: Executive Health Officer, Municipal Cooperation of Greater Mumbai (MCGM, F South), Pranita Tipre, Additional Health Officer, Public Private Partnership (PPP), MCGM, F South, N Sutar, District TB Officer, MCGM, F South
Duration	: 2019-2022

Childhood tuberculosis accounts for approximately 27% of the global burden of tuberculosis (TB) and contributes to 8-20% of TB-related deaths in India. Data from the Revised National TB Control Programme (RNTCP) reveals a decline in the prevalence and mortality rates of TB in the past 10 years. WHO's End TB Strategy aims for 90% reduction in TB deaths and an 80% reduction in the TB incidence rate by 2030. The diagnosis and treatment of Latent TB Infection (LTBI) forms an important pillar in this regard. Routine screening for LTBI is not done currently in RNTCP. The present study is a community-based study in urban slums in collaboration with tertiary hospital, aiming to screen under-five children at risk for LTBI as per WHO guidelines using Interferon Gamma Release Assay (IGRA-Quantiferon Gold Plus) and the proportion/fraction of LTBI children developing active TB. The study also aims to assess adherence to treatment and progression

of disease during follow up among diagnosed patients and assess acceptability of testing and treatment of LTBI among parents. This will be useful for policy makers on interventions for End TB Strategy in under-five age group. To compare LTBI Status in two different age groups i.e. 0-5 and 5-12 years. A total of 489 (369 - under five and 120 in the age group of 5-12 years) children are to be recruited for the study. 22 under-five children and 6 children in 5-12 year age group were recruited from April 2021 to March 2022. Hence, the total children recruited so far from phase 1 (n=369) and 2 (n=6) are 375 so far. Preliminary analysis of data from 369 under-five children revealed IGRA positivity in 29.41% (n=15) whereas total Tuberculin Skin Test (TST) positivity (>10mm) of 27.45% (n=14). Total 46 children suspected for LTBI on basis of IGRA and TST were evaluated at BJ Wadia Hospital-TB Clinic and screened for active tuberculosis by work up (CBC with ESR, X-ray Chest, Gene Xpert). None of the children had active tuberculosis infection. Children confirmed of having LTBI have been started on INH prophylaxis. Till date, 41 under-five children have completed their treatment. Children are also being provided with the nutritional advice and other ancillary care related to malnutrition. Children with LTBI are being followed up at the clinic as per the protocol. Repeat IGRA after completion of treatment is planned to understand the status of latent TB infection

Table 16: Bifurcation of IGRA and/or TST positive patients

Bifurcation	April 2021 to March 2022 (n=28)
IGRA Positive + TST Positive	6 (21.4%)
IGRA Positive + TST Negative	2 (7.1%)
IGRA Negative + TST Positive	1 (3.5%)

6.5 Role of Kisspeptin Mediated Signalling in Onset of Puberty

Principal Investigator : **Antara Banerjee**
 Project : Suchitra Surve, Bhakti Associates Pathak
 Clinical : Sudha Rao, Bai Jerbai
 Collaborator : Wadia Hospital for Children, Mumbai
 Duration : 2021-2024

Kisspeptin-1 (KISS1), a neuropeptide, signals through its cognate G-protein Coupled Receptor (GPCR), the Kisspeptin-1 Receptor (KISS1R) and heralds the onset of puberty. Downstream to KISS1/KISS1R signalling, the secretion of gonadotropin releasing hormone is stimulated, which activates the hypothalamic-pituitary-gonadal axis, resulting in sexual maturation. Environmental, lifestyle factors as well as mutations contribute to the timing of pubertal onset and any alteration in these result in early or delayed puberty. Mutations in KISS1/KISS1R are implicated in several pubertal disorders. However, functional studies for some of these known mutations are lacking.

In the reporting year, functional characterization of few mutations in cases of delayed puberty has been carried out. Four naturally occurring mutations R38P, P46Q, S125L and R198G have been reported in clinical cases of delayed puberty reported in literature. Using the Wild type Flag tagged KISS1R construct as a template and site directed mutagenesis as a tool, four substitution mutants of KISS1R have been generated. Expression of the wild type and four mutant constructs of KISS1R was confirmed by western blotting post transient transfection of CHO cells and results

showed similar total expression of WT KISS1R and the point mutants (Fig. 73).

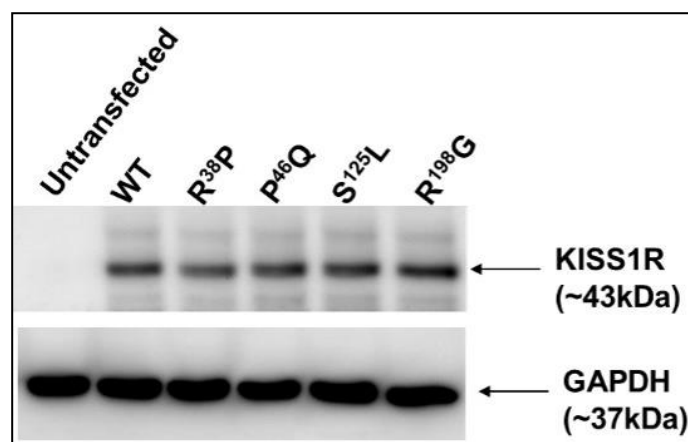


Figure 73: Expression of WT Flag KISS1R and four mutants twenty four hours post transfection in CHO cells. The panel represents results of western blotting using an anti-KISS1R antibody; GAPDH served as a loading control.

To be functional, a receptor must traffick to the cell surface so as to bind its ligand and trigger downstream signaling pathways. Hence, the cell surface KISS1R expression of the four mutants was assessed by immunofluorescence and flow cytometry (Fig. 74).

Results revealed that all the four mutant receptors demonstrated a lower cell surface localization as compared to WT KISS1R. The cell surface expression of the mutant R198G was found to be lowest. Thus, the reduced cell surface localization of mutant receptors indicates their reduced function, which could result in delayed puberty. Further, KISS1 stimulated signaling pathways like inositol phosphate production and phosphorylation of ERK are being investigated in order to assess the loss-of-function phenotype of these KISS1R mutations.

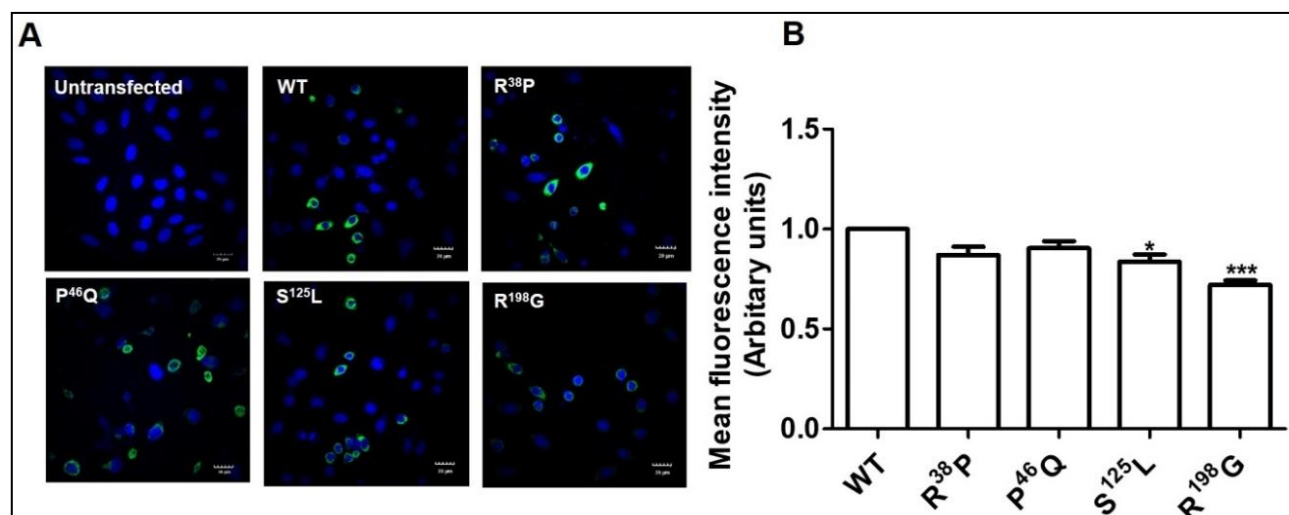


Figure 74: Cell surface expression of WT Flag KISS1R and its point mutants twenty-four hours post transfection in CHO cells as visualized by A) Indirect immunofluorescence and B) Flow Cytometry using an anti-KISS1R antibody. Confocal microscopy images (Scale bar=20 μ M) demonstrated KISS1R (green) with nuclei stained with DAPI (blue). For flow cytometry results, the statistical analysis was performed using one-way ANOVA, Tukey's post hoc test; *P < 0.05 and ***P < 0.001 with respect to WT KISS1R represent statistical significance.

6.6 Exploring Clinical and Therapeutic Relevance of Novel Biomarkers among the Children Presenting with Idiopathic and Incomplete Precocious Puberty at Tertiary Hospital Mumbai (Funded By ICMR)

Principal Investigator : **Suchitra Surve**
 Principal Co-Investigator : Antara Banerjee, Sudha Rao, BJ Wadia Hospital for Children, Mumbai
 Co-Investigators : D Modi, Beena Joshi, Anushree Patil, Shahina Begum, S Pande, Deepti Tandon
 Project Associates : Varsha Trayambake, Rachana Dalvi, Sharmila Kamat, Shital Bhanarkar, Shweta Bombe
 Duration : 2021-2024

Precocious Puberty (PP) is premature reactivation of the hypothalamic-pituitary-gonadal axis leading to GnRH secretion. PP is considered as the onset of breast stage II development before the age of 8 years in girls

and genital stage II development before 9 years in boys. It is classified as Central PP (CPP) wherein about 90% of the cases are idiopathic (ICPP), Peripheral or Incomplete Precocious Puberty. PP is mainly associated with adverse outcomes such as risk of short stature, PCOS, Diabetes mellitus-1, metabolic disorders in later life and most detrimental effect is psychosocial stress. Though GnRH-stimulated gonadotropin level remains the gold standard for diagnosis of CPP, currently ICPP poses significant diagnostic and therapeutic challenges. The lack of conclusive diagnosis may cause delay in initiation of treatment affecting health outcomes. Based on growing evidence in the literature, novel potential factors in the hypothalamo-pituitary-gonadal axis and upstream to GnRH secretion, such as Kisspeptin, Neurokinin B, Neuropeptide Y and MKRN3 can be used for the diagnosis of ICPP and Early Puberty and help in distinguishing these two forms of PP. The present study aims to explore the clinical and therapeutic relevance of the above mentioned markers. It will also evaluate factors contributing to ICPP and incomplete PP among children. This study is

being implemented at a tertiary hospital in Mumbai and Child Health Clinic of the institute at Abhyudaya Nagar over a period of three years. Girls in the age group of 6-9 years with probable diagnosis of ICPP and those with Incomplete variants of PP as well age matched controls will be included.

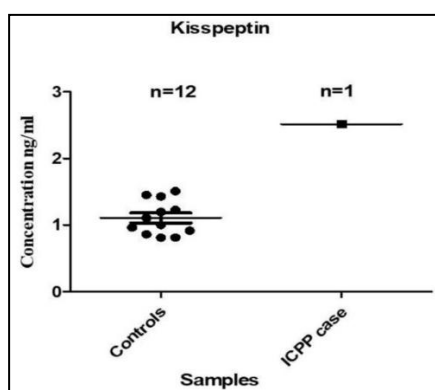


Figure 75: Estimation of Kisspeptin-10 levels by a competitive ELISA method. Each dot represents mean $\pm$ SEM value of each of the control participants and square represents the ICPP case assayed in duplicates.

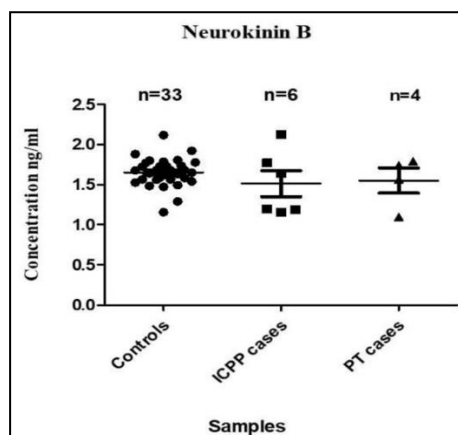


Figure 76: Estimation of Neurokinin B levels by a competitive ELISA method. Each dot represents mean $\pm$ SEM value of each of the control participants, squares represent the ICPP cases and triangles represent the PT cases assayed in duplicates.

During the reporting year, out of two hundred girls who were screened and thirty-three control participants have been recruited. From BJ Wadia Hospital for Children, ten cases of PP have been recruited so far. Out of these ten

cases, six cases are probable ICPP cases and four cases are probable PT (Premature Thelarche). The circulating levels of Kisspeptin-10 were found to be significantly higher in the plasma sample of the ICPP case as compared to controls. However, there were no differences in the plasma levels of Neurokinin B across the controls, ICPP and PT groups. Estimation of Neuropeptide Y and MKRN3 levels is ongoing.

6.7 Genetic and Biochemical Characterization of Mitochondrial Oxidative Phosphorylation (OXPHOS) Disorders in Children

Principal Investigator : **DK Das**

Project Associate : **Debolina Saha**

Collaborators : **Sonam Kothari, Shilpa Kulkarni, Jerbai Wadia Hospitals for Children, Mumbai**

Duration : **2021-2026**

Oxidative phosphorylation (OXPHOS) disorders, also known as mitochondrial disorders, are a complex family of disorders with a vast array of clinical manifestations that can be caused by mutations in mitochondrial DNA (mtDNA) as well as nuclear DNA that encode mitochondrial proteins. The prevalence of mitochondrial diseases is difficult to establish, predominantly because of their clinical and genetic heterogeneity. Phenotypic variations arise due to rearrangements and point mutations in the mtDNA and the prevalence of individual mutations is much higher, approaching 1 in 200 live births. Hence, OXPHOS diseases should be ruled out during differential diagnosis of familial diseases. The objectives are to classify mitochondrial disorders into biochemical and histopathological phenotypes and further identify genetic variations in mitochondrial and nuclear genomes.

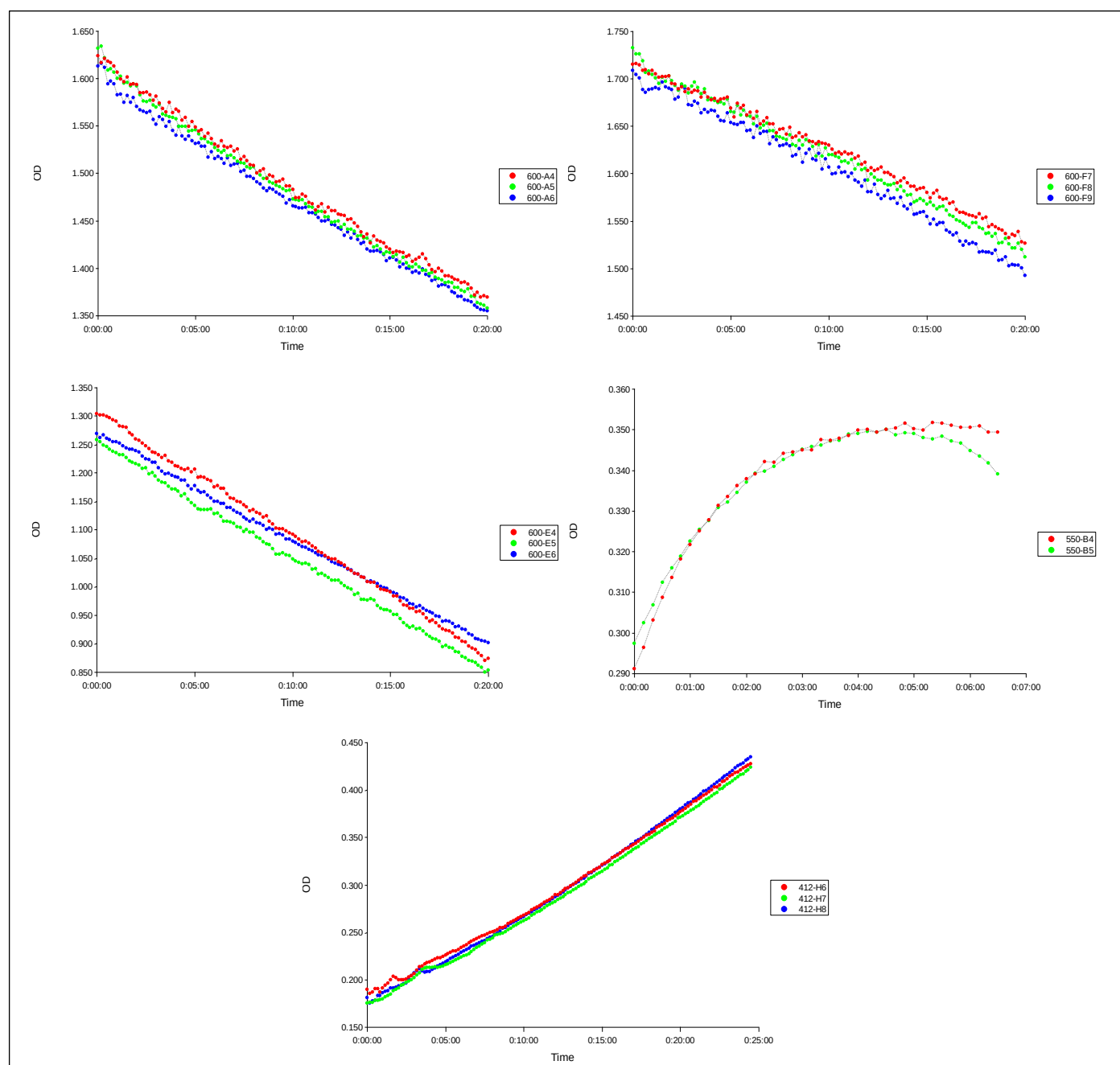


Figure 77: Standardization of biochemical assays on muscle samples of control participants: Michelis-Menton plot showing complex 1 enzyme activity without Rotenone (A); complex 1 enzyme activity with Rotenone (B); complex II enzyme activity (C); complex III enzyme activity (D); Citrate Synthase activity (Internal control) (E)

During the reporting year, 30 control participants were recruited from the orthopedic department at BJ Wadia Hospital for children. Estimation for all the mitochondrial enzyme complexes have been standardized in control muscle samples (Fig. 77). This average value of each complexes will be used further to

determine the enzyme defect in patients muscle sample. Further, second part of the tissue was processed for histopathological analysis including H&E stain and MGT for ragged red fibers. Again, snap frozen muscle tissue was also used for various enzymatic stains like NADH-TR for complex I deficiency, SDH stain

for CII deficiency, COX staining for CIV deficiency (Fig. 78). Muscle biopsy and blood samples were collected from 3 patients suspected of mitochondrial disorder. Biochemical analyses have been carried out on the tissue homogenates of these patients and it was observed that patient 1 (S1) and patient 2 had complex II + III deficiency. The enzyme activity of complex II of patient 1 was 6.1% and activity of complex III was 7.7%. Biochemical

activity of patient 2 was 26.9% for complex II and 4.9% for Complex III. Genetic analysis of both these patients have revealed no large deletions detected. Again, the histopathological findings were also found to be normal in these two patients. Further, these samples will be subjected to whole exome and mitochondrial sequencing for identification of any genetic defect. Analysis of patient 3 is currently ongoing.

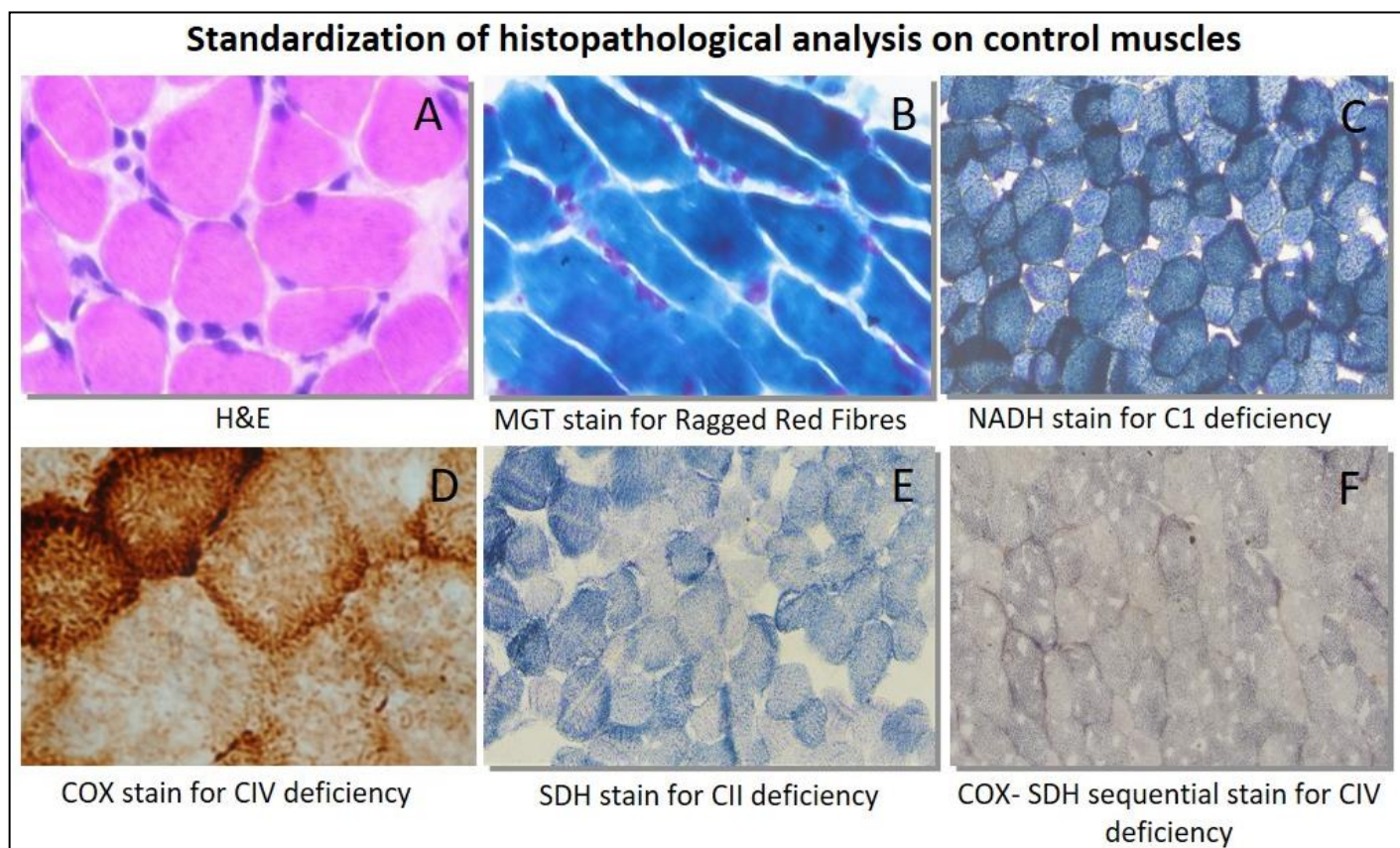


Figure 78: Representative bright-field images of muscle cryosections stained with A- H&E stain; B- Modified Gomori's Trichrome (MGT) for Ragged Red Fibres; C- NADH-TR staining for complex I deficiency; D- COX stain for complex IV deficiency; E- SDH staining for complex II deficiency F- Sequential COX-SDH stain.

7. STEM CELL BIOLOGY

The Stem Cell Biology department previously developed and characterized two human embryonic stem cells lines, KIND1 and KIND2. These cell lines have been successfully used for their differentiation into different lineages. These embryonic stem cells have been used in-house to differentiate into pancreatic progenitors and cardiac progenitors. These two cell lines have been shared with many researchers across India for research. The department has expanded its horizon to the area of adult tissue-resident stem cells. VSELs (Very Small Embryonic-Like stem cells) have been extensively studied in various adult tissues in mouse model. Their presence has been demonstrated in mouse bone marrow, testes, ovary, prostate and uterus. Our studies showed that the neonatal endocrine disruption can alter the number of VSELs and thereby lead to various reproductive pathologies. Recently, the department has embarked into the area of 'Induced Pluripotent Stem Cells' (iPSCs). Various projects were initiated to generate iPSCs from patients with neuro-developmental and neuro-psychiatric disorders in order to develop cellular models.

7.1 Studies using In-house Derived Human Embryonic Stem Cell Lines: Evaluating the Role of Hypoxia in hESCs Differentiation towards Trophoblast Lineage

Principal Investigator : **Sandhya Anand**
Principal Co-Investigator : **DK Das**
Collaborator : **Deepa Bhartiya, DN Modi**
Duration : **2019-2025**

Placenta is an important organ for normal well being of a developing foetus. Improper placentation could lead to pregnancy related

complications like intra-uterine growth restriction, pre-eclampsia, miscarriage etc. Currently, no in-vitro model system is available to study the normal/abnormal development of human placenta. Hypoxia is a key event that governs many cellular processes including cell differentiation and migration. The role of hypoxia in trophoblast invasion is well established; however, information about its role in specification of trophoblast lineage and early stage placentation is limited. This study aims to determine the effects of hypoxia on differentiation of human embryonic stem cells (hESCs-KIND1) into trophoblast lineage and understand the molecular mechanisms of the process. During previous years, the differentiation of hES cells into trophoblast lineage and expression of trophoblast cell specific transcripts using specific media (Annual report 2019-2020, pp. 86; Annual report 2020-2021, pp. 106) were reported.

During the reporting year, protocols were optimized to differentiate KIND1 cells to syncytiotrophoblasts (STBs). Briefly, the cells were primed to trophectoderm lineage using trophoblast differentiation media (TBD media), comprising of BSA, ITS, BMP4, A83-01 and PD173074. Following 6 days of induction in TBD media, cells were cultured in trophoblast organoid media (TOM media, comprising of N2, B27, Glutamine, A83-01, CHIR99021, EGF, Rspo-1, Y27632, N-Ac-L-Cys, PgE-2, HGF and bFGF) for 8-10 days. Differentiated cells showed expression of STB specific marker E-Cad (Fig. 79A) and hCG (Fig. 79B). Another STB marker CGB was shown to be expressed at transcript level (Fig. 79C). Further characterization of the differentiated cells and optimization of differentiation protocols are underway. Further, effect of hypoxia on differentiation towards trophoblast lineage will be evaluated.

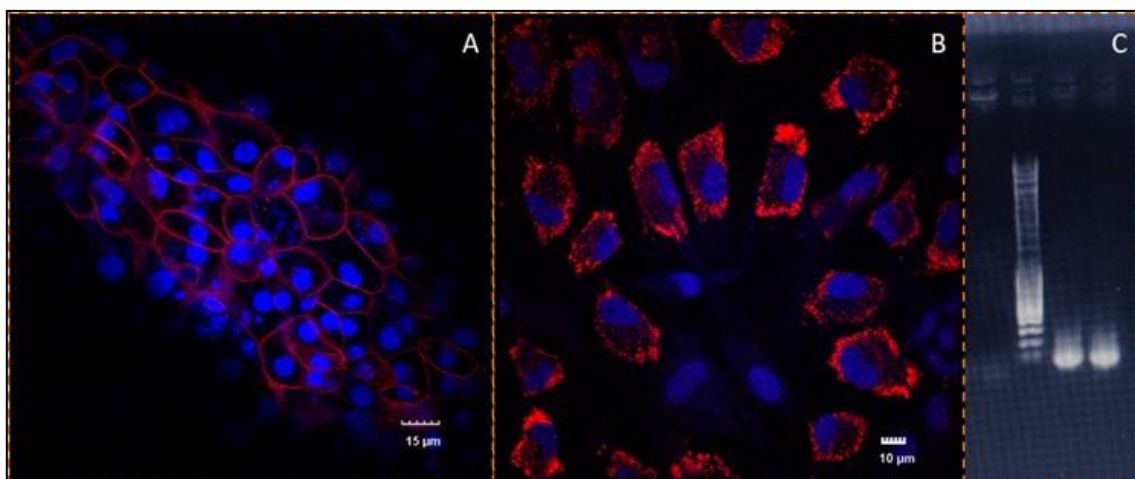


Figure 79: (A) Expression of E-Cad (red) was observed following differentiation of KIND1 hESCs into STBs. (B) Expression of STB marker hCG (red) following differentiation of KIND1 hESCs. Cells were counterstained with DAPI (blue). Bar=10μm. (C) Expression of CGB by RT-PCR following differentiation of KIND1 hESCs into STBs.

7.2 Neonatal Exposure to Estrogen and its Effects on Adult Mouse Prostate Stem Cell Biology

Principal Investigator : DK Das
 Project Associate : Reshma K Chopra (DST-WOSA)
 Collaborators : Deepa Bhartiya, SM Metkari
 Duration : 2019-2022

Prostate resident stem cells have been shown to exist, however, their location remains controversial till date. Pluripotent, very small embryonic-like stem cells (VSELs) expressing nuclear OCT-4A exist in multiple adult tissues along with slightly bigger, tissue-specific progenitors expressing cytoplasmic OCT-4. VSELs and tissue-specific progenitors function together in a subtle manner to maintain life-long tissue homeostasis and their dysfunction results in various pathologies. Present study was undertaken to assess the presence of VSELs and their characterization in adult mice prostate. During previous two years we isolated and characterised the VSELs from mice prostate. FSH treatment led to proliferation of the VSELs where as DHT treatment brought about the regeneration of the organ (Annual report 2020-2021, pp. 115). During the reporting

year, we studied the side population cells (SP) from mouse prostate cells by efflux of Hoechst 33342. Verapamil sensitive side population cells were enumerated by staining with Hoechst dye in both presence and absence of verapamil (Fig. 80A and B). SP was not visualized in presence of verapamil (Fig. 80B). SCA-1+ cells within SP were gated as P5 population (Fig. 80C). The percentage of SP cells within the total prostate cell population was 0.128%. Further, the percentage SP cells that were positive for SCA-1 was found to be 0.062% in normal prostate. Detection in side population indicates the primitive nature of these cells. We further studied the role of VSELs in prostasphere formation. CD49f is a marker expressed by prostate stem cells and renders stem cells the ability to form prostaspheres. Enumeration of CD49f+ population within the VSELs revealed that 0.637% cells of the VSELs (2.380%) were CD49f+ indicating a higher sphere forming capacity (Fig. 81B-C). MACS sorted cells as well as FACS sorted cells were used for the prostasphere formation assay. Prostaspheres were propagated till fifth generation so as to yield true prostaspheres (Fig. 81D). We are in the process of characterising the prostaspheres to decipher if the spheres formed are hollow or packed structures.

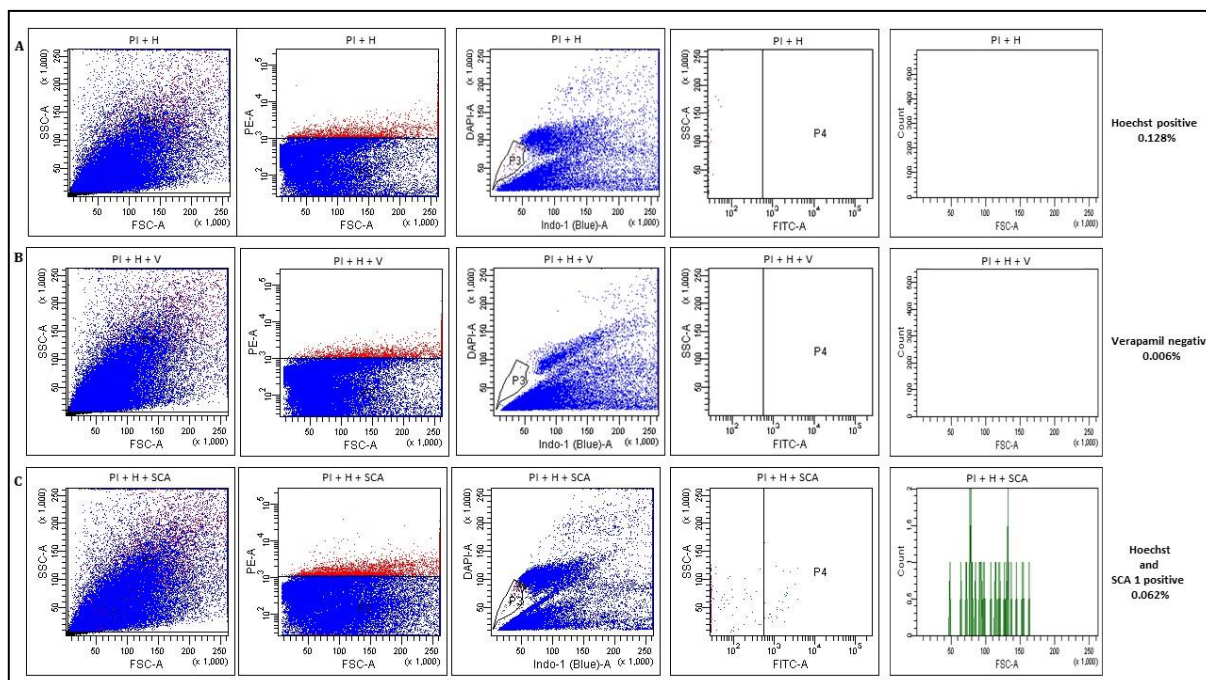


Figure 80: SCA-1+ cells present in verapamil sensitive prostate side population in adult Swiss mice. (A) Population P3 represents the side population gated based on Hoechst effluxing potential after exclusion of debris (P1) and gating live population (P2). (B) Side population was not visualized in presence of inhibitor verapamil. (C) SCA-1+ cells (P4) were visualized within the side population (P3) and they comprised 0.062% of the side population

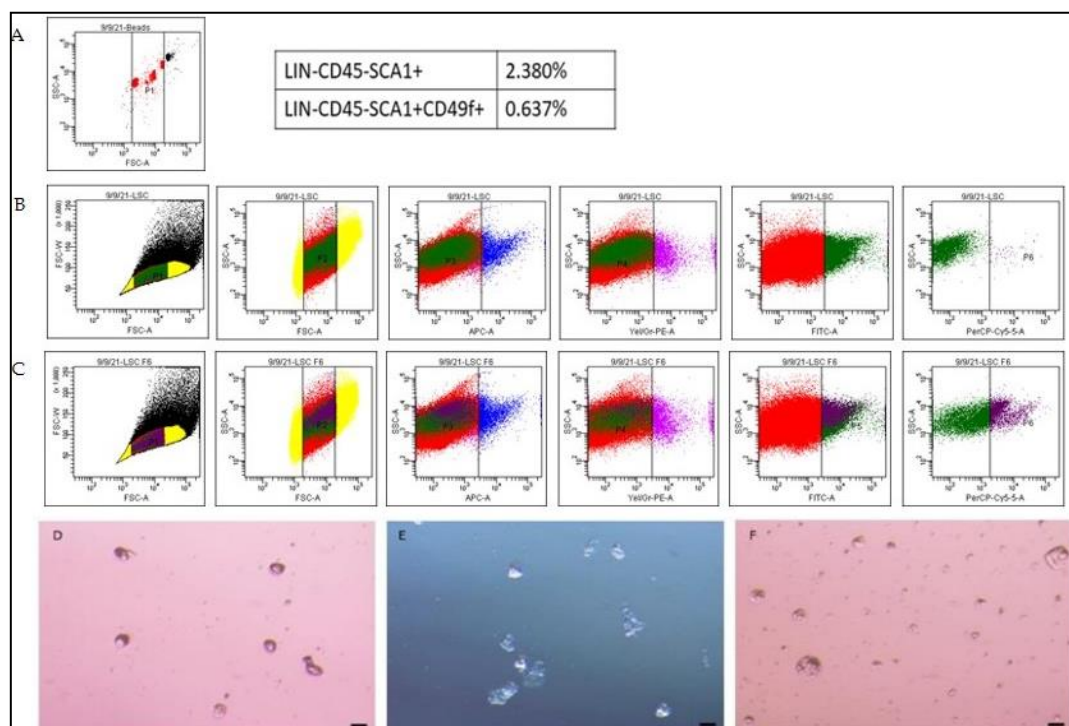


Figure 81: CD49f+ cells present in VSEL population in adult Swiss mice and their ability to form prostaspheres. (A) Size calibration beads to select for cells between 2-6 μ m sized cells. (B) P5 population shows the VSEL population comprising of 2.380% of the cells. (C) P6 population shows the CD49f+ cells with the VSEL population comprising of 0.637% cells. (D-F) Representative images of the spheres formed various generations under 10X magnification. G3 in D; G4 in E and G5 in F. Scale bar 100 μ m.

7.3 Functional Study of Voltage-gated Calcium Channel Gene Mutations in Schizophrenia using Induced Pluripotent Stem Cells (iPSCs): A New Approach for Developing a Cellular Model *(Funded by Science and Engineering Research Board)*

Principal Investigator : **DK Das**
 Project Associates : Shyla R Menon, BR Shekhar
 Collaborator : Ajita Nayak, Seth GS Medical College & KEM Hospital
 Duration : 2017-2022

Schizophrenia (SCZ) is a heritable complex neuropsychiatric disorder. Although, it is the most common mental disorder, its pathophysiology is still elusive. Various causative theories have been put forth; however, a widely accepted theory is the dysregulation of neurotransmitters in the brain, and the treatment is solely based on this theory. Genome-wide Association Studies (GWAS) have identified 108 loci associated with schizophrenia, helping researchers to study different molecular mechanisms contributing to the onset of the disorder. Genes involved in synaptic transmission and neuronal migration have been studied for a long time; however, the contribution of genes related to glycosylation has not been explored. There are numerous pieces of evidence suggesting dysregulated glycosylation in SCZ, mostly from the post-mortem brain. These studies suggested that aberrant glycosylation was one of the contributors to the onset of SCZ. To date, it is not clear whether aberrant glycosylation is the result of environmental exposures or genetic vulnerability. Improper glycosylation leads to neural and neuronal defects in the brain that may lead to the development of SCZ.

The aim of the study is to find out the causative genetic link to adolescent onset Schizophrenia and further develop a cellular model through Induced Pluripotent Stem Cells (iPSCs) to study the effect of aberrant glycosylation in neuronal differentiation. During the reporting year, we successfully differentiated control iPSCs into neural progenitor cells (Fig. 82a) which was further differentiated into cortical neurons (Fig. 82b). These differentiated neurons will be used in future for functional analysis. Further, these cortical neurons were characterized using immunofluorescence for the expression of stage specific markers.

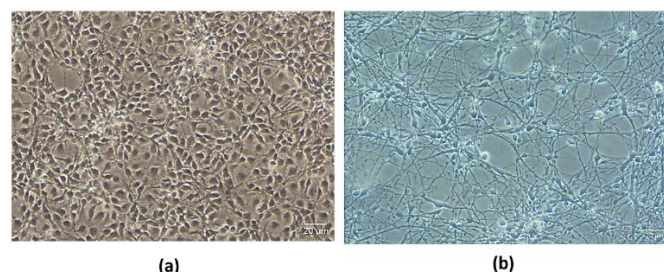


Figure 82: Differentiation of control iPSCs line to cortical neurons through generation of neural progenitors. (a) Phase-contrast images of neural progenitors (b) phase-contrast images of differentiated cortical neurons showing the neuronal connectivity.

The co-localization of NESTIN, a neural progenitor cells marker and PAX6, a neuroectodermal marker (Fig. 83a) revealed that the directed differentiation protocol is robust in differentiating iPSCs into ectodermal lineage. Further, these cells were differentiated to mature neuronal lineage through directed differentiation and presence of MAP2 marker (Fig. 83b) indicated their maturation. Functional characterization was carried out in matured neurons using calcium imaging method. The intensity of native calcium influx was recorded in neurons without any stimulant. This data indicated that the differentiated neurons were functionally active neurons.

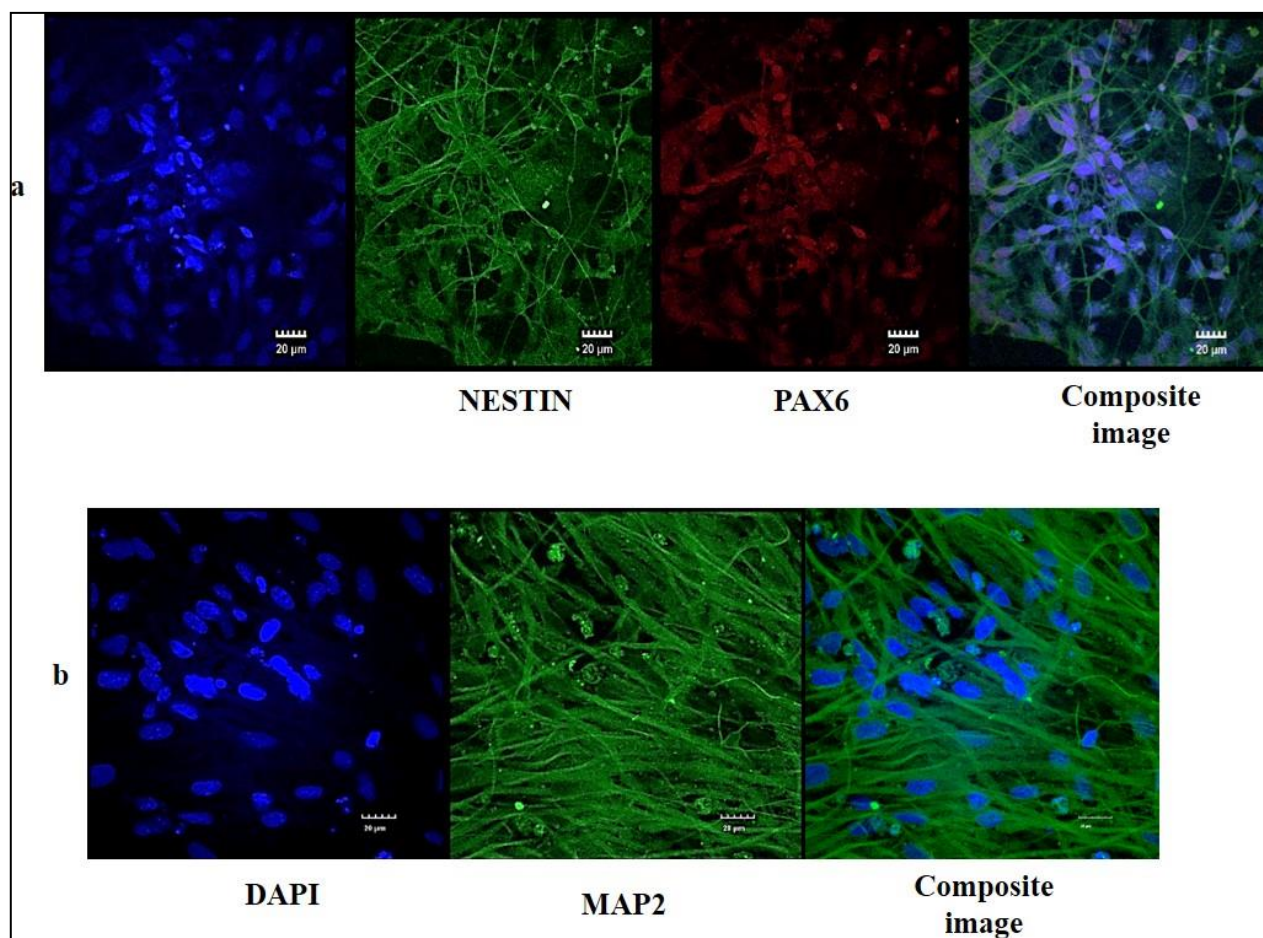


Figure 83: Directed differentiation of neuronal progenitor to cortical neurons (a) co-localization of neural progenitor marker (NESTIN) and Neuroectodermal marker (PAX6) (b) mature neuronal marker (MAP2).

7.4 Establishment of Bioinformatics and Computational Biology Centre (Centre for Advanced Research in Bioinformatics and Computational Biology for Woman and Child Health) (Partly Funded by Department of Biotechnology)

Principal Investigator : **Susan Thomas**
Principal Co-Investigators : Suchitra Surve, RS Barai
Project Associates : Ulka Gawde, Karishma Desai
Duration : 2021-2026

The activities of the DBT funded bioinformatics centre include developing high quality, manually curated, online databases and customizable algorithms that contribute to advancement in disease informatics and research. Genome-wide association studies (GWAS) have helped in identifying single nucleotide polymorphisms (SNPs) associated with several diseases. SNP analysis using pathway and disease enrichment algorithms can provide valuable insights on the underlying disease pathophysiology. Schizophrenia (SCZ) is a multigenic neuropsychiatric disorder and individuals with SCZ develop several comorbidities as the disease progresses. Hence, timely diagnosis of these comorbidities is important for improvement of treatment outcomes and quality of life. The risk of

8. REPRODUCTIVE CANCERS

8.1 Investigating the Key Elements in Estrogen Signalling in the Context of Prostate Cancer

Principal Investigator : **Geetanjali Sachdeva**
 Project Associates : Junita Desouza, Sushama Gadkar, S M Metkari, V Patel, U Chaudhari
 Collaborators : G Bakshi, S Menon, M Pal and N Sable, Tata Memorial Hospital, Mumbai
 S Patwardhan, A Joshi, G Fernandes, GS Medical College & KEM Hospital, Mumbai
 Duration : 2021-2027

Hormones such as androgens play a key role in Prostate Cancer (PCa) pathogenesis. However, androgens act as weak carcinogens and recent evidences have demonstrated that initiation of PCa is mediated through synergistic effect of androgen and estrogens. Estrogenic effects are elicited through a myriad of estrogen receptors (ERs) - conventional ER α / β and non-conventional GPR30, localized in the nuclear compartment and on the plasma membrane respectively. These receptors are known to activate genomic and non-genomic estrogen signalling cascades. Our previous investigations demonstrated the presence of estrogen binding sites on the plasma membrane in prostate cells *in vitro* and *in vivo*. Further, activation of the plasma membrane localised estrogen binding sites *in vitro* with cell-impermeable estradiol led to increased invasion and migration (Annual report 2018-2019, pp. 94-95). However, the ER subtype as well as the mode of estrogenic signalling mediating the pro-metastatic phenotype on activation of plasma membrane estrogen binding site is yet to be identified. Thus, the present study was

undertaken to delineate the role of different ER proteins / estrogen binding proteins in PCa pathophysiology. Towards this, we initially assessed the GPR30 expression in different prostate epithelial cell lines at the transcript level. GPR30 expression was found to be higher in LNCaP (androgen-dependent, tumorigenic) and PC3 cell line (androgen-independent, tumorigenic) followed by RWPE (non-tumorigenic) and least in DU145 cell line (androgen-independent, tumorigenic). We also generated stable cell clones of PC3 cells, knocked down for GPR30 expression (G2.8 and G3.9). The impact of GPR30 silencing on invasion and migration studies was evaluated. We found that GPR30 knockdown PC3 clones were more invasive (Fig. 86) and migratory, compared to cells transfected with empty vector alone. This hinted at the role of GPR30 in regulating metastasis. To study *in vivo* tumorigenicity, GPR30 knockdown PC3 clones were xenografted in NOD- SCID mice. No significant difference in tumor size was observed in mice xenografted parental and GPR30 knockdown PC3 cells. This demonstrated that GPR30 may not be involved in proliferation of PC3 cells *in-vivo*. Further studies will be undertaken to determine whether GPR30 silences PC3 cells respond differentially on activation of non-genomic estrogenic signalling. These investigations will reveal whether GPR30 is of any consequence in non-genomic estrogen signalling in PC3 cells.

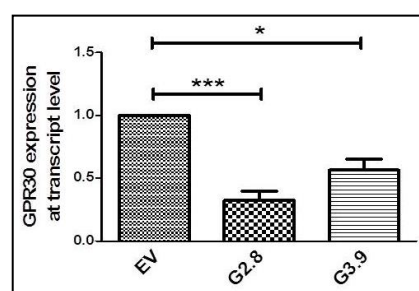


Figure 85: Silencing of GPR30 expression in PC3 cells at the transcript level. Expression of GPR30 was

found to be significantly lower in the cells transfected with shRNAs against GPR30 (shRNA clones G2.8 and G3.9), compared with the cells transfected with empty vector alone (EV). * $P < 0.05$, *** $P < 0.005$

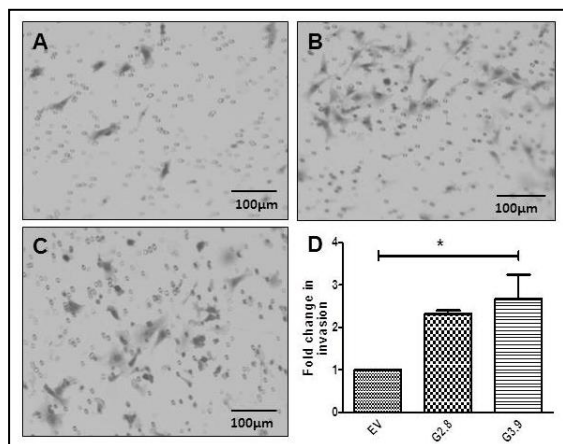


Figure 86: Transwell migration of PC3 cells stably transfected with empty vector (panel A) or shRNA targeting GPR30 expression [G 2.8 (panel B) and G3.9 (panel C)]. Panel D depicts fold change in invasion of GPR30 knockdown cells. * $p < 0.05$

8.2 PSP94 as an Adjunct Marker for Serum PSA for Differentiating between Benign Prostatic Hyperplasia (BPH) and Prostate Cancer (PCa)

Principal Investigator : **Dhanashree Jagtap**
 Principal Co-Investigators : Smita Mahale, Bhakti Pathak
 Project Associate : B Kulkarni, NIIH, Mumbai
 Collaborators : Sujata Patwardhan, Seth G S Medical College and KEM Hospital, Mumbai
 M Andankar, TN Medical College and BYL Nair Hospital, Mumbai
 A Sawant, LTM Medical College and LTMG Hospital, Mumbai
 Duration : 2017-2022

Serum PSA is used globally for prostate cancer screening. However, it lacks specificity which

causes diagnostic dilemma and large number of patients have to undergo unnecessary biopsies as serum PSA concentrations overlap in their ranges in some Benign Prostatic Hyperplasia (BPH) and Prostate Cancer (PCa) cases and this necessitates invasive biopsy for confirmation of prostate cancer. Furthermore, in patients with PSA values between 4- 20 ng/ml (diagnostic grey zone), there is a 75% chance of not finding malignancy and thus there is a need to identify additional markers to effectively discriminate PCa and BPH.

Prostate Secretory Protein of 94 amino acids (PSP94) found in serum appears to be a promising marker along with PSA for differentiating between BPH and PCa. Our pilot study indicated that ratio of serum PSP94/PSA could be of value in reducing the number of biopsies in patients in the diagnostic grey area. This study aimed to check whether PSP94/PSA ratio (PP Index) could help in reducing the number of referrals for prostate biopsies in the lower urinary tract symptoms (LUTS) patients with PSA values between 4-20 ng/ml. Patients having LUTS (n=833) were recruited and serum PSP94 and PSA estimations were done. Age wise distribution of PSP94 and PSA levels was done to ascertain age dependent changes in PSP94 and PSA (Fig. 87). LUTS patients, when stratified based on their age, showed that patients belonging to the age group of 60-80 years had higher PSP94 levels as compared to those in the age group of 51-60 years. When stratifying LUTS patients based on PSA levels, an increase in PSP94 levels with rise in PSA values was observed. Out of 833 LUTS patients studied, 226 patients (27.1%) had PSA levels between 4-20 ng/ml (target group) and biopsy results were available for 139 patients (61.5%). Receiver Operating Characteristic (ROC) curves were plotted to compare the diagnostic sensitivity and specificity of PSA, PSP94 and PP Index in differentiating between BPH and PCa for the target group (Fig. 88).

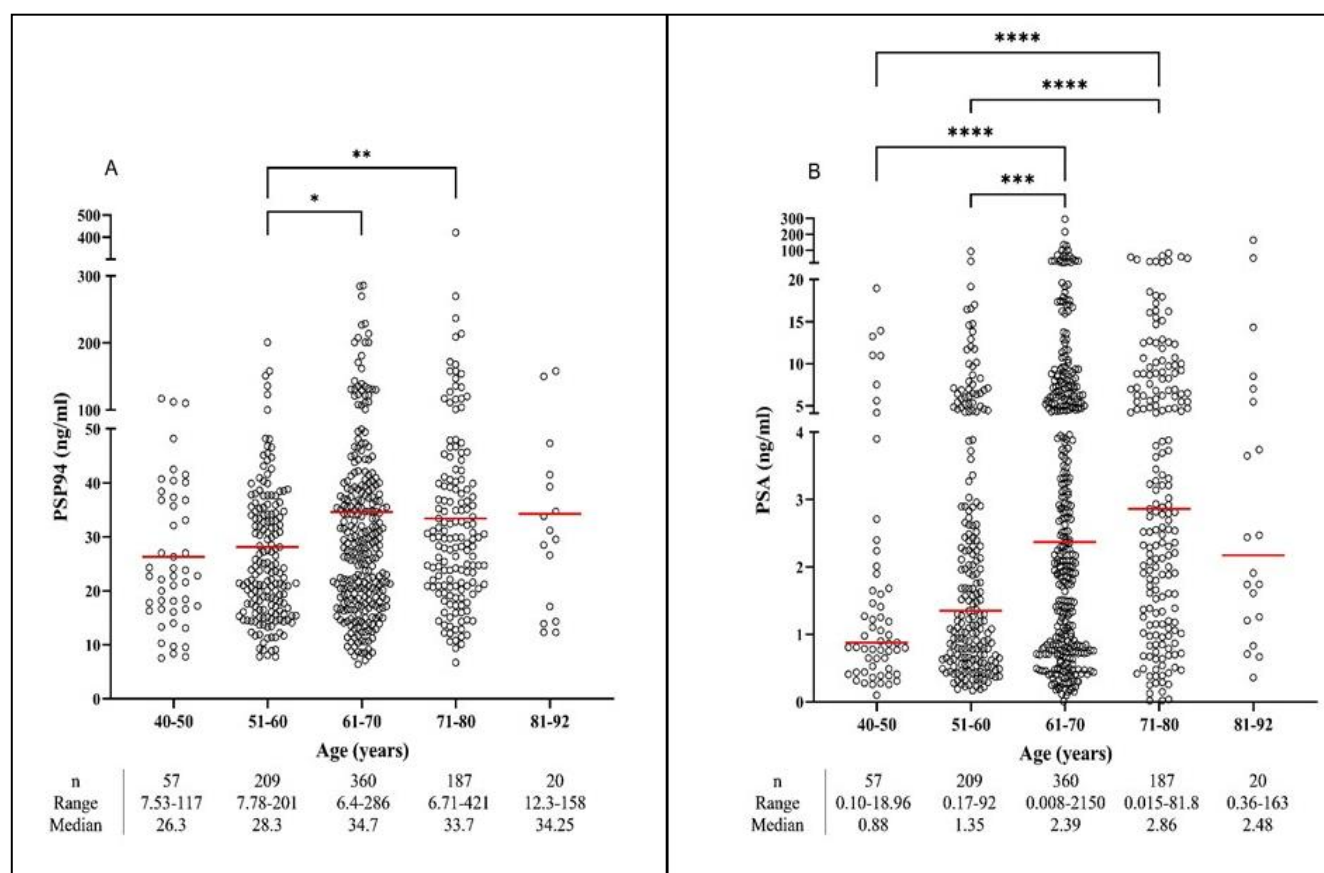


Figure 87: Scatterplots showing age-wise distribution of PSP94 (A) and PSA (B) in LUTS patients. Non-parametric one-way ANOVA (using Kruskal Wallis correction with Dunn's test for multiple comparisons) was carried out and $P < 0.05$ was considered statistically significant. The values significantly different between different age groups are denoted by '*' and accompanying brackets connecting the significantly different groups.

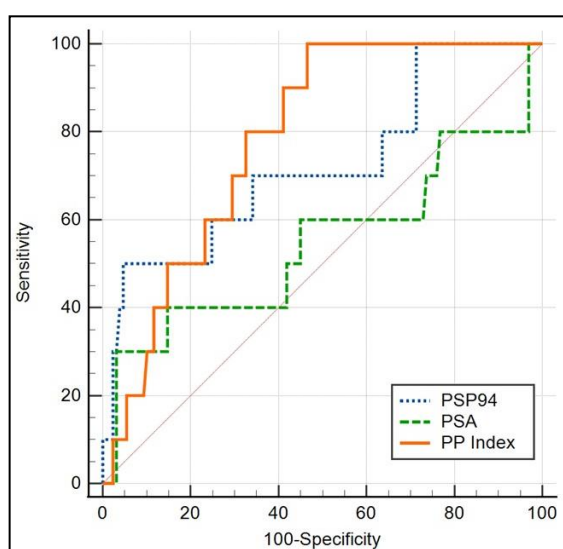


Figure 88: Receiver Operating Characteristic (ROC) curve analyses of PSA, PSP94 & PP Index for differentiating between BPH and prostate cancer when PSA levels are between 4-20 ng/ml.

The AUC for PP Index was observed to be higher than that for both PSP94 and PSA. At PP Index cut off value of ≤ 5.26 , the sensitivity was 100% (not missing any prostate cancer cases) and specificity was 53.49%. When comparing PP index to PSA and PSP94 at 100% sensitivity, a specificity of 3.10% and 28.68% respectively, was observed. When PSA alone (cut off > 4 ng/ml) was used to recommend biopsies, all 139 patients had to undergo biopsy. However, if PP Index (cut off ≤ 5.26) had been used instead of PSA, there could have been 49.6% ($n=69$) reduction in number of unnecessary biopsies. Thus, PP Index has the ability to differentiate between PCa and BPH at PSA levels between 4-20 ng/ml and can help clinicians in better decision making.

8.3 Deciphering the Mechanisms of Innate Immune Surveillance in Prostate Cancer for Immunotherapy

Principal Investigator : **Taruna Madan**

Project Associate : Kasturi Ganguly

Collaborator : U Kishore, Brunel University, London, UK

Duration : 2018-2023

Prostate cancer (PCa) is one of the deadliest cancer in men with second highest incidence rate globally. Immune suppression and low immunogenicity contribute to the “cold” immune unresponsive prostate tumour microenvironment (TME) leading to a sub-category of poor-responders to adaptive immune therapies. This necessitates consideration of tumour antigen-independent, innate immune responses mediated by pattern recognition receptors (PRRs) as an alternative or complementary means to target cancer cells.

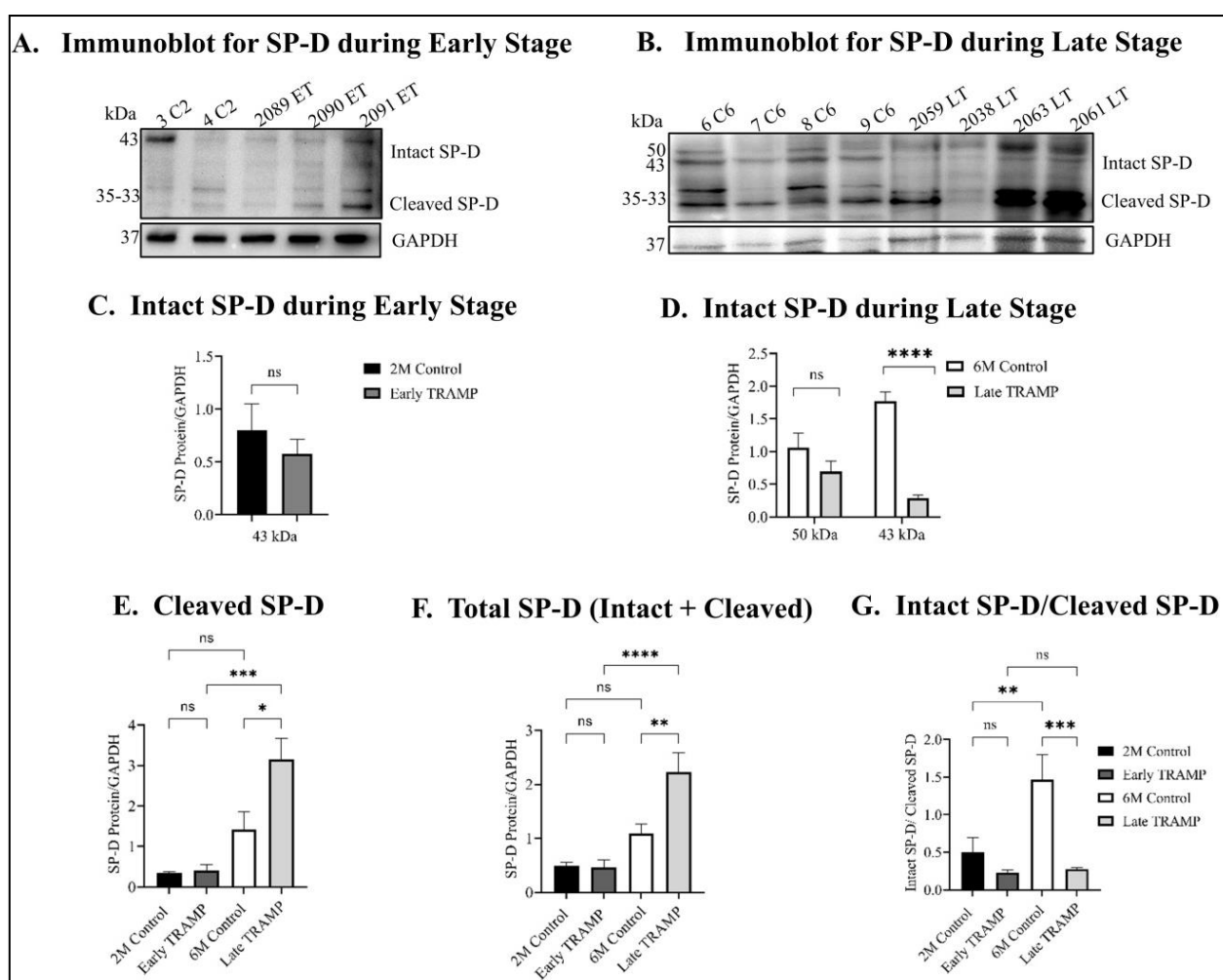


Figure 89: Impaired expression of the intact form of SP-D throughout the tumour progression. (A) Immunoblot analyses of intact monomeric form of SP-D (~45kDa) and cleaved SP-D fragments (~35kDa~33kDa) in the Early TRAMP (ET, Panel A, Lane no. 3,4) compared with the corresponding two month controls (C2, Panel A, Lane no. 1,2). (B) Immunoblot analyses of intact monomeric form of SP-D (~43kDa), differentially glycosylated form of SP-D (~50kDa) and cleaved SP-D fragments (~35kDa~33kDa) in the Late TRAMP (LT, Panel B, Lane no. 5-8), compared with the corresponding 6M controls (C6, Panel B, Lane no. 1-4). GAPDH was used as a loading control. (E-G) Densitometric analysis of proteins normalized with GAPDH.

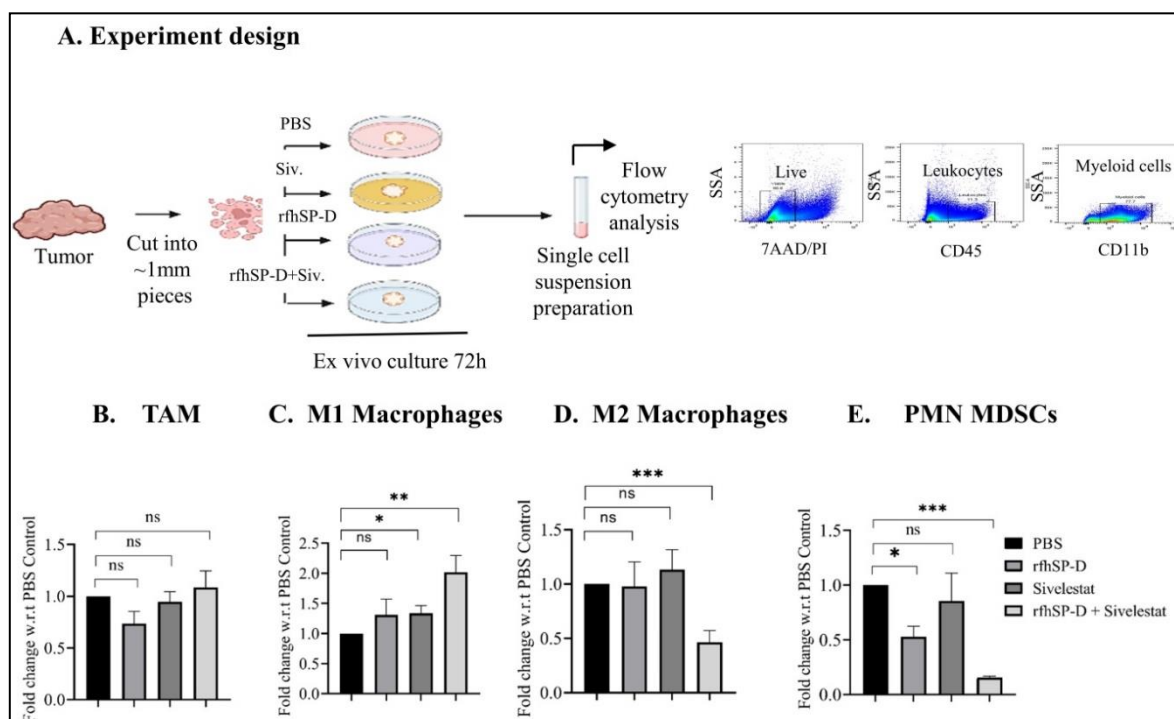


Figure 90: Treatment with rfhSP-D+sivelestat for 72h induced polarisation of macrophages in *ex vivo* cultured TRAMP tumour explants. (A) Study design. (B-E) Flow cytometric analysis immune cells. Fold change of (B) tumour-associated macrophages (TAM), (C) M1; (D) M2 and, (E) PMN-MDSCs population in *ex vivo* cultured TRAMP tumour explants were calculated with respect to PBS control.

Surfactant protein D (SP-D), a collagen-containing calcium dependent C-type lectin or collectin, contribute significantly to lung surfactant homeostasis and lung defense. The extra-pulmonary existence and innate immune role of SP-D is now well documented. This soluble pattern recognition molecule is also emerging as a potent anti-tumoural defense molecule in a range of cancers. In context to the prostate cancer (PCa), a significant reduction in the SP-D protein expression was found at the malignant sites of human PCa tissues and associated with an increasing Gleason score and severity. Also, a recombinant fragment of human SP-D (rfhSP-D) induce intrinsic apoptotic pathway in human prostate cancer cell lines PC3 and LNCaP and human PCa biopsy explants via interacting with glucose regulated protein 78 (GRP78). In the present study, we evaluated the expression of SP-D in early (PIN: prostatic intraepithelial neoplasia)

and advanced stages (PD: poorly differentiated adenocarcinoma) of PCa using transgenic adenocarcinoma of mouse prostate (TRAMP) model (Annual report 2019-20, pp. 112). Both stages of PCa exhibited significantly decreased SP-D mRNA expression along with proteolytic degradation of SP-D protein (Fig. 89). Immunophenotyping of TRAMP mice showed increased accumulation of serine protease producing granulocytes and polymorphonuclear myeloid-derived suppressor cells (PMN MDSCs) in the advanced staged tumour tissue that were plausibly involved in the SP-D proteolysis. The susceptibility of rfhSP-D to elastase-mediated proteolysis provided the rationale to use an elastase-inhibitor to sustain intact rfhSP-D in the TME. In *ex-vivo* cultured TRAMP tumor explants, treatment with rfhSP-D and elastase inhibitor, sivelestat, caused dynamic macrophage polarization towards M1 with

downregulation of PMN MDSCs (Fig. 90). In addition, rfSP-D also induced immunogenic cell death in the murine PCa cells and in the TRAMP explants. This study highlights yet another novel innate immune surveillance mechanism of SP-D by inducing immunogenic cell death and immunomodulation in PCa, which will eventually help in the transformation of an immunologically “cold” prostate tumour into a “hot” prostate tumour.

8.4 Investigating the *in vitro* and *in vivo* Potential of rfSP-D to Inhibit Prostate Cancer Metastasis and Mechanistic Involvement of GRP78 (Partly Funded by ICMR-RA)

Principal Co- : Taruna Madan
Investigator

Collaborators : U Kishore, Brunel University,
London, UK
SM Metkari

Duration : 2021-2024

A specific and significant impact of Surfactant protein D (SP-D), a soluble pattern recognition receptor, on the survival of tumour cells was discovered by our group. Previously, it was reported that the recombinant fragment of human SP-D (rfSP-D) induced apoptosis in the tissue explants from patients of metastatic prostate cancer (Annual report 2017-18, pp. 95).

Principal : R Subedi
Investigator

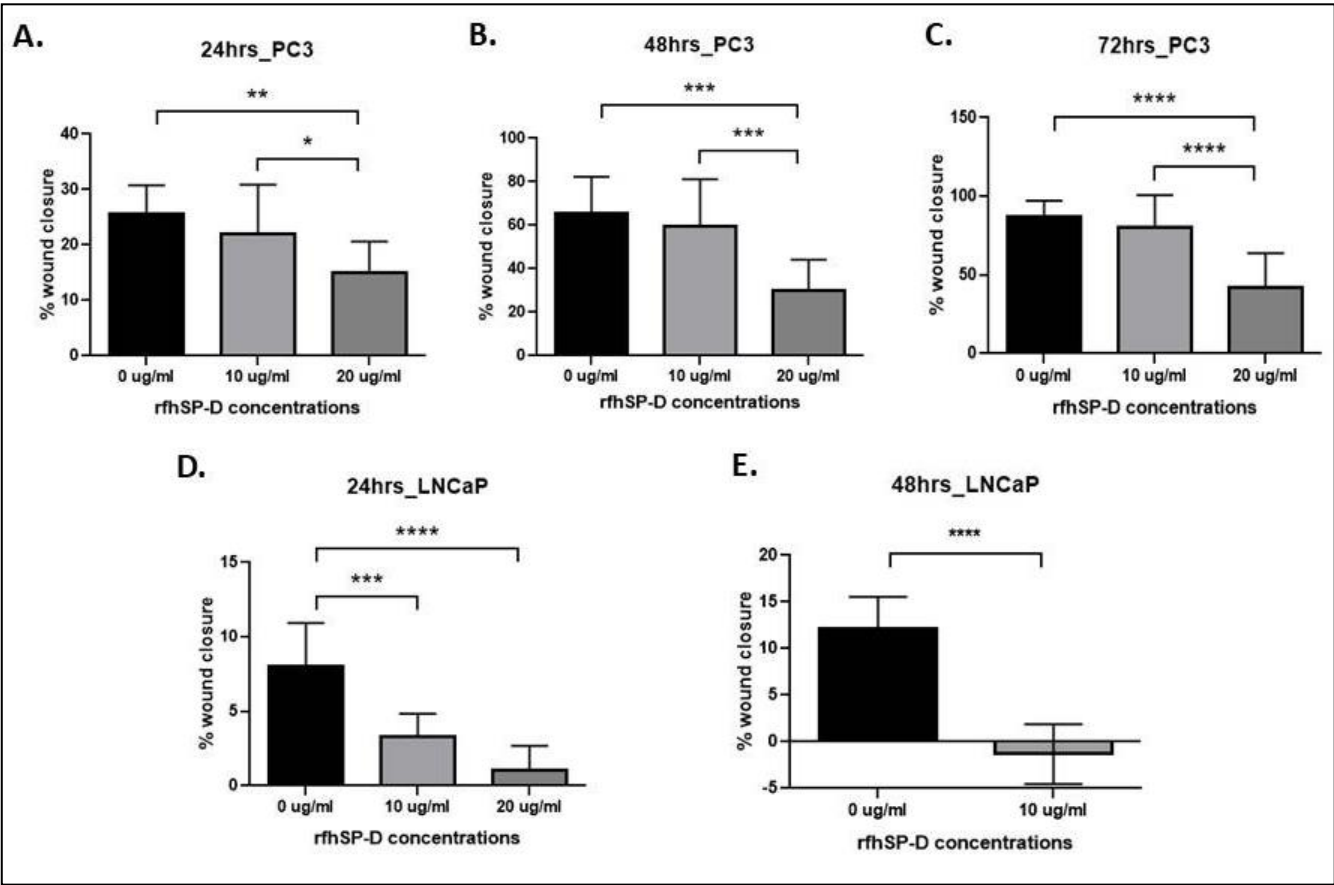


Figure 91: Recombinant fragment of human SP-D (rfSP-D) reduces PC3 and LNCaP cell migration in a concentration dependent manner. Percent wound closure was calculated and compared for each time point 24hrs, 48hrs and 72hrs (A-C: PC3 cells, D-E: LNCaP cells). The mean values are plotted with error bar

representing standard deviation of measurements at 8 sites for Control and 16 sites for 10 μ g/ml and 20 μ g/ml rfhSP-D treatment. * p <0.05, ** p <.01, *** p <0.001, **** p <0.0001

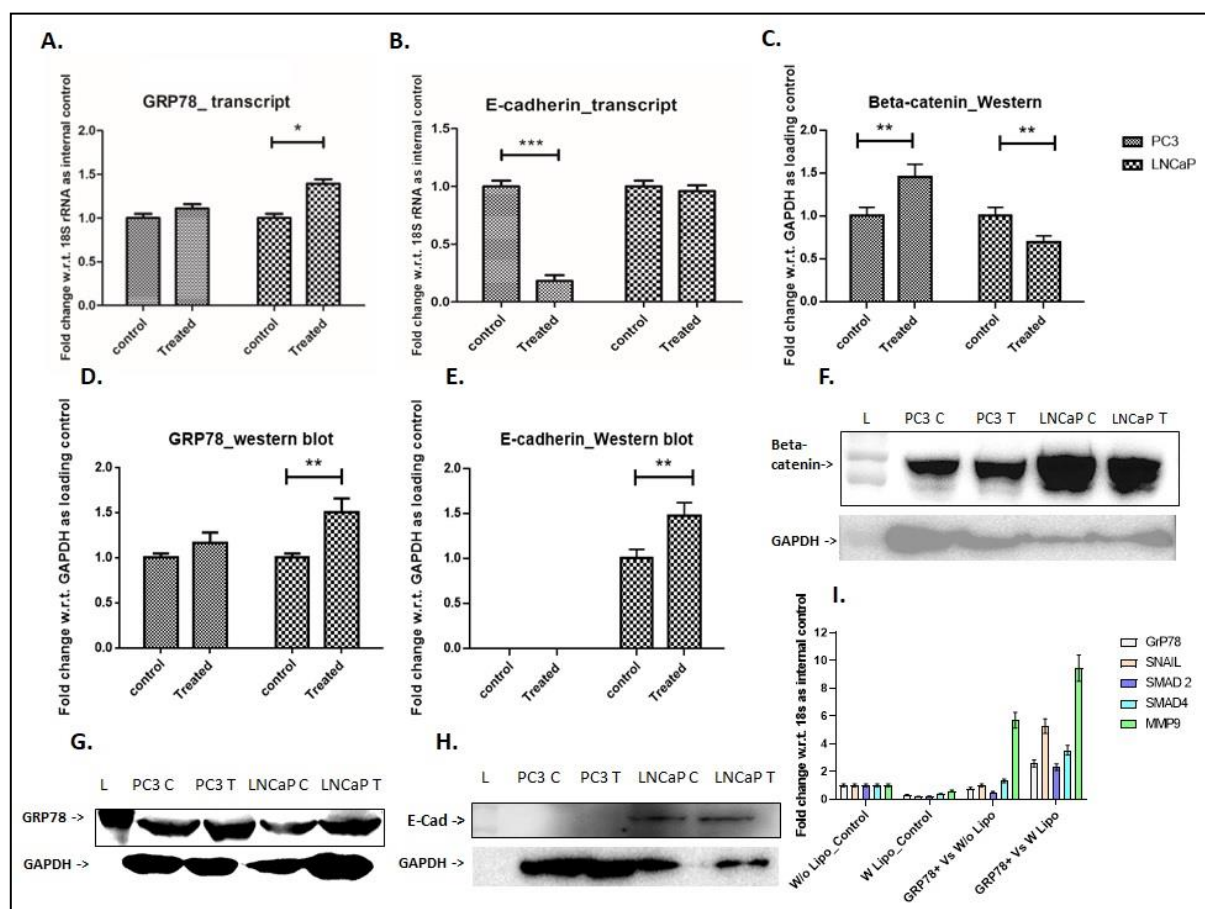


Figure 92: A-H: Altered transcript and protein expression profile of GRP78, E-cadherin and beta-catenin in PC3 and LNCaP cells on treatment with 10 μ g/ml rfhSP-D. GAPDH was used for normalizing protein load. Densitometry was performed using image J software. I: PC3 cells were transfected with pcDNA3.1 (+)-GRP78/BiP plasmid and analyzed for expression of GRP78, SNAIL, SMAD2 and SMAD4 expression after 72hrs in cells not treated with lipofectamine (W/o Lipo_Control), Cells treated with lipofectamine but without plasmid (W Lipo_Control), Cells transfected with plasmid (GRP78+). * p <0.05, ** p <.01, *** p <0.001

Proteomics of interactome of SP-D on PC3, prostate cancer cells, resulted in identification of Glucose Regulated Protein 78 (GRP78) as a major interacting partner. GRP78-knockdown-PC3 cells showed a decreased expression of the adhesion markers E-cad and N-cad resulting in reduced metastatic potential. In view of the plausible SP-D mediated suppression of EMT transition via its interaction with GRP78, it was hypothesised that rfhSP-D treatment may hamper metastasis. The efficacy of the rfhSP-D to inhibit cell migration was analysed using

wound healing/scratch assay. Treatment with 10 μ g/ml and 20 μ g/ml rfhSP-D resulted in significantly decreased cell migration of PC3 and LNCaP cells in a concentration dependent manner (Fig. 91). PC3 and LNCaP cells were treated with rfhSP-D and the changes leading to metastasis at the transcript and protein level were analysed. Treatment with 10 μ g/ml rfhSP-D for 48hrs significantly increased the transcript and protein expression of GRP78 in the LNCaP cells but marginally in the PC3 cells (Fig. 92 A, D and G). LNCaP cells also showed a distinct

increase in the E-cadherin at the protein level (Fig. 92, B, E and H), however, E-Cadherin was undetectable in the PC3 cells at protein level. Additionally, a decrease in the protein expression of β -catenin was observed for LNCaP cells (Fig. 92 C and F). On the contrary, PC3 cells showed an increase in the levels of β -catenin. To study the effect of overexpression of GRP78, transient transfection of the PC3 cells with GRP78 expressing plasmid was performed. GRP78 overexpression was confirmed by the increased transcript levels of GRP78. With the increase in GRP78 levels, an increase in the expression of SNAIL1, SMAD2, SMAD4 and MMP9 (associated with cancer progression and metastasis) transcripts was observed (Fig. 2I). Further investigations are ongoing to elucidate the mechanistic details of action of rfhSP-D in prostate cancer.

8.5 Role of Toll-like Receptors and TLR Agonists in Modulating Response to Chemotherapy in TNBC Patients (Funded by DBT-BioCare)

Principal Investigator : **Nupur Mukherjee**
 Co- Investigator : Taruna Madan
 Project Associate : M Jhondhale
 Collaborators : Shalaka P Joshi,
 Tata Memorial Hospital,
 Mumbai
 Duration : 2019-2022

Triple-Negative-Breast Cancer (TNBC) is a hormone receptor negative (ER/PR/Her2)-subtype of breast cancer that in response to hormone receptor-based therapies ineffective. This aggressive form of breast cancer has an immunogenic character and shows extensive molecular heterogeneity. TLRs (Toll-like-receptors) are known to modulate tumor microenvironment. The objective of the present study was to compare TLR expression in TNBC

tumors and TNBC cell lines representing distinct molecular subtypes. Also, the correlation of TLR expression with distinct cell types infiltrating TNBC tumours was analysed using publicly available transcriptome datasets on TNBC patients. In addition, the effect of TLR agonists on TNBC cells and immune cells was analyzed using in-vitro proliferation assays. Differences in the transcript expression of TLR genes (TLR1-10) were observed in TNBC cell lines mimicking different molecular subtypes of TNBC.

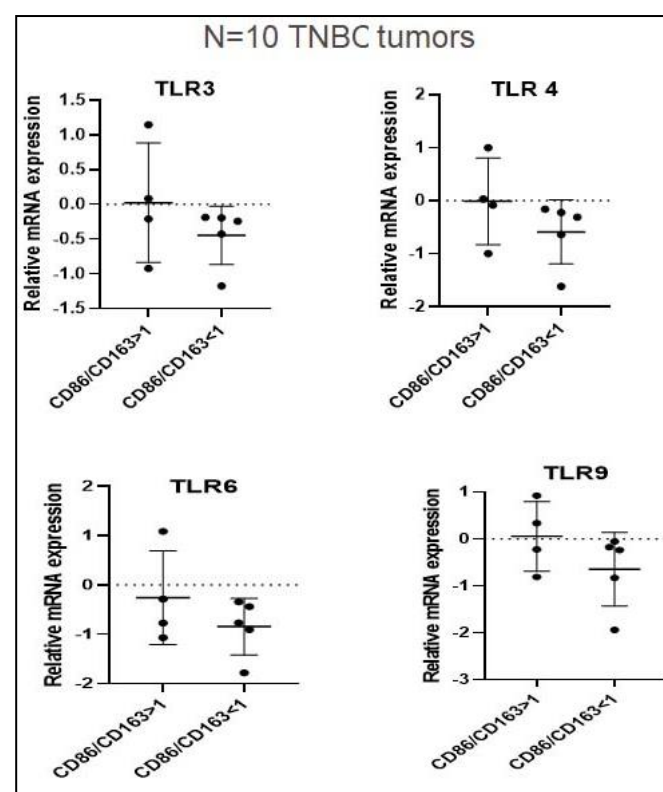


Figure 93: Pattern of TLR expression in chemo-naïve TNBC tumors with high/low CD86/CD163 (M1/M2 macrophage markers) expression index, determined by q-RT PCR analysis. Fold change in expression was calculated using $2^{-\Delta\Delta Ct}$ method with 18S rRNA as reference.

The levels of TLRs (TLR 3/4/6/9) in TNBC tumors varied for Indian patients (n=13) with comparably higher expression in tumors with high (>10%) Tumor Infiltrating Lymphocytes (TIL) infiltration compared to TNBC tumors

with moderate/low TIL infiltration. These findings suggest that TLR3/4/6/9 could play a role in regulating the immune landscape of TNBC tumors. TNBC tumor tissues showed differential pattern of TLR4 expression within TNBC tumor cell (CDH1+) and TNBC stromal cell (CDH1-) compartments in the TNBC-tumor microenvironment.

Interestingly, higher expression (in terms of florescent intensity) of TLR4 was observed in TNBC tumor cells compared to stromal cells (CDH1-) in TNBC tumors suggesting that downregulation of TLR4 in the stromal compartment could be a contributing factor for induction of immune suppressive environment in the TNBC Tumor Microenvironment (TME).

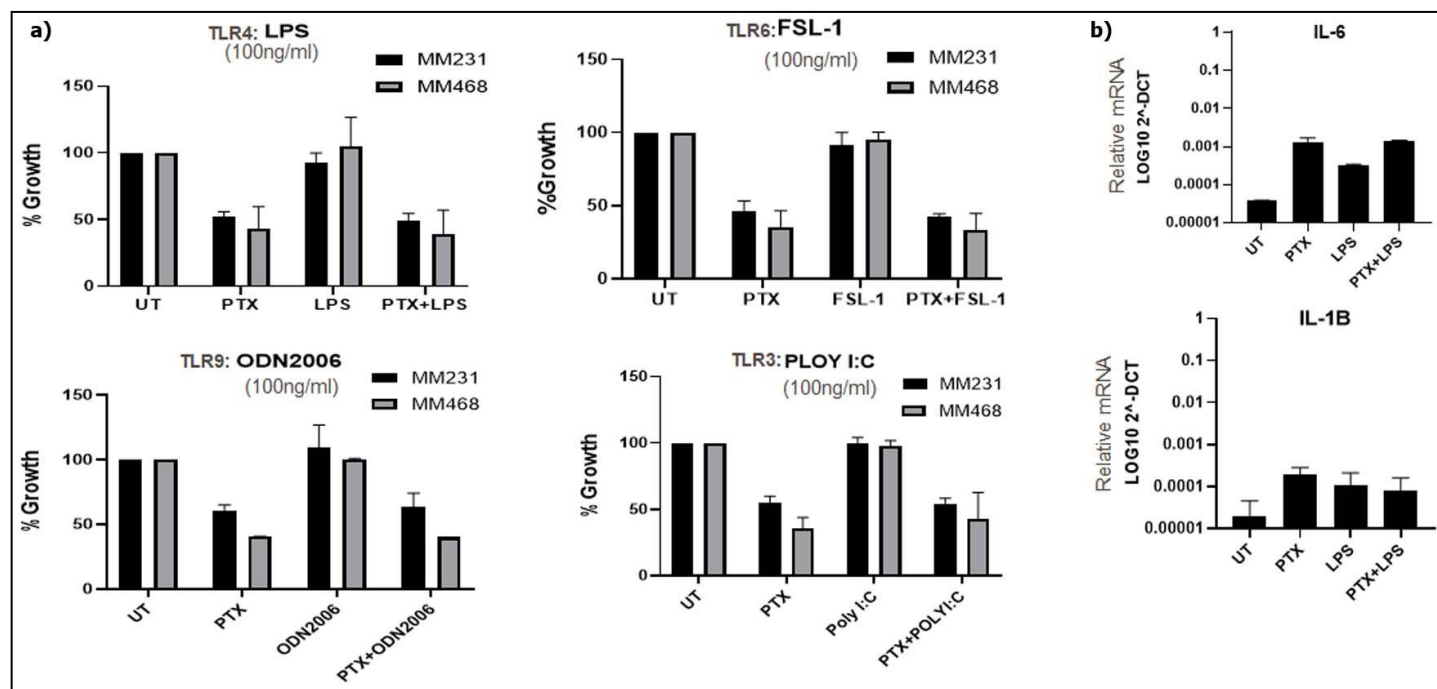


Figure 94: Growth pattern of TNBC cells during combination treatment with synthetic agonists targeting TLR genes (TLR3: POLY I:C/TLR4: LPS/TLR6: FSL-1/TLR9: ODN2006) and paclitaxel, analysed by proliferation assay; (b) Cytokines (IL-6 and IL1-B) expression in response to TLR4 agonist (LPS) treatment in TNBC cells analyzed by qRT-PCR.

Furthermore, TLR expression (TLR 3/4/6/9) was found to be higher in TNBC tumors having enrichment of M1-type macrophage markers compared to M2-type macrophage marker as evident from higher TLR gene expression in TNBC tumors with higher CD86/CD163 ratio (Fig. 93), evaluated by qRT-PCR analysis and analysis of transcriptome datasets TCGA database on TNBC tumors. However, combination treatment with TLR3/4/6/9 agonists and chemotherapy drug paclitaxel did not significantly increase chemosensitivity of the TNBC cell lines (MDA MB 231, MDA MB 468) (Fig. 94).

On the other hand, treating PBMCs isolated from TNBC patients (n=5) with TLR4 agonists caused a change in the expression of surface markers for M1 (CD86) and M2 (CD163) macrophages indicating that TLR4 agonist treatment was capable of inducing polarization of peripheral immune-suppressive M2 type macrophages towards antitumor M1-type macrophage phenotype. The study suggests that targeting the TLR pathway could contribute to regulation of tumor-immune cell cross talk especially in TIL enriched TNBC tumors.

8.6 Deciphering the Placental-Breast Epithelial Cell Cross Talk in Pregnancy Associated Breast Cancer (PABC)

Principal Investigator : Nupur Mukherjee

Co-Principal Investigator : D Modi, Geetanjali Sachdeva

Collaborators : Jyoti Bajpai, Tata Memorial Hospital, Mumbai
Vandana Bansal, Nowrosjee Wadia Maternity Hospital, Mumbai
S Gupta, DY Patil Medical College, Pune

Duration : 2021-2024

Breast Cancer (BC) is one of most common and aggressive reproductive tract associated cancer. BC diagnosed during pregnancy or within two years postpartum is called Pregnancy Associated Breast Cancer (PABC). Although PABC is a relatively rare form of BC, the incidence of PABC cases is increasing over the years as more women are delaying age of first pregnancy to ≥ 30 years. The risk factors and molecular mechanisms that potentially contribute to development/progression of BC during pregnancy are poorly understood from the existing studies on PABC. Previous evidence indicates a potential role of pregnancy associated factors including the placental tissue secretions in pathogenesis of PABC. Placental secretions are known to affect several physiological processes in the mother, including mammary gland development for lactation with the potential to regulate BC cell functions. However, detailed studies to understand the role of placental tissue in pathogenesis of BC during pregnancy are missing. This study aims to characterize BC cell (MDA-MB-231) phenotype in response to exposure to spent culture medium from

different placental cell types, using in-vitro studies. The MDA-MB-231 BC cells were exposed to placental secretions of two placental cell lines HTR-8/SVneo and BeWo cells to investigate the cellular changes undergone by the BC cells via measuring the clonogenicity potential of BC cells (CFU assay), proliferation potential (PNPP Assay), and associated molecular mechanisms. The BC cells did not show any difference in cell proliferation and clonogenic abilities when treated with spent media (condition media) of BeWo cells. However, when treated with HTR-8-derived conditioned media, BC cells showed a dose-dependent decrease in cell proliferation and clonogenic abilities (Fig. 95). Also, expression of β -catenin was downregulated in MDA-MB-231 cells when treated with HTR-8 conditioned media (Fig. 96) indicating a potential involvement of Wnt/ β -catenin pathway in placental cell driven pathogenesis of BC. These preliminary experiments indicate the regulatory role of placental cells associated factors on progression of BC. Detailed analysis is needed to understand the role of placenta in development and progression of PABC.

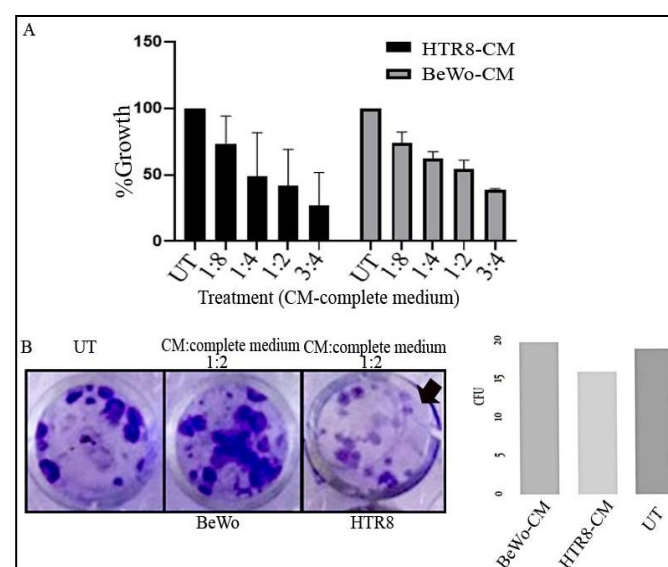


Figure 95: (A) Growth pattern of BC cells (MDA MB 231) to treatment condition medium (spent culture medium/CM) of HTR8 or BeWo trophoblast cells, analyzed by proliferation assay. (B) Colony Forming

Assay Unit (CFU): BC cells (MDA MB 231) were treated with conditioned medium of HTR8/BeWo trophoblast cell lines. A decrease in clonogenic potential (number of colony forming units) was observed in MDA MB 231 cells post exposure to HTR8 derived CM.

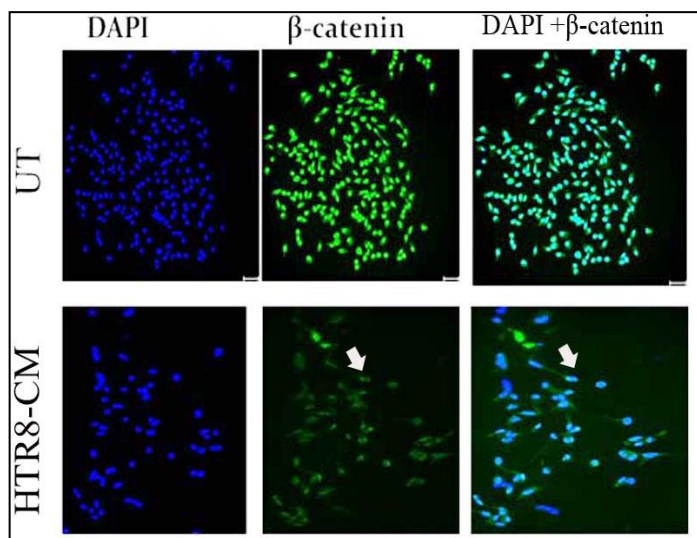


Figure 96: Status of β -catenin expression in BC cells (MDA MB 231) exposed to HTR8 placental trophoblast cells derived conditioned medium (CM) and untreated (UT) controls. Downregulation in β -catenin expression observed in MDA MB 231 cells co-cultured with HTR8-CM compared to controls. Arrow denotes downregulation in expression.

8.7 Identification of Circulating MicroRNA Signatures as Diagnostic Markers for Early Stage and Metastatic Breast Cancer (Funded by ICMR)

Principal Investigator : **Sadhana M Gupta**
 Project Associate : Samruddhi Ranmale
 Collaborators : S Mehta, J Anam, V Maniar
 Duration : 2021-2024

The aim of this study is to identify differentially expressed miRNAs in breast cancer (BC) and determine their utility as minimally invasive diagnostic markers and prognostic markers for

monitoring patient response to drug therapy and treatment outcome.

A total of 120 breast cancer patients and 30 healthy controls will be recruited for this project. Blood samples from 37 breast cancer patients and 10 healthy controls have been completed from July 2021 to date. Patients have been divided into four groups based on the subtype as Luminal A- ER and PR positive (Group 1), Luminal B- ER positive and PR-negative (Group 2), HER2 positive (Group 3) and Triple negative (Group 4). Significant downregulation in the relative expression of serum miRNAs-miR-20b, miR-141, miR-205, miR-143, miR-26a, miR-374a-5p ($p < 0.05$) was observed in BC patients ($n=35$) as compared to healthy subjects ($n=10$) (Fig. 97). The diagnostic potential of these differentially regulated miRNAs was evaluated using ROC analysis. The ROC curve analysis revealed significant p-values for six significantly dysregulated miRNAs (Fig. 98).

The study is expected to be beneficial for identifying subtype-specific miRNA signatures as blood-based markers for early diagnosis of breast cancer. The correlation of microRNA expression with clinicopathological features of breast cancer patients was also determined. The expression of miRNAs- miR-26a-5p ($p=0.0462$) and miR-374a-5p ($p=0.0320$) correlated significantly with the presence of lymph node metastasis in BC patients. Significant association of miR-20b-5p was detected with distant metastasis ($p=0.0298$) and estrogen receptor expression ($p=0.0138$). Further, the expression of miR-141-3p ($p=0.0423$) and miR-205-5p ($p=0.0155$) correlated with age at the time of diagnosis. miR-26a-5p expression correlated with body mass index (BMI) ($p=0.0148$) and miR-205-5p expression correlated with menopausal state ($p=0.0350$) of breast cancer patients.

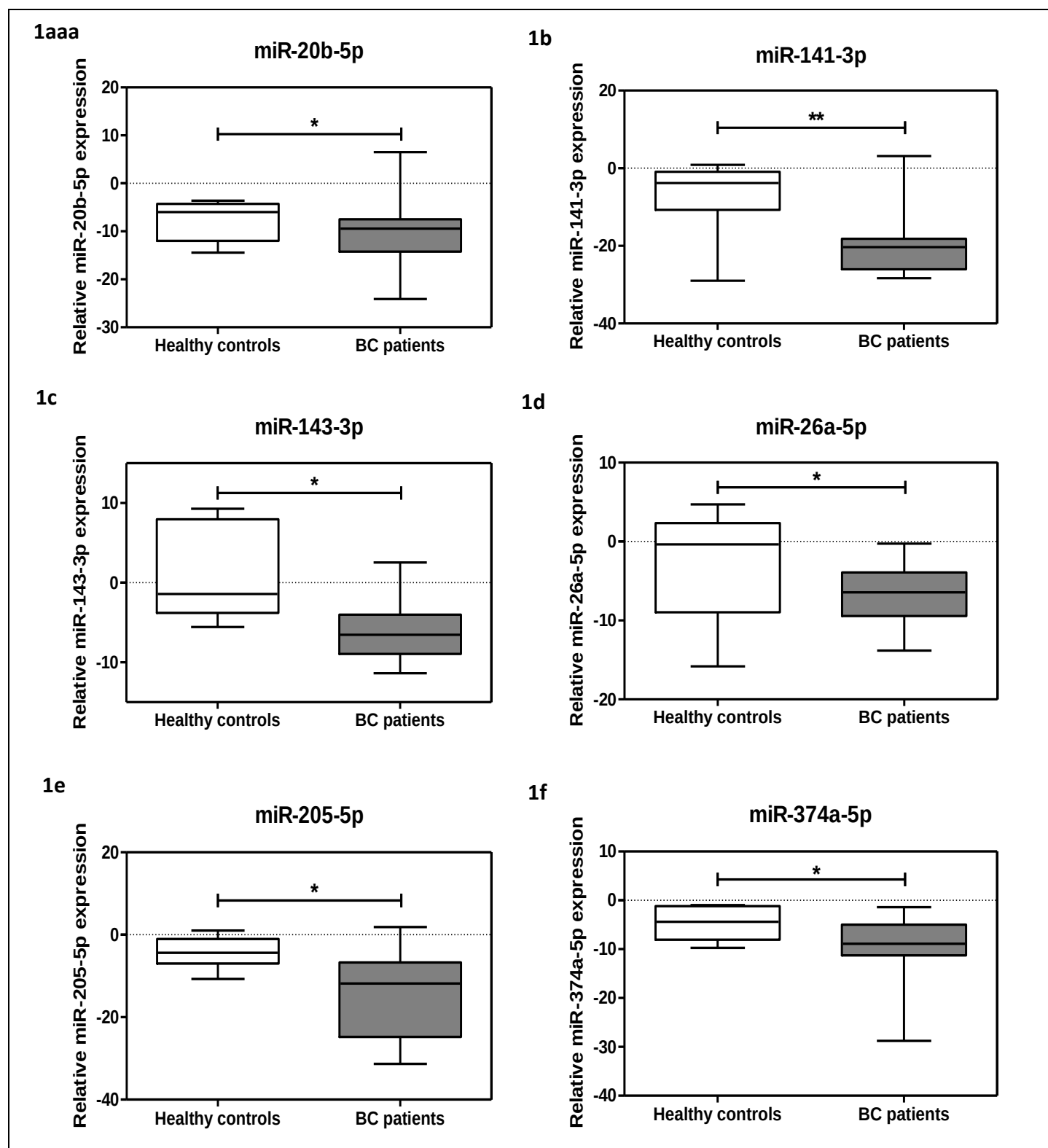


Figure 97: Relative levels of a) miR-20b-5p, b) miR-141-3p, c) miR-143-3p, d) miR-26a-5p, e) miR-205-5p, and f) miR-374a-5p in breast cancer (BC) patients and healthy controls.

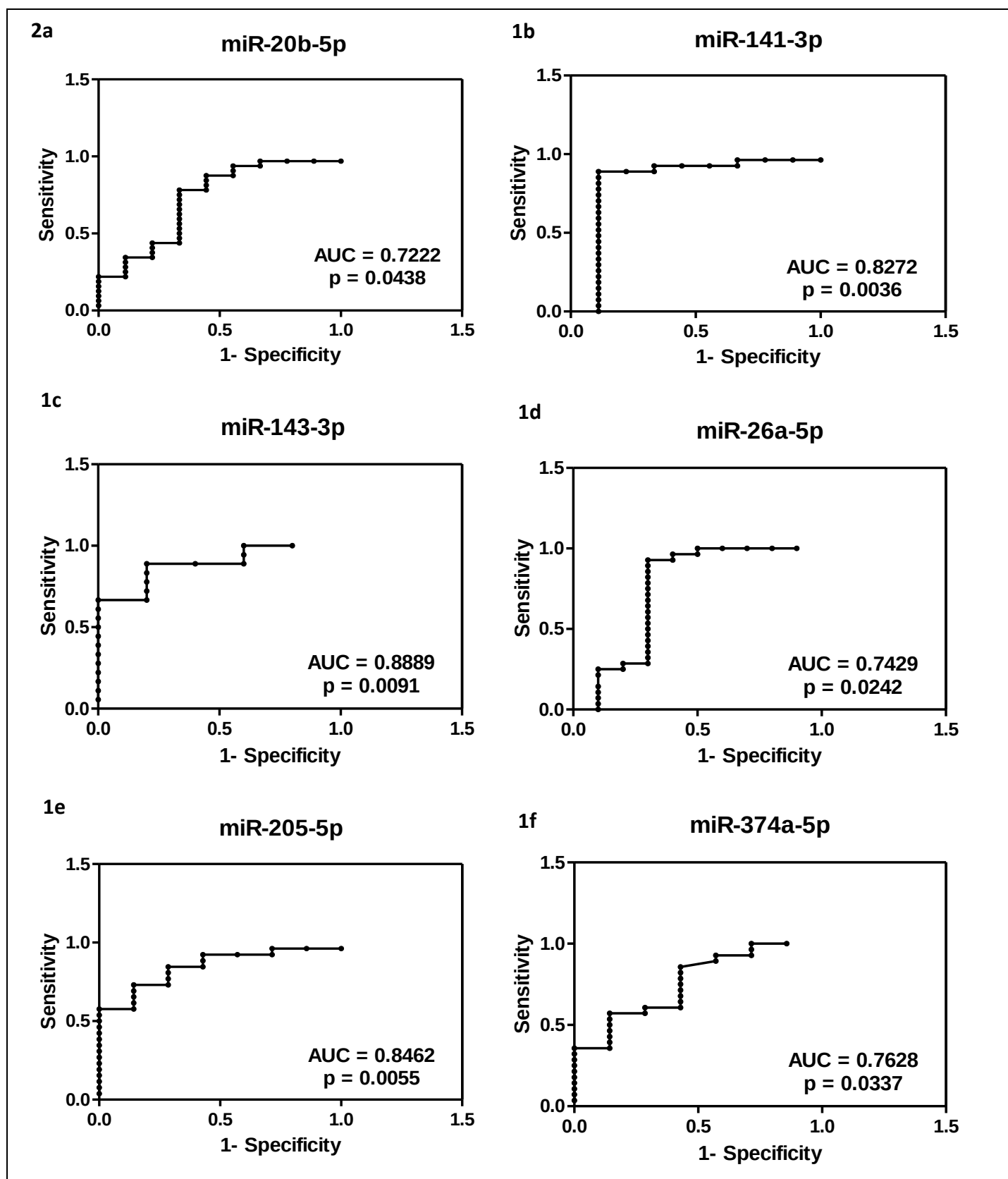


Figure 98: ROC curve analysis of a) miR-20b-5p, b) miR-141-3p, c) miR-143-3p, d) miR-26a-5p, e) miR-205-5p, and f) miR-374a-5p for breast cancer detection in serum. The ROC curve analysis differentiates between breast cancer patients and healthy controls.

8.8 Analysis of Molecular Cargo and Paracrine Effects of Extracellular Vesicles Secreted by Ovarian Cancer Cells

Principal Investigator : **Bhakti R Pathak**

Project Associates : Meghali Borkotoky, Ananya Breed, Dhanashree Jagtap, A Banerjee

Duration : 2021-2026

Extracellular Vesicles (EVs) are known to play an important role in mediating cell-to-cell communication in both normal physiology as well as pathological conditions like cancer. Exosomes are a type of extracellular vesicles of 50-150nm in size and are reported to transfer biologically active cargos such as proteins, RNAs, lncRNAs miRNAs etc. to recipient cells which can mediate different downstream effects. The exosomal cargo can have potential utility in cancer diagnosis as well as therapy. Under different conditions or stages in cancer exosomal cargo are either differentially or uniquely expressed. However, studies concerning extensive characterization of this exosomal cargo in ovarian cancer are limited. 3D or spheroid cultures compared to monolayer cultures, offer multiple advantages since they better mimic the *in vivo* conditions of a tumor, like cell-cell interaction, oxygen gradient and heterogeneity in terms of different proliferative

potential and metabolism. Thus, the present study aims at characterization of proteomic and transcriptomic profile of exosomes derived from ovarian cancer cell lines grown in 2D versus 3D cultures. During the reporting year, scaffold free agarose overlay method was used to establish 3D culture of three different ovarian cancer cell lines namely OVCAR3, OVCAR4 and OVCAR5. It was observed that morphology of the spheroids generated from these cell lines vary greatly from each other (Fig. 99) and time taken for spheroid formation (spheroidization time) varies from cell line to cell line. Spheroidization time for OVCAR5 was approximately 10 days while OVCAR4 started forming spherical clusters along with hollow spheres from day 4 onwards. However, OVCAR3 did not display prominent spheroid morphology and instead showed cellular aggregates even on day 10. Exosomes were isolated from these spheroids as well as from the cells in monolayer via differential centrifugation. Isolated exosomes were probed for known exosomal protein markers like CD9 and CD63 and it was evident during immunoblotting that number of exosomes derived from spheroids were comparatively less than that of monolayer. This suggests a possibility of cells undergoing quiescence during spheroidization or the cells in non-adherent condition tends to liberate less exosomes. Further, exosomes isolated from less invasive OVCAR4 and high invasive OVCAR3 were outsourced for RNA-seq analysis.

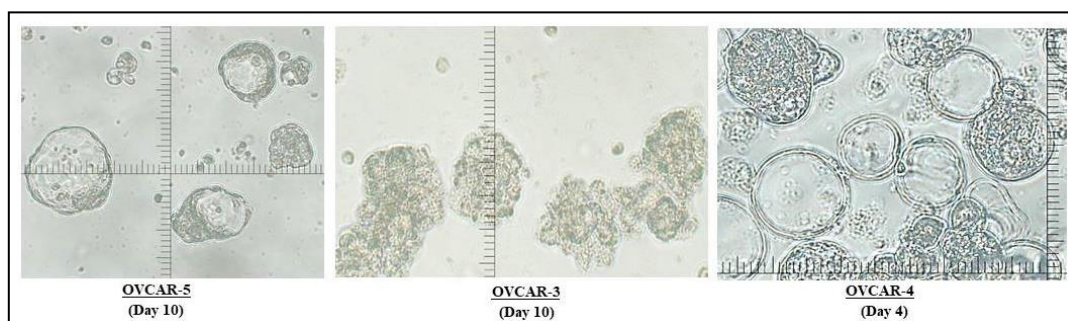


Figure 99: Three ovarian cancer cell lines showing different morphology of spheroids under same culture conditions. OVCAR5 showing compact spheroid morphology at day 10 whereas OVCAR3 forming mere cell aggregates at same day point. OVCAR4 forming spherical clusters along with hollow spheres from day 4

onwards distinct from the other two cell lines. All images were taken under bright field microscope at 40X magnification.

8.9 Assessing Trop2 Expression and its Correlation with the Anti-Trop2 Immune Status in Ovarian Cancer Patients

Principal Investigator : **Bhakti R Pathak**

Project Associates : Ananya Breed, B Kulkarni, Pradnya Kamble, Smita Mahale

Collaborator : Amita Maheshwari, Tata Memorial Hospital, Mumbai

Duration : 2017-2023

Trop2 is a transmembrane glycoprotein, which is upregulated in ovarian cancer as compared to the normal ovaries. Overexpression of Trop2 in ovarian cancer tissue is associated with poor prognosis. Trop2 undergoes proteolytic cleavage and extracellular domain of Trop2 may get released from the cancer cells. We as well as others have reported Trop2 expression in exosomes. We hypothesized that apart from cleaved Trop2, full length Trop2 would also be present in circulation in the ovarian cancer patients. Most of the ovarian cancer patients are diagnosed at an advanced stage with presence of peritoneal ascites. The current study was designed to detect cell associated and soluble Trop2 in the ascitic fluid of ovarian cancer patients, which would be correlated with their autoantibodies against Trop2. Apart from Trop2, its known binding partner claudin-7 was also detected in the cells and exosomes derived from the ascitic fluid. During the reporting period, recruitment of treatment naïve ovarian cancer patients from Tata Hospital (collaborative center) was continued. Ascitic fluid collected from the patients was processed for isolation of ascitic cells and exosomes. Presence of Trop2 and claudin-7 in the ascitic

cells and exosomes was determined by western blotting (Fig. 100 A and B). Densitometry was performed using ImageLab software (BioRad) and the normalized values were used for determining the ratio of exosomal to cell associated Trop2 in the ascitic fluid of ovarian cancer patients. It was observed that exosomal Trop2 normalized to cell associated Trop2 varied widely from 0.5 to 10 among ovarian cancer patients highlighting the need to perform such analysis in more patients and correlating Trop2 expression with other clinical parameters as total ascitic fluid volume, type and grade of tumor, CA125 levels. Additionally, Trop2 expression can also be compared in chemo naïve vs chemoresistant cases.

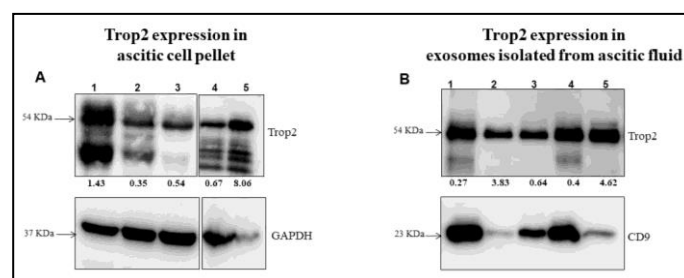


Figure 100: Trop2 expression in ascitic cells (A) and in exosomes derived from ascitic fluid of ovarian cancer patients (B). Trop2 expression was normalized with GAPDH and CD9 for cell associated and exosomal Trop2 respectively. Normalized values for Trop2 expression are indicated below each lane of both the top panels (A and B)

8.10 Primary Screening of High Risk HPV DNA by a Low Cost Molecular HPV Test for Early Detection of Cervical Precancers and Cancers among Women in Urban and Rural Community of Maharashtra (Funded by Department of Health Research)

Principal Investigator : **Kiran Munne**

Co-Principal : Anushree Patil, Sharmila
 Investigators : Pimple, S Biswas
 Project : Suchitra Surve, Shahina
 Associates : Begum, Deepti Tandon,
 Varsha Trayambake,
 Rachana Dalvi, Sharmila
 Kamat
 Duration : 2019-2023

Cervical cancer and premalignant lesions constitute a major problem in women's health. In this community-based study, the eligible women are being screened for cervical precancers and cancers by a low cost molecular careHPV test, HC2 test, PAP smear and VIA test. Objectives of the study are to estimate the prevalence of high risk HPV infection for early detection of cervical precancers and cancers by careHPV test among women aged 30-59 years; identify various high risk HPV genotypes; compare the results of genotyping, VIA and cytology with careHPV positive subjects; compare the results of NIRRCH HPV PCR test with care HPV, hybrid capture 2 and geneXpert test. This study uses low cost alternative (careHPV test) to gold standard Hybrid Capture II (HC2) HPV DNA technique for primary screening of cervical precancers and cancers in urban (Abhyudaya Nagar, Mumbai) and rural (Dahanu, Palghar) communities of Maharashtra. Out of 1038 women recruited in the project, 680 women were from the urban site and 353 women from the rural site. HPV DNA positivity by HC2 test was seen in 5.45% women and careHPV test was 4.08%. Most common genotype by geneXpert was HPV16 followed by HPV18 and other high-risk HPVs. Acetowhite lesion after application of 5% acetic acid (VIA test) was seen in 5.63% women. PAP results revealed atypical squamous cells of undetermined significance (ASCUS) in 10 women; high grade squamous intraepithelial lesion (HSIL) in 4 women; and low-grade squamous intraepithelial lesion (LSIL) in 4 women. All the screen positives from urban site

have been followed up at preventive oncology department, Tata Memorial hospital, Mumbai. Follow up has been completed for 41 women. Out of these cervical biopsy was advised for 15 women. Three women have been treated by cryotherapy and two were treated with Loop Electrosurgical Excision Procedure (LEEP) at Tata Memorial Hospital.

8.11 Improving Access for Screening of Common Cancers and Non-Communicable Diseases among Women in Tribal Block of Maharashtra: Challenges in Implementation (Funded by ICMR)

Principal : **Anushree Patil**
 Investigator
 Principal Co- : Beena Joshi, Kiran Munne,
 Investigators : Neha Salvi
 Project : A Chavan, Pranjali Patil,
 Associates : Deepti Tandon, Shahina
 Begum, RK Prusty, D Patil,
 Anamika Akula, Sonu
 Babu, Ganga Bhikare
 Collaborator : Sharmila Pimple, Tata
 Memorial Hospital,
 Mumbai
 Duration : 2019-2022

This is a joint collaborative project between ICMR-NIRRCH and the district health system. In the first phase, training of Master trainers, formative research using qualitative research tools like Focus Group Discussions (FGD) of eligible women and ASHAs, key informant's interview of ANMs and facility survey were done to assess the readiness of health system. Screening was done for cervical, breast and oral cancers as well as for common non-communicable diseases (NCDs), such as hypertension, anaemia and high random blood sugar. During speculum examination, endo-cervical samples were collected for HPV tests and RTI screening for Chlamydia, Candida and

bacterial vaginosis. A visual inspection with acetic acid (VIA) was done and acetowhite areas were observed. The implementation phase was delayed due to COVID pandemic. Due to the fear of COVID, women were hesitant to come for screening. Until now, three hundred and sixty (360) participants have been screened. Of these 89.7% (n=323) belonged to the Warli tribe. About 60.8% (n=219) were illiterate and 31.6% (n=114) women were labourers in Chickoowadi. A total of 61.1% (n=220) had monthly income less than Rs. 10,000. Dal and rice were the staple food for 78.8% (n=284) of the women. We found 5.8% (n=21) women were unmarried but sexually active. A total of 8.6% (n=31) consumed tobacco (Tapkiri) and 9.7% (n=35) consumed alcohol (Tadi). Out of 360 participants 34.1% (n=123) women were underweight, 75% (n=270) women had low Hb, 19.44% (n=70) women were hypertensive, and 8.05% (n=29) women had high random blood sugar. During gynecological examination, we found 5.27% (n=19) women were positive by VIA and 6.06% (n=20) women were positive for HPV. The gram staining reports of 182 women revealed that 7.69% (n= 14) were positive for Candida and 29.67% (n= 54) were positive for bacterial vaginosis. Through oral screening, melanoplakia was observed in 3.61% (n= 13) of women and among them 10 women were using smokeless tobacco. Cyst under gum line were found in 0.55% (n= 2) women. During breast examination, we found 1.11% (n=4) women had nipple discharge (other than milk) and 0.55% (n= 2) had fibroadenoma of breast. Screen-positive women were not ready to travel to Tata Memorial Hospital, Mumbai (TMH) for follow

up. Hence, follow up were arranged at MRHRU (Model Rural Health Research Unit), Dahanu. Out of these 10 women, seven women were positive in VIA and eight women were positive in VILI test. In addition, we found one patient had uterine prolapse, two women had breast lump, four were HPV positive and only one woman was willing for follow up at TMH, Mumbai for further evaluation. Cervical biopsy was performed and report confirmed it to be Cervical Intraepithelial Neoplasia 1 (CIN1). Cryotherapy was performed and follow up was advised after six months for further evaluation.

Table 17: NCDs screening in Ashagad PHC and their sub centres at Dahanu

NCDs Screening	No.	Percentage
Random Blood Sugar (n=360)		
RBS >200mg/dl	4	1.11%
RBS 140-199mg/dl	25	6.94%
Hypertension (n=360)		
Systolic >140mm of Hg or Diastolic >90 mm of Hg	70	19.44%
Anemia (n=360)		
Mild 11 - 11.9	96	26.66%
Moderate 8 - 10.9	160	44.44%
Severe < 8	14	3.88%
Gram stain Report (n=182*)		
Candida	14	7.69%
Bacterial Vaginosis	54	29.67%
Cervical Screening (n=360)		
VIA Positive	19	5.27%
HPV Positive (n=330)#	20	6.06%
Oral Screening (Benign lesion) (n=360)		
Melanoplakia	13	3.61%
Cyst under gumline	2	0.55%

*Gram stain report of 178 are awaited. #30 reports are awaited for cervical screening

9. HEALTH TECHNOLOGY ASSESSMENT AND DRUG DISCOVERY

In 2017, National Institute for Research in Reproductive Health was chosen as one of the “Regional Resource Hubs” for Health Technology Assessment (HTA) in India, by HTAIn (Health Technology Assessment in India) under Department of Health Research, Ministry of Health and Family Welfare, Government of India. Its objectives are i) to conduct and report robust HTAs, on topics allotted by user departments; ii) to liaise with officials of the state and local governments and sensitize them about the need for Health Technology Assessment (HTA) for health interventions; and iii) dissemination of final outcomes/policy briefs generated by HTAIn.

9.1 Health Technology Assessment for Use of Intravenous Tranexamic Acid in Management of Primary Post-Partum Haemorrhage in India (Partly Funded by SHR HTAIn)

Principal Investigator : **Beena Joshi**

Project Associates : S Shetty, K Moray, H Chaurasia
Collaborator : Government of Maharashtra
Duration : 2020-2021

The Government of Maharashtra proposed a question to HTAIn DHR to assess cost-effectiveness of including IV Tranexemic Acid (IV TXA) in managing post-partum haemorrhage (PPH). Accordingly the study objectives were framed i) to collate evidence on clinical efficacy and cost-effectiveness of intravenous Tranexamic acid use in women who experience PPH; and ii) to assess the financial implications for introduction of Tranexamic acid into the public health program. Based on input parameters derived from the review of literature, a decision analytic model was built using a decision tree to determine cost-effectiveness of administering IV TXA within 3 hours of birth to the existing management with uterotonics and supportive care to women with PPH. The final report was approved by the technical advisory committee.

Table 18: Costs and health outcomes of adding IV TXA to standard of care in management of PPH

	Standard of care + TXA	Standard of care	Increment /Averted with TXA
Health system cost per patient	INR 5,934	INR 5,782	INR 152 (increment)
Societal cost per patient	INR 6,607	INR 6,486	INR 121 (increment)
Total surgeries (for annual cohort)	19,387	20,293	905 (averted)
Total number of ICU admissions	27,181	27,836	655 (averted)
Total number of maternal deaths	13,923	15,913	1990 (averted)

Results revealed that with an estimated annual cohort of 510,915 PPH cases in India, addition of IV TXA would result in a per-patient disaggregated societal cost of INR 6607 with discounted gain of 20.25 quality-adjusted life-years (QALYs) as compared to INR 6486 with discounted gain of 20.17 QALYs with existing standard care for PPH management. At an

incremental cost-utility ratio (ICUR) value of INR 1470 per QALY gained (USD 21), addition of IV TXA is cost-effective in Indian public health settings. The intervention is likely to prevent 389 maternal deaths, 177 surgeries, and 128 ICU admissions per 100,000 PPH cases. Recommendations submitted to Maternal Health Division, Ministry of Health and Family

Welfare, Government of India and Government of Maharashtra to include it in guidelines, training manuals and facility checklists especially Dakshata.

9.2 HTA for Strengthening Prong 2 Interventions of PPTCT Program at Public Health Facilities through Provision of Linked HIV and Family Planning Services (*Partly Funded by DHR, HTAIn*)

Principal Investigator : **Beena Joshi**

Project Associates : Shahina Begum, S
Oshima, S Shetty,
Revathy Panicker, H
Chaurasia, Namrata
Kharat, P Sanap

Duration : 2020-2022

Government of Maharashtra wanted the HTA Resource Hub to assess cost-effectiveness of a program strategy of linking HIV and Family Planning Programs to reduce unintended pregnancies among people living with HIV (PLHIV). The specific objectives were i) to collate evidence on clinical and cost-effectiveness of providing linkage of HIV to family planning services in reducing unintended pregnancies among PLHIV women; and ii) estimate quality of life and health utility scores for PLHIV women in India. The Population Intervention Comparator and Outcome (PICO) was defined and the decision analytical model was finalised to be a Markov Model. Input parameters to populate the model were derived from the review of literature for effectiveness parameters and from primary studies for estimating costs and Quality of Life. Accordingly, a systematic review and meta-analysis was undertaken after registering with PROSPERO to synthesize the evidence on the effectiveness of health facility-based interventions in improving uptake of modern

contraception and dual methods among PLHIV.

The results yielded a pooled odds ratio for uptake of dual contraception to be 1.42 [1.03, 1.95] ($p=0.05$; $I^2=90\%$) and that of unmet family planning needs to be 0.75 [0.59, 0.94]. Primary data was collected after ethical approvals from ART centers and OBGYN departments at KEM and Nair hospitals in Mumbai and Thane District hospital for PLHIV. Primary health system costing study estimated the unit costs for delivering contraceptive and pregnancy related services to PLHIV. The unit cost for providing ART services was INR 1729.4. The out of pocket expenditure incurred by PLHIV to avail maternal and contraceptive services was also derived. The median Out of Pocket Expenditure (OOPE) for ART services was INR 1000 (500-1800) and for family planning services was INR 2200 (1050-6000). The OOPE was highest for C-section delivery among all the pregnancies-related conditions at INR 6860 (3170-10740). Utility scores for Health related Quality of Life among PLHIV availing contraceptive and maternal health services were derived using EQ5D5L tool and the Indian QOL tariff values. The mean utility and VAS scores of WLHIV in the reproductive age group were 0.976 (± 0.519) and 82.21 (± 15.77) respectively. Subgroup analyses revealed significantly higher utility scores [0.986 (± 0.029)] among contraceptive users as compared to non-users [0.976 (± 0.028)]. WLHIV who had undergone sterilization had the highest utility score among contraceptive users.

Currently pregnant WLHIV had least utility score [0.959 (± 0.088)] Data on epidemiological parameters was derived from Health Management Information System (HMIS) of Maharashtra State AIDS Control Society (MSACS) and literature review. The study is in the final stage of analysis.

9.3 Identification of Enriched Biochemical Networks and Polypharmacological Targets for Metabolic Syndrome *(Partly Funded by SERB-STAR)*

Principal Investigator : **Susan Thomas**

Project Associate : Indra Kundu

Duration : 2021-2024

Systematic mining of data on disease-associated genes can be useful for prediction of comorbidity risk, polypharmacology and possible adverse effects of new or repurposed drugs for the management of multi-morbid diseases. There are online databases such as OMIM, DisGeNET, ClinGen, ClinVar, HPO, and OrphaNet with information on diseases and their causal genes. Researchers have to currently retrieve data from multiple sources due to missing or inadequate annotation in the individual databases. A major impediment in collating the data from these sources is the lack of standardized disease vocabulary. For example, to retrieve gene associations for ovarian cancer, association of variants of DPH1, FAM107A and MRE11 is documented for the disease term "malignant neoplasm of ovary" in DisGeNET; and association of MRE11 is captured in OrphaNet for the disease term "ovarian cancer". The association of the above three genes with ovarian cancer is absent in ClinVar, OMIM, and HPO databases. The association of CHEK2 with "ovarian cancer" is only present in OrphaNet. Although DisGeNET has a very good coverage of gene-disease associations; it has over 37 disease terms synonymous for ovarian cancer and users will have to manually review these terms for collating the relevant gene-disease associations. Therefore, to address these lacunae we have developed a webserver GeDiPNet, which is an integrated knowledgebase for genes, pathways and networks associated with diseases. Gene-

disease associations were fetched from multiple data sources and manually curated to reduce redundancy and data loss. Tools were added so that the data of disease-associated genes, biochemical pathways and molecular networks can be further analysed using comorbidity prediction, enrichment analysis and polypharmacological target prediction algorithms. The tools were used for studying metabolic syndrome (MetS). Disease enrichment analysis based on the genes associated with MetS identified syndromes related to bilirubin metabolism - Crigler-Najjar syndrome, Lucey-Driscoll syndrome and Gilbert disease (Fig. 101A). Comorbid conditions of MetS such as hyperinsulinemia, leptin deficiency syndrome, hypercholesterolemia, obesity, hypertension and diabetes were found to be most significantly enriched (Fig. 101B). The outcome of the disease enrichment analysis was consistent with our current understanding of MetS. Oxidative stress in MetS is known to be caused due to hyperuricemia and hypobilirubinemia. Therefore, MetS individuals are at higher risk of developing syndromes related to bilirubin metabolism. Individuals with MetS are clinically known to suffer from multimorbidities; primarily linked through obesity and insulin resistance. Hence, it is expected that diseases such as cardiovascular disorders, diabetes, and fatty liver disease be identified to have a higher comorbidity risk with MetS. The utility of the polypharmacology prediction algorithm was established using the existing knowledge of popular polypharmacological drugs and targets (Table 19). GeDiPNet is the first webserver developed for prediction of polypharmacological targets using gene-disease networks. The database can be used to draw insights and generate hypothesis for complex diseases. The database and algorithms can be freely accessed at <http://gedipnet.bicnirrh.res.in>.

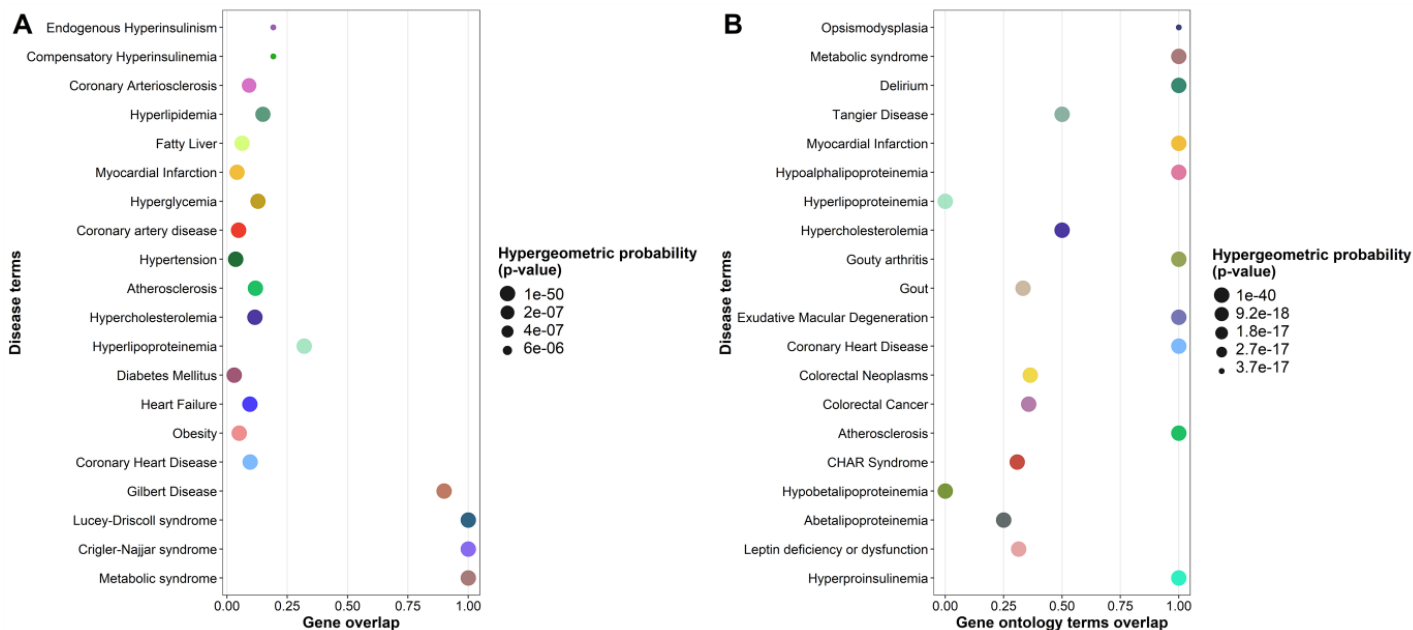


Figure 101: Top 20 enriched diseases (in descending order of significance) identified for MetS based on shared A) genes and B) gene ontology terms associated with MetS.

Table 19: List of popular polypharmacological drugs with associated targets and diseases used for validation of the polypharmacological prediction tool in GeDiPNet

Diseases	Polypharmacological drug	Polypharmacological target
Hypertension and Erectile dysfunction	Sildenafil (Viagra)	Phosphodiesterase type 5
Polycystic ovary syndrome, and Diabetes mellitus	Metformin	Phosphatidylinositol 3-kinase
Rheumatoid arthritis, Crohn's disease, and Psoriasis	Methotrexate	Adenosine deaminase
Multiple sclerosis and Psoriasis	Dimethyl fumarate	Nuclear factor (erythroid-derived 2)-like 2 - Kelch-like ECH-associated protein 1

5.4 Identification and Preparation of Radio-Labeled Modulators of hFSHR for Therapeutic Applications (Funded by Board of Research in Nuclear Sciences)

Research Centre, Mumbai

Duration : 2018-2021

Principal Investigator : Susan Thomas
Co-investigator : V Dighe
Project Associates : Karishma Desai, C Kumar
Colaborators : Drishty Satpati, Bhabha Atomic

Ovarian cancer is the most lethal female reproductive cancer with a poor prognosis and survival rate. The high incidence of mortality can be attributed to lack of specific early-stage biomarkers for detection and ineffectiveness of current treatment therapies. The current treatment therapies lack specificity and thus a targeted therapy approach is being explored.

Follicle stimulating hormone receptor (FSHR) binders are touted as promising candidates for selectively delivering chemotherapeutic drug as several tumors overexpress FSHR. This study aims to identify FSHR modulating agents and study their potential to be used as radiolabelled therapeutic anti-cancer agents. Previously, the

putative binding sites of experimentally known FSHR modulators were predicted using molecular docking studies. These putative binding sites were used to screen Asinex peptidomimetic library to identify novel peptidomimetic modulators of FSHR.

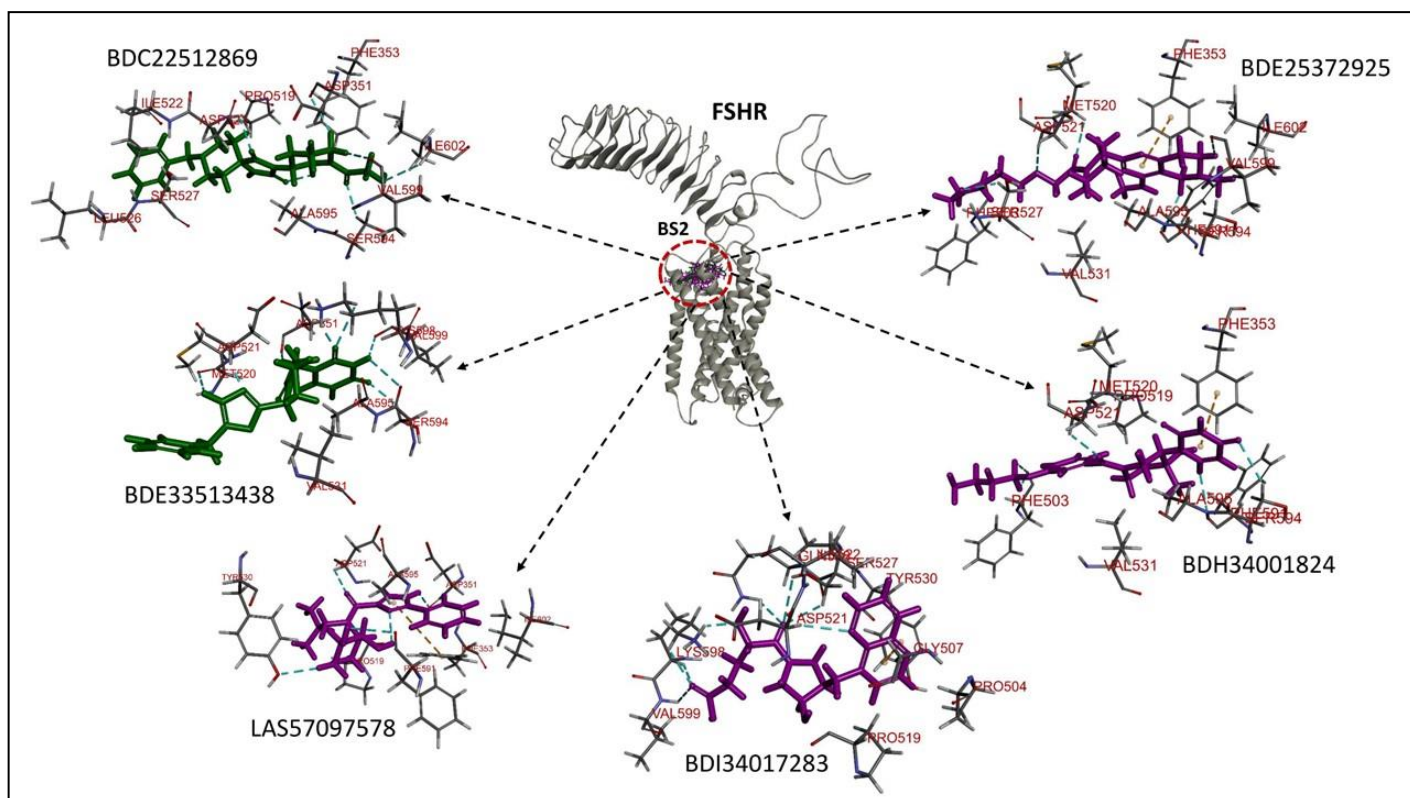


Figure 102: Molecular docking interactions of six peptidomimetics (stick model) with hFSHR (cartoon-ribbon). NAMs and PAMs are depicted in magenta and green respectively. The residues of hFSHR, participating in H-bonds and π - π interactions with docked peptidomimetics are depicted.

Six peptidomimetics were short-listed from the docking studies and further validated using *in vitro* cAMP assay. These peptidomimetics were observed to interact at binding site 2 (BS2) similar to the experimentally known modulators (Fig. 102). Detailed inter-molecular interaction analysis was performed to compare the interactions of the validated six peptidomimetics in our study and experimentally known hFSHR modulators. Four of the six peptidomimetics (BDE 25372925, BDI 34017283, LAS 57097578 & BDH 34001824) behaved as negative allosteric modulators

(NAMs) and interacted similar to the reported NAM ADX61623; two peptidomimetics (BDE33513438 & BDC22512869) behaved as positive allosteric modulators (PAMs) and showed similar interactions as reported agonist (Compound 6) (Fig. 103). These peptidomimetics interacted at transmembrane domain (TMD) pocket encircled with transmembrane helices (TMH4-6) and extracellular loop 2 (EL2) through H-bonds, π - π stacking and hydrophobic interactions (Fig. 103). It is noteworthy that NAMs and PAMs occupied the same binding site in TMD;

however, with distinct interaction pattern. The next objective of this study is to evaluate the therapeutic potential of FSHR modulators in

ovarian cancer by developing a xenograft mouse model using OVCAR-3 cell line.

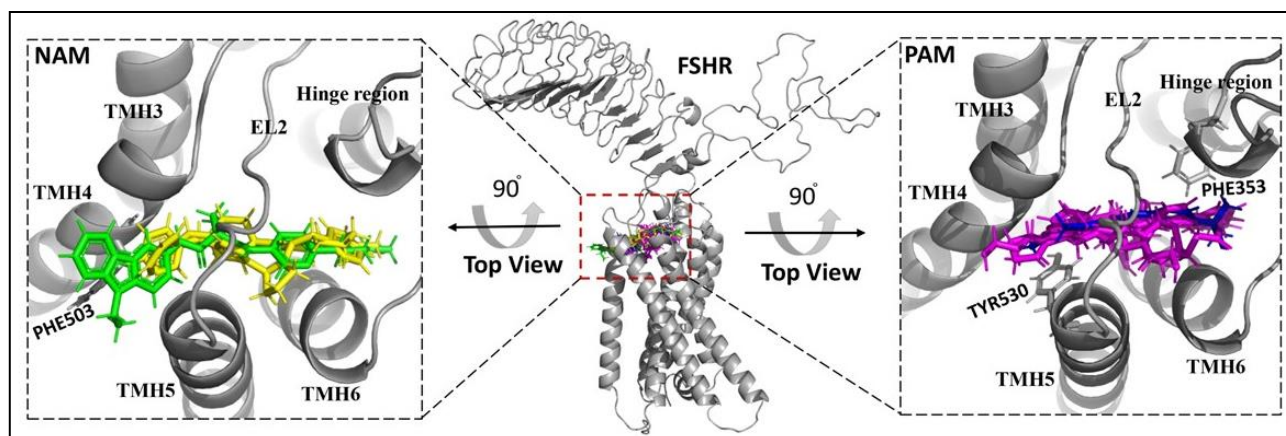


Figure 103: Comparison of binding poses of A) LMW PAM (compound 6; green stick) and peptidomimetic PAMs (BDC 22512869, BDE 33513438; yellow stick); B) LMW NAM (ADX61623; blue stick) and peptidomimetic NAMs (BDE 25372925, BDI 34017283, LAS 57097578, BDH 34001824; magenta stick) with hFSHR (grey ribbon).

9.5 Evaluating the Potential of Trop2 as Immunotherapeutic Target for Ovarian Cancer

Principal : **Bhakti Pathak**
 Investigator
 Principal Co- : Smita Mahale
 Investigator
 Project : Pradnya Kamble, Ananya
 Associates Breed, B Kulkarni,
 Dhanashree Jagtap
 Duration : 2015-2022

Trop2 (Trophoblast antigen 2) is a tumor antigen overexpressed in multiple solid tumors. Its membrane localization and multiple roles in tumorigenesis make it a potential candidate for immunotherapy. Targeting Trop2 by passive immunotherapies has been attempted by various groups. Anti-Trop2 monoclonal antibody-drug conjugate is also in early phase clinical trials for treating different solid tumors including ovarian cancer. However, active immunotherapy in the form of Trop2 peptides or proteins, which is a cost-effective approach, is not yet investigated. The current study was undertaken to identify the immunodominant

regions of Trop2 for their active immunotherapeutic applications against ovarian cancer. We earlier reported that anti-peptide antibodies generated against single peptide epitopes from different regions of Trop2 ECD (Annual report 2017-2018, pp. 90-91) could not detect endogenous protein expressed by cancer cells. Therefore, a truncated region (Trop2¹⁷²) from the Trop2 ECD displaying multiple epitopes in their native conformation was selected to evaluate its ability to elicit a humoral immune response against Trop2 expressing ovarian cancer cells.

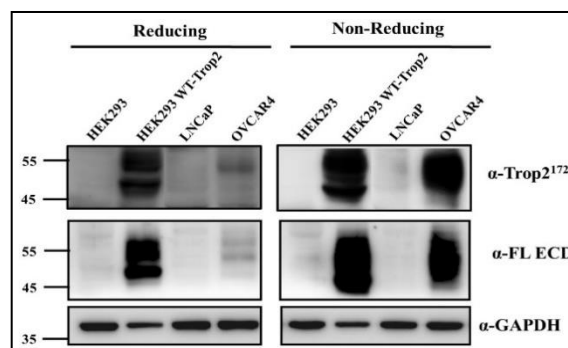


Figure 104: Characterization of the anti- Trop2¹⁷² and anti-FL ECD antibodies by western blotting Total cell lysates from HEK293, HEK293 cells transiently expressing WT Trop2HA (WT Trop2),

LNCaP and OVCAR4 cells were subjected to western blotting with indicated antibodies under reducing or non-reducing conditions. GAPDH was used as a loading control.

Truncated Trop2¹⁷² having a C-terminal His tag was cloned, expressed *in-vitro* and purified. Antisera generated against Trop2¹⁷² and commercially processed full length Trop2 ECD (FL ECD) were characterized for their ability to bind ectopically and endogenously expressed Trop2 in HEK293 and OVCAR4 cells respectively. Both the antisera recognized ectopic as well as endogenous Trop2 and the detection was much stronger under non-reducing conditions (Fig. 104) suggesting that the majority of the anti-Trop2¹⁷² and anti-FL

ECD antibodies were generated against the conformational epitopes of Trop2. In concordance with the western blotting data, both the antibodies recognized Trop2 present on the surface of HEK293-Trop2 and OVCAR4 cells as revealed by flow cytometry (Fig. 105). These antibodies were then tested in proliferation & invasion assay as well as for their ability to induce antibody-dependent cell cytotoxicity (ADCC). They inhibited the proliferation of ovarian cancer cells and also induced ADCC. Thus, truncated Trop2¹⁷² possesses the ability to elicit a humoral immune response against Trop2 expressing ovarian cancer cells. Hence, Trop2¹⁷² can be pursued further in preclinical studies for active immunotherapy against ovarian cancer.

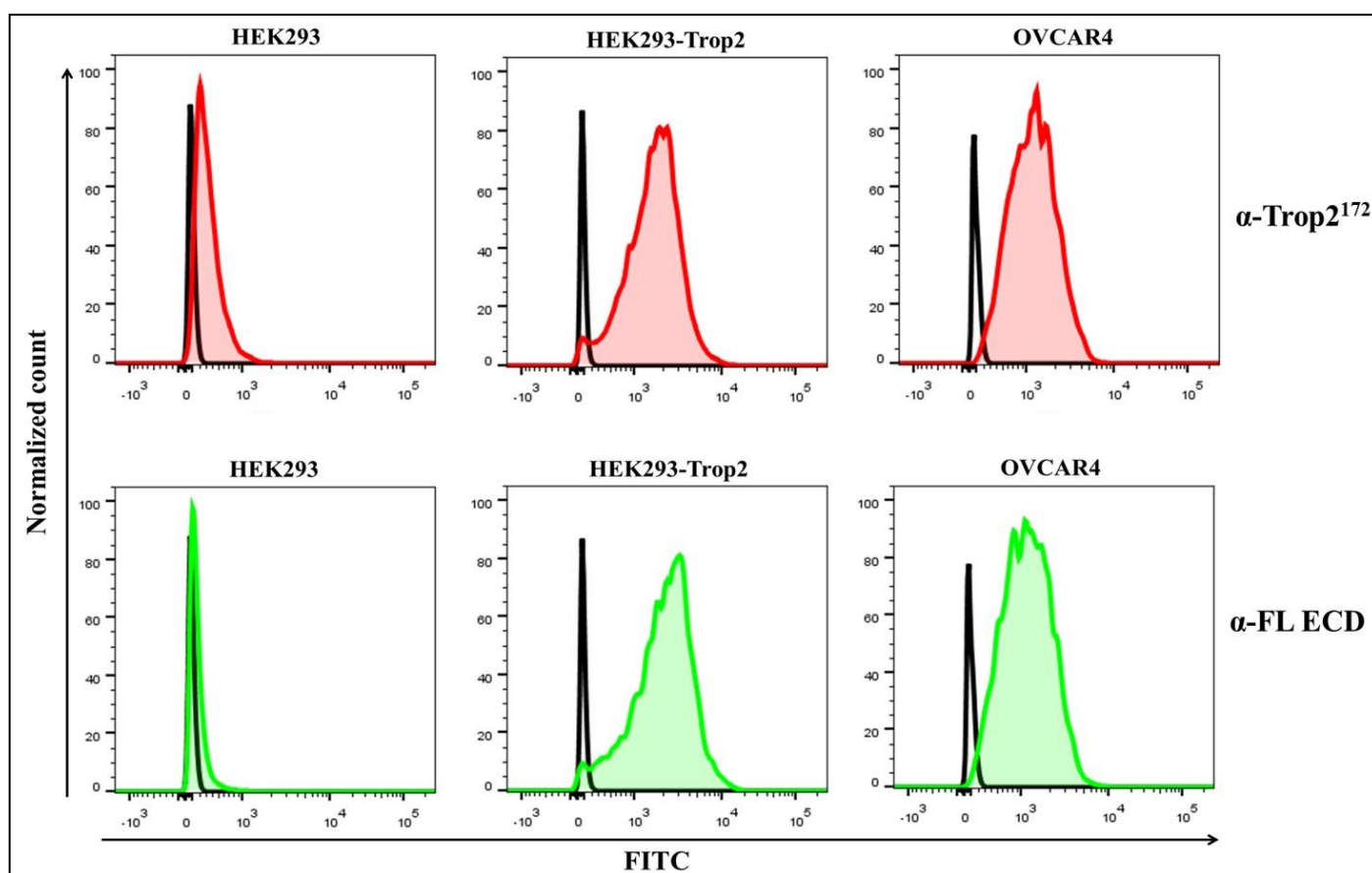


Figure 105: Flow cytometry for detection of Trop2 expressed on the cell surface by anti-Trop2¹⁷² and anti-FL ECD antibodies. Representative flow cytometry overlay histogram of HEK293, HEK293 stably expressing WT Trop2 (HEK293-Trop2) and OVCAR4 cells stained with indicated anti-Trop2 antisera. Black: unstained cells, Red and Green: cells stained with anti- Trop2¹⁷² and anti-FL ECD antisera respectively.

9.6 Exploring the Therapeutic Potential of Peptides Targeting Lysophosphatidic Acid Receptors in Ovarian Cancer (Partly Funded by Department of Biotechnology)

Principal : VD Dighe
Investigator
Principal Co- : Taruna Madan
Investigator
Project : Bhavana Bhat,
Associates Yugandhara Jirwankar, S
Jadhav, P Salunke
Duration : 2021-2024

Ovarian cancer is one of the leading gynaecological cancers affecting women globally. The survival rate is very low due to late diagnosis. Lysophosphatidic acid (LPA) is one of the key molecules elevated in ovarian cancer. LPA receptors (LPARs) are present in six different isoforms LPAR (1-6) in the body and are involved in various cellular processes. LPA -LPAR binding activates the signaling pathways leading to cell proliferation, cytoskeletal changes, angiogenesis, cell adhesion, and survival in normal conditions. In ovarian cancer, LPA and LPARs (2 and 3) are overexpressed. LPA-LPAR interaction activates the downstream signaling cascades resulting in cell proliferation, invasion, and migration leading to cancer metastasis. We hypothesize that targeting LPAR with LPAR3 binding peptides may disturb LPA-LPAR interaction and might delay ovarian cancer progression and metastasis.

So, the present study aims to identify the peptides binding to LPAR3 using phage display library approach. In the previous year, the effect of LPA on the expression of LPARs in human and mouse ovarian cancer cell lines was analysed. OVCAR3 and ID8 cells treated with 1 μ M LPA for 24 hours showed upregulation of LPARs compared to control cells. This study

elucidated that LPA influenced LPAR expression in human and mouse ovarian cancer cell lines.

In the reporting year, the LPAR3 binding peptide was identified using a phage display library approach. Three rounds of *in vitro* panning were carried out using rLPAR3 as bait by Ph.D.-12 phage display library. After each round, there was an increase in the titre of the enriched phages. For sequence identification of the peptides, the phage DNA pools of rounds 1 and 3 were subjected to NGS sequencing. After NGS analysis, the unique peptides obtained in round 1 were ~24,000; for round 3, the count was ~7000. The diversity of the peptides was reduced in round 3 due to the enrichment of the phages. Top 10 peptides were screened after NGS analysis on the basis of the frequency count.

The peptide sequences which have occurred at higher frequency among the total peptide sequences will be selected for further validation. Three to four peptides will be synthesized commercially and validated for the specificity of the peptides to human ovarian cancer cell line, OVCAR3 *in vitro* and *in vivo* in ovarian cancer xenograft mice model.

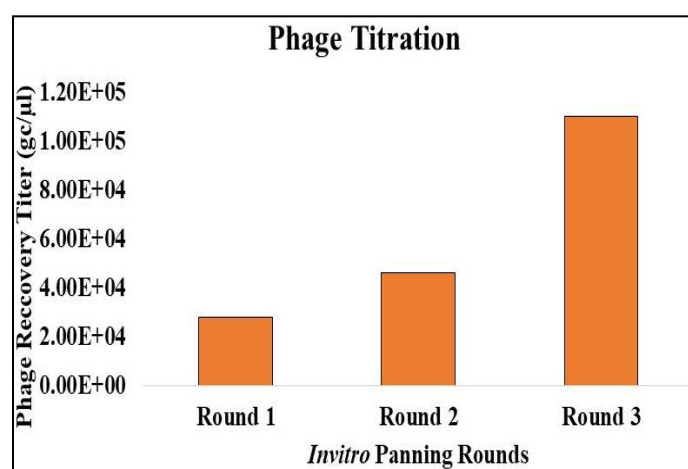


Figure 106: Phage titre of three rounds of *in vitro* panning

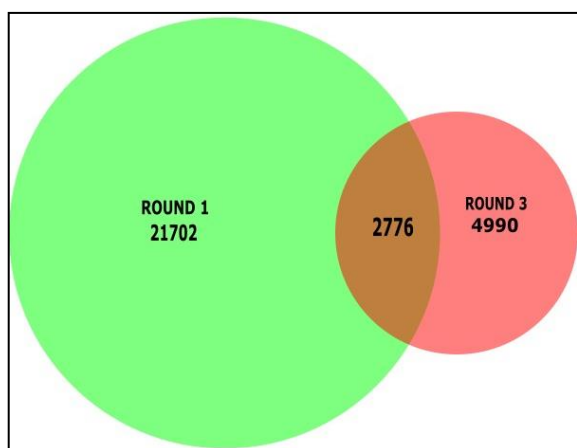


Figure 107: Venn illustration of peptides derived from rounds 1 and 3 of *in vitro* panning. 2776 peptides were commonly identified in both rounds.

9.7 Evaluation of Drugs-Cytochrome P450 Enzyme Interaction through Fluorometric High Throughput Screening Assays (Partly Funded by CCRAS)

Principal Investigator	: Vikas Dighe
Principal Co-Investigator	: Susan Thomas
Project Associates	: Amruta Gadade, S Jadhav, Shilpa Kerker, P Salunke
Collaborator	: Pallavi Deshmukh, RARI, Pune
Duration	: 2021-2023

Cytochrome P450 superfamily has been implicated as one of the most critical drug-metabolizing enzymes because it metabolizes a wide range of pharmaceuticals. In the proposed study, ayurvedic formulations are studied for their herb drug interaction (HDI) against the key marker, human CYP isoforms CYP1A2, CYP2C9, CYP2D6, CYP2E1, and CYP3A4. It is vital to include preclinical (*in-vitro*) and clinical (*in-vivo*) interaction studies during the drug development process. Different formulations were validated by *in-vivo* and *in-vitro* studies for

their HDI. Out of 100 formulations, 36 formulations were analyzed. The crystal structures of four human cytochrome P450 isozymes, i.e., Cyp3A4, Cyp1A2, Cyp2D6, and Cyp2C19 with PDB ID: 2V0M, 2HI4, 4GQS and 4WNV, respectively were retrieved from RCSB PDB. The retrieved co-crystal structures of Cyp3A4, Cyp1A2, and Cyp2D6 contained Ketoconazole, Alpha-naphthoflavone, and Quinidine inhibitors, respectively and were considered reference molecules. Miconazole, the inhibitor of the Cyp2C19 isozyme, was not present in the co-crystallized PDB structures; thus, the structure was retrieved from PubChem (ID: 4189). The structure for 45 active principles of 36 traditional medicinal formulations was downloaded from the PubChem database. The structures of four Cyp450 isozymes were prepared. The retrieved active principles were prepared using the 'prepare ligands' module of DS 2021. All active principles were docked with all 4 Cyp450 isozymes at their inhibitor binding sites. The molecular docking was performed using the CDOCKER module of the DS 2021. A total of 10 conformations were generated for each active principle. Top active principles were selected based on their interaction with key residues (in order of hydrogen bonding, pi-stacking, and hydrophobic interactions), similar to their respective inhibitors and CDOCKER interaction scores. In the case of Miconazole, there was no interacting key residue in the receptor Cyp450 2C19. So, conformations of active principles similar to Miconazole with similar or higher CDOCKER interaction scores and similar hydrophobic interactions were considered. Dhatri lauha, Parthadyarishta, Amrita guggulu, Sukumara ghrita and Gandharvahasta are five main formulations that showed higher interaction scores and interactions with key residues similar to respective inhibitors in all four crystal structures of human cytochrome P450 isozymes (Table 20).

Table 20: List of formulations exhibiting *in-silico* interactions with key residues of Cyp450 isozymes

Sr. No.	Formulation	Cyp450 Isozymes			
		Cyp3A4	Cyp1A2	Cyp2D6	Cyp2C19
1	Vyaghriharitaki	✓			
2	Dhatri lauha	✓	✓	✓	✓
3	Parthadyarishta	✓	✓	✓	✓
4	Rasnasaptakakvathachurna	✓	✓		
5	Haritaki churna	✓	✓	✓	
6	Amrita Guggulu	✓	✓	✓	✓
7	Sarpagandha vati	✓		✓	
8	Ashvagandhadyarishta	✓		✓	
9	Sukumara Ghrita	✓	✓	✓	✓
10	Navayasa Churna	✓	✓	✓	
11	Gandharvahasta	✓	✓	✓	✓
12	Trayodashanga Guggulu	✓		✓	✓
13	Kaishoraguggulu	✓	✓	✓	
14	Kalyanaka Ghrita	✓	✓	✓	
15	Sarpagandha Mishrana	✓		✓	
16	Tryushandi Guggulu	✓	✓	✓	
17	Kushmandakarasayana	✓		✓	
18	Punarnava Guggulu	✓	✓	✓	
19	Vatari Guggulu	✓		✓	
20	Simhanaada Guggulu	✓		✓	
21	Brihad manjisthadi		✓		
22	Mandura vataka		✓		✓
23	Punarnavadi mandura		✓		✓
24	Haridrakhand		✓		✓
25	Jatamamsi arka		✓		
26	Dashmoola Ghrita		✓		
27	Hingvashtaka Churna		✓		✓
28	Panchasama Churna		✓		✓
29	Madhusnuhi Rasayana		✓		✓
30	Bramhi Ghrita		✓		✓
31	Khadirarishta		✓		
32	Ashvagandha			✓	
33	Ashwagandhadi Lehya			✓	✓
34	Kanakasava			✓	
35	Simhanaada Guggulu				
36	Bilvadileha				✓

10. COVID-19 RESEARCH

10.1 National Registry of Pregnant Women with COVID-19 in India (PregCovid Study)

Principal Investigators	: R Gajbhiye N Mahajan , TNMC & BYL Nair Charitable Hospital, Mumbai R Waghmare , Medical Education and Drugs Department (MEDD), Government of Maharashtra
Principal Co-Investigators	: D Modi, Suchitra Surve
Project Associates	: Sarika Zala, Divya Balgoniwar, Kalyani Jadhav, S Parperlli, C Jadhav, Supriya Jawale, Samruddhi Kothe
Collaborators	: Department of Obstetrics and Gynecology, Department of Pediatrics of 18 Government Medical Colleges under MEDD in Maharashtra State & BYL Nair Charitable Hospital, Mumbai
Duration	: 2020-2022

For addressing the public health emergency of COVID-19, ICMR-National Institute for Research in Reproductive and Child Health initiated a hospital-based pregnancy registry to capture socio-demographic, clinical presentations, treatment outcomes, obstetric and neonatal outcomes in Indian women with COVID-19. The objectives of the study are: i) to understand the socio-demographic, clinical presentations, reproductive characteristics of pregnant and post-partum women diagnosed with SARS-CoV-2 infection; ii) to determine the pregnancy and fetal outcomes in women with SARS-CoV-2 infection and study the effect of

treatment on pregnancy outcomes; and iii) to evaluate the proportion of maternal to fetal transmission of SARS-CoV-2 infection in pregnancy. During the reporting year, we conducted analysis of a total of 1530 pregnant and postpartum women admitted during the first wave (April 1, 2020 to January 31, 2021) and the second wave (February 1, 2021 to May 14, 2021) of the COVID-19 pandemic. In our cohort, symptomatic cases were significantly higher in the second wave compared to the first wave (28.7% Vs 14.2%) ($p < 0.001$). The case fatality rate (CFR) was significantly higher (5.7%) during the second wave as compared to the first wave (0.7%) of pandemic ($p < 0.001$). During the second wave, significantly higher cases of severe COVID-19 (8.5%), intensive care units (ICU)/high dependency units (HDU) admissions (11.6%) were observed as compared to the first wave ($p < 0.001$). The majority of the maternal deaths (93%, 28/30) were due to COVID-19 pneumonia and respiratory failure. The data on women with sickle cell anemia (HbSS, $n = 6$), sickle cell trait (HbS, $n = 24$), HbS with β thalassemia ($n = 1$) was compared with women with COVID-19 but without any sickle cell disease ($n = 1551$) from the same study cohort. Our results indicate an increased risk of pregnancy complications and adverse outcomes (including death) in women with SCD and COVID-19 than non-SCD. The results of our study will help the obstetricians practicing in regions with a high prevalence of SCD. During the second wave of the COVID-19 pandemic, five times higher severe COVID-19 cases, 8 times higher maternal deaths, 2 times higher stillbirth rate per 1000 births, 3 times higher spontaneous abortion rate per 1000 births, 3 times higher very spontaneous preterm births (28 to 32 weeks of gestation) were observed in pregnant and postpartum women with COVID-19. A retrospective analysis of 2524 neonates born to SARS-CoV-2-infected

mothers was conducted during the first wave (n=1782) and second wave (n=742) of the COVID-19 revealed that risk of birth asphyxia and prematurity were 3.8 and 2.1 times higher in the second wave. It was also observed that higher proportion of neonates required intensive care admissions in the second wave. To share the research findings with scientific community, policy makers and general populations, we analyzed the data and published articles in peer reviewed national and international journals and provided links for PregCovid registry publications (<https://pregcovid.com/publications/>). The findings were also reported in national and international media (Media - (pregcovid.com)). The evidence generated from PregCovid registry was useful for policy decision on COVID-19 vaccination for pregnant women in India.

10.2 Incidence and Dynamics of SARS-CoV-2 Infection in Unvaccinated and Vaccinated Populations of Various Categories in Mumbai, India

Principal Investigator : V Bhanothu

Principal Co-Investigator : S Pande

Project Associates : Priyanka Singh, S Bhagat, Reshma Gaonkar, Shilpa Kerkar

Collaborators : Kiran Munne, Dhanashree Jagtap, Clara Aranha, D Gogoi, K Shah, Nupur Mukherjee, V Bhor, V Patel, Smita D Mahale, Geetanjali Sachdeva, Shahina Begum

Duration : 2020-2021

As the emergence of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), variants may compromise vaccine efficacy (VE)

with re-infections in vaccinated individuals, continuous re-evaluation and monitoring of the VE with current evolving scenario is essential. This study was undertaken to understand the occurrence and dynamics of SARS-CoV-2 infections during the second wave in unvaccinated and vaccinated populations in Mumbai, India. RT-PCR cycle threshold (Ct) data of confirmatory genes obtained between December 28, 2020, and August 30, 2021 were analyzed. A total of 42,415 samples suspected of SARS-CoV-2 infection, received from unvaccinated cases (96.88%) and vaccinated cases (3.12%) were analyzed. A lower incidence of SARS-CoV-2 in fully vaccinated cases (5.07%) compared to partially vaccinated cases (only one dose) (6.5%) and unvaccinated cases (13.453%) was recorded. The VE in clearing the SARS-CoV-2 was highly significant after the 1st dose of vaccination (ORF gene p-value=0.003429, and E/N gene p-value=0.000866). Within 30 days of the first dose of vaccination (ORF gene p value = 0.0094 and E/N gene p value = 0.0023) to 60 days of the second dose of vaccination (ORF gene p-value = 0.0132 and E/N gene p-value = 0.0387), the SARS-CoV-2 infection was significantly suppressed. The emergence of the SARS-CoV-2 new infections even after vaccination was evident (odds ratio =0.34; 95% confidence interval =0.27-0.43). High viral loads in females, symptomatic cases, and cases over 61 years old vaccinated with the first dose compared to the second dose and the unvaccinated population were evident (Table 21). The analysis of this study covered the real-world setting of second wave of COVID-19 data of the general population of various categories in Mumbai, India. A significant risk of SARS-CoV-2 infections in males (Odds ratio [OR] =0.86; 95% confidence interval [CI] =0.81-0.91), asymptomatic cases (OR =2.35; 95% CI =2.13-2.60) and cases with advance age (OR =1.01; 95% CI =1.01-1.02) were reported (Table 22).

Table 21: The characteristic distribution of Ct values for SARS-CoV-2 genes in unvaccinated and vaccinated cases

Characteristics	Unvaccinated (n=41092)				Partially vaccinated (n=692)				Fully vaccinated (n=631)			
	Ct of ORF1 gene		Ct of E/N gene		Ct of ORF1 gene		Ct of E/N gene		Ct of ORF1 gene		Ct of E/N gene	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Gender												
Female	26	6.34	26.7	6.73	23.58	5.64	23.84	5.728	28.23	5.05	27.91	5.45
Male	26.61	6.27	27.27	6.65	23.65	6.11	23.56	6.332	25.63	6.4	25.88	6.79
Overall Ct value	26.38	6.3	27.05	6.69	23.62	5.85	23.682	6.01	26.69	5.943	26.45	6.32
Age group in years												
<20	26.24	6.24	27.01	6.66	22	0	22	0	0	0	0	0
21-40	26.34	6.34	27.01	6.72	23.93	4.89	23.23	3.723	28.54	6.64	29.08	7.48
41 -60	26.4	6.36	27.03	6.73	25	6.38	25.44	7.071	26.31	5.72	25.09	5.26
>61	26.71	6.08	27.35	6.43	21.86	6.25	22.21	6.52	23.5	3.62	23.67	3.93
Clinical status												
Symptomatic	29.05	5.14	29.69	5.67	19.67	1.03	19.67	1.033	27.5	4.21	26.86	3.67
Asymptomatic	26.02	6.36	26.69	6.74	24.23	6.05	24.32	6.23	26.42	6.47	26.31	7.03

Table 22: Odds Ratio and 95% confidence intervals of the factors affecting the incidence of SARS-CoV-2 infection during the second wave in unvaccinated and vaccinated populations

Variables	Odds Ratio (OR)	95% Confidence intervals (CI)	
		Lower (LCI)	Upper (UCI)
Gender (female versus male)	0.86	0.81	0.91
Age (continuous)	1.01	1.01	1.02
Clinical presentation (asymptomatic versus symptomatic)	2.35	2.13	2.6
Underlying medical conditions (with versus without)	2.12	1.68	2.69
Vaccination status (non-vaccinated versus vaccinated)	0.34	0.27	0.43

10.3 An Integrated Approach towards Delineating Determinants of Viral Pathology and Disease Progression of SARS-CoV-2 Infection (Partly Funded by MCGM)

Project : Smita Mahale, Geetanjali
 Coordinators: Sachdeva
 Principal : V Patel, V Bhor, Dhanashree
 Investigators Jagtap

Principal Co- : Jayanthi Shastri, Sachee
 Investigator Agarwal, O Khandkar, Nair
 Hospital & Kasturba
 Hospital

Project : D Gogoi, Clara Aranha,
 Associates Nandini Kasarpalkar, Shilpa
 Bhowmick, AK Singh

Duration : 2020-2022

Immune cell dysregulation and lymphopenia
 characterize COVID-19 pathology in moderate

to severe disease. While underlying inflammatory factors have been extensively studied, homeostatic and mucosal migratory signatures remain largely unexplored as causative factors. In this study, we evaluated the association of circulating IL-6, soluble mucosal addressin cell adhesion molecule (sMAdCAM) and IL-15 with the cellular dysfunction characterizing mild and hypoxemic stages of COVID-19.

A cohort of SARS-CoV-2 infected individuals (n=130) at various stages of disease progression together with healthy controls (n=16) were recruited from COVID Care Centres (CCCs) across Mumbai. Multiparametric flow cytometry was used to perform in-depth immune subset characterization and also to measure plasma IL-6 levels. Distinct depletion profiles, with relative sparing of CD8 effector memory and CD4⁺ regulatory T cells, were observed in hypoxemic disease within the lymphocyte compartment. An apparent increase in the frequency of intermediate monocytes characterized both mild as well as hypoxemic disease. IL-6 levels inversely correlated with those of sMAdCAM and both markers showed converse associations with observed lympho-depletion suggesting opposing roles in pathogenesis. Interestingly, IL-15, a key cytokine involved in lymphocyte

activation and homeostasis, was detected in symptomatic individuals but not in healthy controls or asymptomatic cases. Further, plasma IL-15 levels negatively correlated with T, B and NK counts suggesting a compensatory production of this cytokine in response to the profound lymphopenia. Finally, higher levels of plasma IL-15 and IL-6, but not sMAdCAM, were associated with longer duration of hospitalization.

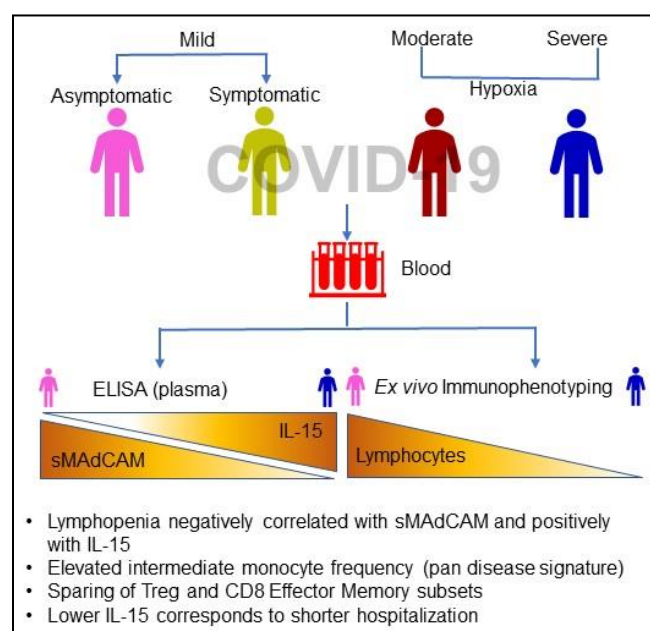


Figure 108: Summary of findings: Opposing roles for sMAdCAM and IL-15 in COVID-19 associated cellular immune pathology

Table 23: Abridged clinical data of study participants

	Seronegative Healthy	Asymptomatic mild	Symptomatic mild	Moderate	Severe
Number of Participants	16	47	62	10	11
Age ^a (years)	26 (24-46)	39 (18-75)	37 (18-71)	64 (47-75)	66 (31-81)
Gender (M/F)	8/8	33/14	41/16	6/4	9/2
Duration of Hospitalisation (days)	-	8 (1-41)	7 (1-41)	12 (2-27)	18 (8-38)

^a Data are expressed as the median (range).

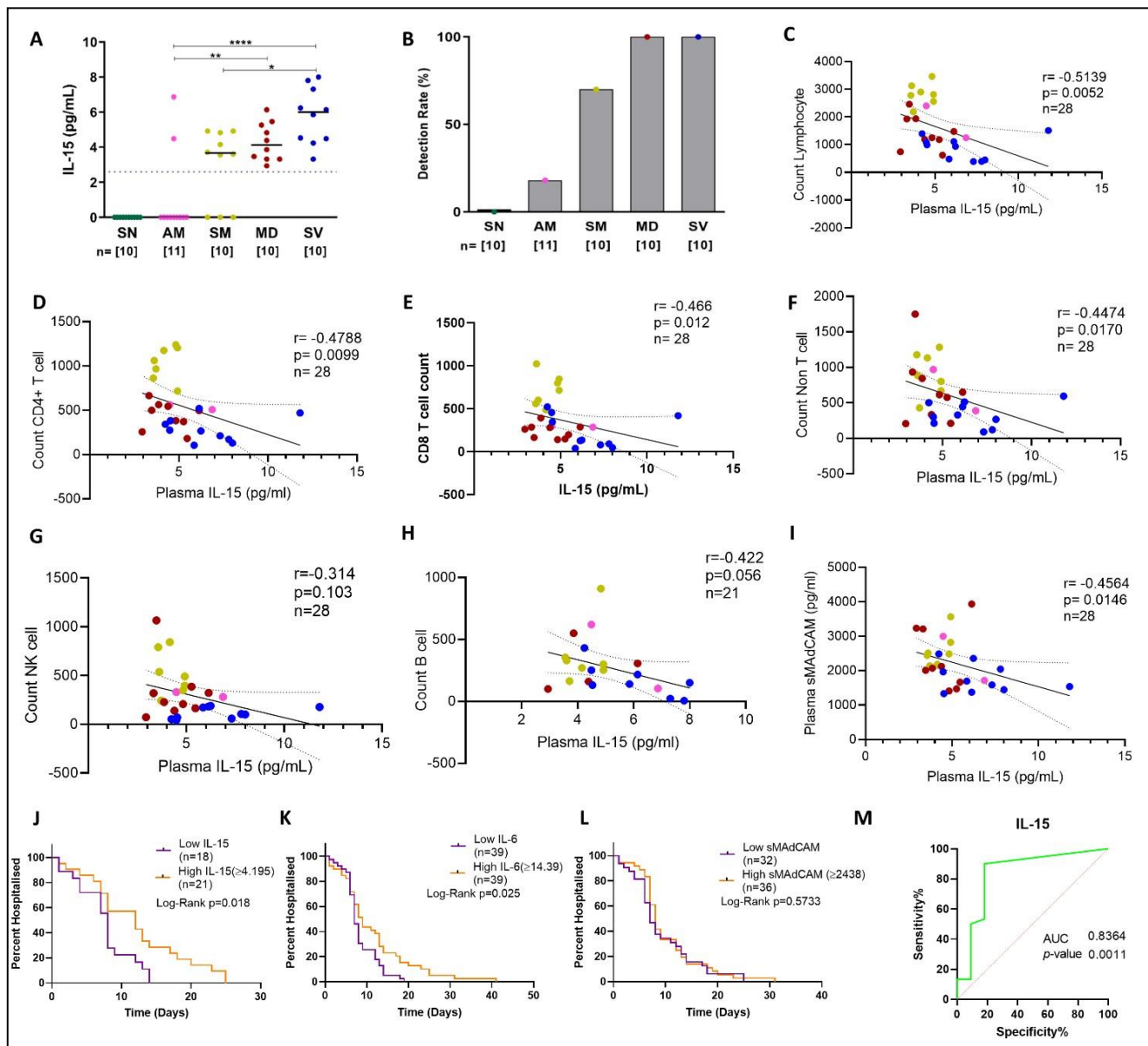


Figure 109: Distribution of IL-15 and its correlation with immune cells among study participants. (A) Variation in levels of IL-15 in Seronegative (SN), asymptomatic mild (AM), symptomatic mild (SM), moderate (MD) and severe (SV) groups. Dotted line indicates limit of detection. (B) Responder rate. Association of IL-15 with different cellular subsets (C) Lymphocyte count (D) CD4+ T cell count (E) CD8+ T cell count (F) Non-T cell count (G) NK cell count (H) B cell count (I) Soluble MAdCAM. (J-L) Kaplan Meier plots depicting hospitalization duration post recruitment associated with plasma levels of (J) IL-15 (K) sMAdCAM and (L) IL-6. Individuals were categorized into a low and a high group on the basis of median levels. (M) Receiver operating characteristic (ROC) curve analysis of IL-15 level to discriminate between asymptomatic (AM) and symptomatic (SM, MD and SV) COVID-19 infected patients. Area under curve (AUC) 0.8364; 95%CI 0.6716 to 1.000; $P = 0.0011$. Statistical significance was calculated by non-parametric one way ANOVA (Kruskal-Wallis test) followed by post hoc Dunn's test; *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$; and ****, $p < 0.001$. Correlation analysis was performed using non-parametric Spearman Rank Correlation test. For Kaplan Meier comparisons, Log-rank (Mantel-Cox) test was used.

11. RESEARCH SUPPORT FACILITIES

11.1 Family Welfare Clinics

Two family welfare clinics of NIRRCH, one located in Wadia Hospital, Parel and another in community at Abhyudaya Nagar provide family planning services and serve as models for integration of family planning services with other aspects of reproductive health care. Services offered routinely include IEC on various contraceptives, creating awareness, screening and treatment of reproductive tract infections (RTIs) and cervical cancers and services such as pelvic ultrasonography for women. The women attending these clinics, if willing are then enrolled for clinical studies. These women provide biological samples for basic research studies approved by the institutional ethics committee. The important feature of these clinics is the good rapport of the clinic staff with the women/couples attending these clinics. This results in high rate of follow-ups (more than 95%) for study participants.

Table 24: Clinical services provided by Family Welfare Clinics during 2020-2021

Clinical services provided	Wadia Clinic	Abhyudaya Nagar Clinic
Total clinic attendance:	988	1181
Women		
Number of women counselled for contraception	1028	194
Number of women tested and treated for RTI/STIs	26	223
Number of women tested for urine infection	38	13
Women accepted CuT- 380A	11	5
Women accepted Oral Contraceptive Pills (OCPs)	8	16
Total condoms distributed	4666	1666
Total women accepted injectable contraception	08	30

11.2 Child Health Clinic

Child health services were initiated at Family Welfare clinic located at Abhyudaya Nagar in 2015. The objectives of the Clinic are growth monitoring and nutritional guidance, screening for diseases and deficiencies, child development and mental health and health education. Services such as height, weight monitoring and growth parameters, learning disorders and behavioral issues, counseling and referral and sex education are being provided. Parents are advised mainly on new born care and breastfeeding, growth and development of children, nutrition, issues related to menarche and puberty. During the reporting year, 533 children visited the clinic. Various community outreach program on Poshan Abhiyaan, Health and Hygiene, Swachh Bharat were also conducted in Anganwadis and schools.

Table 25: Clinical services provided by Child Health Clinic

Child health services	Number
Total number of children visited	533
Total number of new children	135
Children treated for deworming	85
Children treated for anemia	55
Dietary counseling for children	175
Children treated with Calcium and Vitamin D supplementation	120 (Calcium) 37 (Vit D)
Puberty counseling	32
Children recruited for research	57
Pediatric endocrinology	15

11.3 Infertility and Reproductive Endocrinology Clinic

During the reporting year, clinical services were provided to infertile couples through the Infertility clinic. Infertile males availed the

services of a visiting andrologist to the department. The results obtained helped in management and prognosis of infertile couples. Telephonic consultations were offered to infertile couples. The women attending these clinics were enrolled for clinical and basic studies in case they provide consent for participation. The details about the clinical services provided are given in following table.

Table 26: Clinical services provided by Infertility and Reproductive Endocrinology Clinic

Clinical services provided	Numbers
Total Number of telephonic consultation provided	548
Total Infertile couple new, old and follow up	New-265 Old- 1194
Follow up patient visits for diagnosis, counseling and management	1194
Ultrasound for serial follicular monitoring	171
Number of pregnancies reported	19
Number of Multidisciplinary PCOS Clinics	3
Number of PCOS cases detected	46
Number of couples referred for IUI and IVF at Wadia Hospital	56
Total ongoing collaborative projects of the institute	8

11.4 Andrology Clinic

Andrology clinic offers services such as clinical examination, scrotal doppler, semen analysis, diagnosis, treatment, and counseling of infertile men. During the reporting year, following clinical and laboratory services were provided to infertile men.

Table 27: Clinical services provided by Andrology Clinic

Clinical services provided *	Number
Andrology consultations (New)	42
Andrology consultations (Old)	39
Total Andrology Consultations	81

* Semen analysis (n=13). Due to the COVID-19 pandemic, the andrology clinic and semen analysis services were temporarily closed as per the instructions received from the appropriate authorities.

11.5 Bone Health Clinic

The community based Bone Health Clinic is a unique model to address osteoporosis with a special focus on knee/ spine problems and improvement in physical activity/ quality of life by Yoga and stretching exercises. This multidisciplinary and comprehensive health care management may help in compliance of treatment for osteoporosis. In the reporting year, DXA was done in 47 clinic clientele with 21 newly registered cases. The clinic resorted to telephone calls (>2000) by the staff for history taking, follow up services. Total clinic attendees during the reporting year was 735 with referrals as required for cardiac opinion, urology, physiotherapy, diabetologist and physician for hypertensive disorders. Due to pandemic, regular follow up was done for all the registered clients every 2 months via telephonic calls regarding intake of medicines/ adverse events if any. Specialty OPDs like spine OPD (clinic consultation) and endocrine OPD (telephonic consultation) were also started.

11.6 Women's Health Clinic

The Women's Health Clinic, community-based service cum research clinic located at BDD Chawl, Naigaon, offers services for minor ailments, gynaecological complaints, family planning needs etc. Routine health services include clinical examination, breast examination, cytology screening and contraception services. During the reporting year, gynaecology consultations at clinic were done for 132 women. Services included copper-T 380A insertions, condom distribution, and transvaginal ultrasonography (n=25) for any gynaecological complaints. These consultations included women who came with menorrhagia,

incomplete abortion, PCOS, irregular menstruation, post-menopausal bleeding, urinary tract infections, Copper- T users etc.

11.7 Genetic Research Centre

Forty-seven patients visited Genetic Research Centre OPD for consultation. This include 85 new patients with bad obstetric history (BOH), autism, dysmorphic features, sickle cell disease, short stature, neural tube defect (NTD), primary amenorrhea, intrauterine fetal death (IUFD), ambiguous genitalia, omphalocele, arthrogryposis, D'George syndrome, Wilson disease, Y-microdeletions, secondary amenorrhoea, Downs syndrome, fragile X syndrome, hypospadias, Turner's syndrome, ataxia, Duchenne Muscular Dystrophy (DMD), metabolic diseases, Congenital Adrenal Hyperplasia (CAH), cerebral palsy and other genetic disorders etc. and rest were follow-up cases. PCR for 11 STS markers for Y chromosome microdeletion has been standardized at the department for genetic testing of patients with male infertility. The new patients underwent genetic counselling and karyotyping was done whenever necessary. The services and support facilities provided (including services rendered through clinics as well as research laboratories) are given in the table below:

Table 28: Clinical services provided by Genetic Research Centre

Clinical Services Provided	Number
Karyotyping	92
Genetic counselling	247
Molecular genetic testing	37

11.8 National Center for Preclinical Reproductive and Genetic Toxicology

The National Center for Pre-clinical Reproductive and Genetic Toxicity has mandate of research, services and capacity

building. Services to various academic and industry are being undertaken following Organization for Economic Co-operation and Development (OECD) guidelines and Good Laboratory Practices (GLP).

Research projects undertaken in collaboration with academic institutions during 2021-2022:

1. Antitumor efficacy of anticancer nano-formulations after oral and intravenous route of administration in breast cancer induced mice model Mumbai (*Funded by Institute of Chemical Technology, Mumbai*)
2. Investigation of bio-distribution of APIs (UA and OA) using in-vivo imaging (*Funded by Indian Institute Technology, Mumbai*)
3. Pharmacological evaluation of potential therapeutic entities for its anti-Alzheimer's activity (*Funded by Institute of Chemical Technology (ICT), Matunga, Mumbai*)
4. Genotoxicity of cottonseed protein isolated by alkali extraction method (*Funded by ICAR-Central Institute for Research on Cotton Technology, Mumbai*)
5. Subchronic (90 days) toxicity of an Nanocurcumin (*Funded by KIIT-Technology Business Incubator, Bhubaneswar*)
6. Acute, subchronic and genotoxicity of WSSV DNA vaccine (*Funded by ICAR-Central Institute of Fisheries Education (CIFE), Mumbai*)
7. Acute toxicity of a water-soluble and biodegradable plastic using Wistar rats (*Funded by Indian Institute Technology, Mumbai*)
8. Investigation of in-vivo bio-distribution of ultrasound responsive nanoparticles, their potential of ultrasound-triggered targeted therapeutics delivery and effect on tissues (*Funded by Indian Institute Technology, Mumbai*)

9. Study the intra-articular retention time of dye-loaded formulations (*Funded by Indian Institute Technology, Mumbai*)
10. Pharmacokinetics, synovial clearance and efficacy studies of nanoparticles loaded in-situ gel by intra-articular route in rats (*Funded by Indian Institute Technology, Mumbai*)

Research projects undertaken in collaboration with the Industry during 2021-2022:

1. Effect of Streevyadhihari Rasa and Ashotone + Zpter combination in experimental model of polycystic ovary syndrome (*Funded by Soulmiks Herbaceuticals limited, Mumbai*)
2. Effect of a Kaklarakshak Yog (SDLKRY) in experimental model of hypothyroidism (*Funded by Soulmiks Herbaceuticals Limited, Mumbai*)
3. Evaluation of the reproductive function of BeejapustiTM using experimentally induced oligospermia in rats (*Funded by Soulmiks Herbaceuticals Limited, Mumbai*)
4. Acute dermal irritation/ corrosion testing and acute oral toxicity of EczetarTM (*Funded by Roop Herbal Life, Thane*)
5. Reproduction/developmental toxicity of ayurvedic formulation Torch-XV in rats (*Funded by Acuere Biosciences Pvt. Ltd. Acuere Lifescience Foundation*)
6. Developmental and reproductive toxicity studies and 28 day repeated dose oral toxicity studies for Cap.PCOSNIL in rats (*Funded by Acuere Biosciences Pvt. Ltd. Acuere Lifescience Foundation*)

11.9 COVID-19 Diagnostics Testing and Reporting

ICMR-NIRRH, at the behest of ICMR, as well as through synergistic partnerships with Municipal Corporation of Greater Mumbai (MCGM) and Government of Maharashtra was

amongst the earliest responders to the COVID-19 pandemic. The institute responded by initiating immediate public outreach activities; by providing technical guidance and mentoring of neighboring institutes; and validation of COVID-19 testing reagents. In addition, several research programs were initiated, including the setting up of a national biorepository and public-private partnerships to develop novel testing reagents and provide potentially translational findings in the area of COVID-19 pathogenesis and therapy. During the reporting year, a joint ICMR-NIRRH and Nair Hospital (MCGM) center of excellence in Immunology research was established at Nair Hospital for collaborative research programs on COVID-19 immunopathogenesis and other areas of mutual interest.

11.9.1 Preparedness

The existing BSL-2 virology laboratory, which was repurposed for SARS-CoV-2 RT-PCR testing, is being upgraded to a BSL-3 laboratory to further enhance the preparedness for handling pathogens with epidemic and pandemic potential.

11.9.2 Testing and Reporting

During the reporting year, COVID-19 testing facility continued to provide diagnostic services to MCGM, and peripheral regions such as Palghar district and Ulhasnagar and Navi Mumbai municipal corporations using both manual testing as well as the Cobas 6800 high throughput testing. During the reporting year, 1,04,782 samples were tested.

11.9.3 Diagnostic Kit Validation

ICMR-NIRRH was designated as the Centre of Excellence for Validation and Quality Assurance for COVID-19 testing kits on May 8, 2020. During the reporting period, 107 VTM/VLTM/MTM kits and 8 commercial RT-PCR kits were validated.

11.9.4 Reagent Depot

The activities for storage and distribution of COVID-19 diagnostic kits for Maharashtra and Goa were initiated on March 25, 2020. ICMR-NIRRH was designated as Central Depot on June 1, 2020 and Maharashtra State Depot on September 4, 2020. It supplied reagents to 35 testing labs, validation centers and other depots. Since inception up to March 2022, around 44 lakh COVID-19 test kits (VTM, RTPCR & RNA Extraction) have been successfully stored and distributed by the central depot.

11.9.5 National Biorepository

ICMR-NIRRH was identified as one of the biorepository sites in May 2020. This biorepository was established in collaboration with MCGM as a resource of well characterized clinical samples from COVID-19 patients to promote Indian academia, industry and commercial entities for developing novel solutions for COVID-19 prevention, control and treatment. Since inception till March 2022, biological samples (plasma, serum, urine and stool) from 584 cross-sectional and 48 follow-up participants have been accrued amounting to around 15,000 samples.

11.10 Model Rural Health Research Unit (MRHRU), Dahanu

During April 2021 - March 2022, following research projects were undertaken at MRHRU

1. Improving access for screening of common cancers and non-communicable diseases among women in tribal block of Maharashtra: Challenges in implementation
2. Assessment of neonatal screening approaches for sickle cell disease and the effect of early intervention in management of the disease in tribal population
3. Population based birth defect (BD) surveillance in linkage with Rashtriya Bal Swasthya Karyakram (RBSK) programme in

rural blocks of Palghar district in Maharashtra

4. A national model to measure burden and map quality of care for type 2 diabetes mellitus in rural populations in India, involving medical colleges through primary health care setup- a feasibility study
5. Implementation research to explore operational feasibility, acceptability and cost-effectiveness of using IV ferric carboxymaltose (FCM) in management of iron deficiency anemia (IDA) among pregnant women through sub district health system in Maharashtra
6. In addition, Sentinel Surveillance Hospital (SSH) for the diagnosis of dengue and chikungunya fever was established at MRHRU. Leptospirosis diagnostic service were also initiated.

11.10.1 COVID-19 Diagnostic Facility at MRHRU, Dahanu

Due to the joint efforts of ICMR-NIRRH and district health officials, ICMR approved the COVID-19 testing diagnostic facility (RT-PCR) at MRHRU, Dahanu from May 10, 2020. The scientists and technical staff of MRHRU received training on COVID-19 molecular diagnosis from the field unit of National Institute of Virology, Pune in Mumbai. Diagnostic facility was initiated with a testing capacity of 24 samples per day, which was then increased to 96 samples per day after installing a KingFisher flex automated nucleic acid extraction system. Later, additional staff was recruited, and testing capacity of the laboratory was increased to 180 samples per day. Government of Maharashtra supported the facility by providing two microbiologists, three technicians, four data entry operators and one assistant for sample collection. In addition to the KingFisher automated system, one -20°C freezer was also provided by the state government. Realtime PCR kits, RNA extraction kits and consumables required for

the diagnosis are being supplied by the State Government through Directorate of Medical Education and Research and Civil Surgeon, Palghar.

During the reporting period, 31,311 samples were processed for the presence of SARS-CoV-2 virus. Of these, 3362 samples were identified with a positivity rate of 10.73%. During this period, samples were received from 13 sample collection centers spanning across the seven blocks of Palghar district. Positivity rate was high in of April and May 2021 with 27.6% and 19.9%, respectively. A gradually decreasing trend in the positive cases was observed after May 2021. Less than 1% positivity was observed in the months of August to December 2021. Another rise in the number of positives was recorded in January 2022 with a positivity rate of 22.9%.

11.11 Institutional Animal Ethics Committee

The Institute is registered for breeding and experimentation on laboratory rodents including non-human-primates with Committee for the Purpose of Control And Supervision of Experiments on Animals (CPCSEA), Ministry of Fisheries, Animal Husbandry and Dairying, Department of Animal Husbandry and Dairying, Government of India (vide Registration No. 78/GO/ReBi/SL/99/CPCSEA dated 11th March 1999) and this registration is periodically renewed. The Institutional Animal Ethics Committee (IAEC) members including CPCSEA nominees critically review, approve and monitor research protocols employing laboratory animals. The IAEC also conducts inspection of animal house facility periodically to ensure that animal welfare activities are being stringently followed before, during and after animal experimentation. The institute upholds the principals of 3R's - Reduction, Refinement and Replacement principal for

humane use of experimental animals in the scientific research.

During the year 2021-22, three IAEC meetings were convened on April 20, 2021 and October 13, 2021 respectively. Total 19 animal study protocols were reviewed and approved by the IAEC.

11.12 Experimental Animal Facility

The Institute has a well-maintained experimental animal facility distributed over three floors and houses different species of laboratory animals like mice, rats, rabbits, bonnet monkeys and marmosets. The animals are maintained and well taken care of by qualified and trained staff everyday including on weekends and holidays. The details of animals bred and supplied during the year is given in Table 29.

Table 29: Animals bred and supplied after approval of IAEC during April 2021-March 2022

Species	Animals	
	Bred	Supplied
Swiss Mice	1138	443
Balb/c Mice	764	279
C57BL/6	907	479
DBA2	108	20
FVB-NJ	263	98
GFP- BL/6	136	45
GFP- FVB	365	109
Transgenic Mice WB/Rej/Kit	220	56
Transgenic Mice C57BL/6/ Kit	196	67
Transgenic Mice Mgat1 -/-	196	31
Transgenic C57BL/6-Tg (TRAMP)	150	62
Transgenic Mice B6:CBA Tg- Oct4	398	138
Transgenic mice =SP-D+/-, SP-D-/-, SP-D+/-	115	43
Holtzman Rats	431	169
Wistar rats	2159	947
Rabbits	NIL	NIL
Bonnet Monkeys 12 (In house)	NIL	NIL
Marmosets	30	NIL

11.13 NIRRH Ethics Committee for Clinical Studies

The ICMR-National Institute for Research in Reproductive Health Ethics committee for Clinical Research was renamed as ICMR-National Institute for Research in Reproductive and Child Health Ethics committee for Human Studies since February 2022. Prof Shubhada Chiplunkar took over charge as chairperson; Dr Vikrant Bhor assumed the charge as Member Secretary and Dr Suchitra Surve as Joint Member Secretary from January 30, 2022. New committee members have been inducted to the team. Mrs Sudha Sathaye has been included as community representative and Adv Megha Shirke as alternate legal expert. Mrs Vaishali Bhogate, Senior Technical Officer-I from October 2, 2022 and Mrs Zakia Ansari, Technical Assistant from February 7, 2022 joined the ethics secretariat.

GCP refresher training workshop and SOP training was conducted on February 28 - March 1, 2022 for all members and ICMR-NIRRH scientists. A total of 8 full board meetings were held during the reporting period. Seven of these meetings were conducted through virtual platform and one meeting was held on October 27, 2021 in the physical mode. Overall, 41 new projects were reviewed along with a number of amendments, revisions, continuing review reports and completion reports. SIDCER-FERCAP re-recognition survey was conducted during October 25-29, 2021 through virtual mode. Site monitoring visits were also held.

11.14 Library and Information Centre

The Dr GM Phadke Memorial Library and Information Centre houses an exclusive collection of books, journals, databases, reports etc. on subjects encompassing different aspects of reproductive health care. The library houses collection of manuals and books on basic

techniques in molecular biology, immunology, cell biology, etc.

To cater to specific requirements of the researchers at the Institute, the library subscribes to 50 online journals focusing on reproductive health and allied fields. These journals can be accessed via the institutional intranet. The library for the first time introduced video journals to its collection. A subscription of customized package of Journal of Visualized Experiments (JoVE) was made which included modules on core areas, laboratory manuals, science education, encyclopaedia of experiments and the JoVE Journal.

Apart from providing information services to all the staff and students of the Institute and being a hub of specialized collection in reproductive sciences and allied fields, the library also serves the users from other Institutions. The library and Information Centre has an in-house printer and photocopier for its users. The library also collates information for the monthly/quarterly reports on the significant achievements and publications of the Institute to be sent to headquarters. In addition to this, the compilation of the annual highlights and scientific and technical report is done by the library.

In addition to the library activities, the library staff aids in updating the institutional website and maintaining the social media handles of the institute on regular basis including Twitter, Facebook, LinkedIn and YouTube.

11.15 Core Facilities

11.15.1 Confocal Imaging

This facility provided assistance to staff and students of the Institute and other academic/research institutes for co-localization, live cell imaging and 3-D imaging

on laser confocal system. From April 2021 to March 2022, 39 researchers used the facility hours both from our institute and from other institutes.

11.15.2 Droplet Digital PCR (ddPCR)

It is a technology for quantification of nucleic acids. ddPCR is being applied for quantification of mRNA and DNA targets. Several assays are optimized in the system that is now being used by scientists and students. The facility trains the users and allows them to carry out the assay themselves. Support is provided by the laboratory in designing and optimizing the assays. In the reporting year, the system was used by 13 users from five departments at the institute. The equipment usage and applications was also demonstrated to participants of workshops and student visitors. A paper from NIRRCH is published using data generated from this machine where low abundance miRNAs, that were hard to be detected by conventional qPCR, were quantified using ddPCR.

11.15.3 Flow Cytometer and DNA Sequencing

The facility provides services toward institutional projects and inter-institutional projects. During the reporting year, 1630 samples were processed for flow cytometry and DNA sequencing was done for 7765 samples.

11.15.3 Histology Work Station

Central histology facility has been providing services to the investigators at the institute and collaborators.

Table 30: Details of samples processed by the Histology facility

Service provided	Samples
Tissue processing/embedding	876
Tissue sectioning	2696
Slide staining	764

11.15.1 Transmission Electron Microscopy

TEM facility provides the high-resolution EM analysis to the institute's investigators. It also provides research support to other academic/research institutes.

12. PUBLICATIONS

12.1 Peer Reviewed Publications during 2021

1. Aranha C, Patel V, Bhor V M, Gogoi D. Cycle threshold values in RT-PCR to determine dynamics of SARS-CoV-2 viral load: An approach to reduce the isolation period for COVID-19 patients.
J Med Virol 93: 6794-6797, 2021
[IF: 20.693]
2. Bane K, Desouza J, Shetty D, Choudhary P, Kadam S, Katkam R R, Fernandes G, Sawant R, Dudhedia U, Warty N, Chauhan A, Chaudhari U, Gajbhiye R, Sachdeva G. Endometrial DNA damage response is modulated in endometriosis.
Hum Reprod 36: 160-174, 2021
[IF: 6.353]
3. Banerjee AA, Joseph S, Mahale SD. From cell surface to signaling and back: the life of the mammalian FSH receptor.
FEBS J 288: 2673-2696, 2021
[IF: 5.622]
4. Begum S, Chaurasia H, Moray KV, Joshi B. Predictors of discontinuation of modern spacing contraceptives in India.
Asia Pac J Public Health 33(1): 121-125, 2021
[IF: 2.270]
5. Bhadriraja H, Patel V, Singh AK, Savardekar L, Patil A, Surve S, Desai M. Increased frequency of Th17 cells and IL-17 levels are associated with low bone mineral density in postmenopausal women.
Sci Rep 11(1): 16155, 2021
[IF: 4.996]
6. Bhagwat S, Sontakke S, Desai S, Panchal D, Jadhav S, Parte P. N-Formyl L-Aspartate: a novel sperm chemoattractant identified in ovulatory phase oviductal fluid using a microfluidic chip.
Andrology 9: 1214-1226, 2021
[IF: 4.456]
7. Bhanothu V, Venkatesan V, Kondapi AK, Ajumeera R. Restrictions and supplementations effects of Vitamins B6, B9, and B12 on growth, vasculogenesis, and senescence of BG01V human embryonic stem cell-derived embryoid bodies.
Nutrition clinique et métabolisme 35: 297-316, 2021
[IF: 0.704]
8. Bhartiya D. Adult tissue-resident stem cells – fact or fiction?
Stem Cell Res Ther 12: 73, 2021
[IF: 8.079]
9. Bhartiya D. Testicular stem cells survive oncotherapy.
Reprod Sci 28: 1785-1787, 2021
[IF: 2.924]
10. Bhartiya D, Flora Y, Sharma D, Mohammad SA. Two stem cell populations including VSELs and CSCs detected in the pericardium of adult mouse heart.
Stem Cell Rev Rep 17: 685-693, 2021
[IF: 6.692]
11. Bhartiya D, Hinduja I. Fertility restoration in azoospermic cancer survivors from testicular VSELs that survive oncotherapy upon transplanting MSCs.
Hum Reprod Update 27(3): 619-620, 2021
[IF: 17.179]
12. Bhartiya D, Kaushik A. Testicular stem cell dysfunction due to environmental insults could be responsible for deteriorating reproductive health of men.

- Reprod Sci* 28: 649-658, 2021
[IF: 2.924]
13. Bhartiya D, Kaushik A, Singh P, Sharma D. Will single-cell RNAseq decipher stem cells biology in normal and cancerous tissues?
Hum Reprod Update 27: 421, 2021
[IF: 17.179]
 14. Bhartiya, D, Patel H, Kaushik A, Singh P, Sharma D. Endogenous, tissue-resident stem/progenitor cells in gonads and bone marrow express FSHR and respond to FSH via FSHR-3
J Ovarian Res 14: 145, 2021
[IF: 5.506]
 15. Bhartiya D, Patel H. An overview of FSH-FSHR biology and explaining the existing conundrums.
J Ovarian Res 14: 144, 2021
[IF: 5.506]
 16. Bhise S, Jain A, Savardekar L, Shetty NS, Shah I. Bone health in HIV-infected children on antiretroviral therapy: An Indian study.
Indian J Sex Transm Dis AIDS 42: 138-143, 2021
[IF: NA]
 17. Chaaithanya IK, Abnave D, Bawaskar H, Pachalkar U, Tarukar S, Salvi N, Bhoje P, Yadav A, Mahale SD, Gajbhiye RK. Perceptions, awareness on snakebite envenoming among the tribal community and health care providers of Dahanu block, Palghar District in Maharashtra, India.
PLoS One 16: e0255657, 2021
[IF: 3.752]
 18. Chaaithanya IK, Bhor V M. Fungal co-infections in pregnant women with COVID -19: A potential challenge?
J Assoc Physicians India 69: 11-12, 2021
[IF: NA]
 19. Chaaithanya IK, Kulkarni R, Salvi N, Nahire KA, Kantela NN, Patil S, Chauhan S. Sentinel Surveillance of Dengue Virus Infection in a Tribal District of Maharashtra, India.
J Assoc Physicians India 69(9): 11-12, 2021
[IF: NA]
 20. Chakma T, Thomas BE, Kohli S, Moral R, Menon GR, Periyasamy M, U Venkatesh , Kulkarni R, Prusty RK, Balu V, Grover A, Kishore J, Viray M, Venkateswaran C, Mathew G, Ketharam A, Balachandar R, Singh P K, Jakhar K, Singh S, Devi R, Saha KB, Barde P, Singh R, John D, Mishra BK, Yadav J, Agarwal S, Rao VV, Panda S. Psychosocial impact of COVID-19 pandemic on healthcare workers in India & their perceptions on the way forward-A qualitative study.
Indian J Med Res 153: 637-48, 2021
[IF: 5.274]
 21. Colaco S, Chhabria K, Singh D, Bhide A, Singh N, Singh A, Husein A, Mishra A, Sharma R, Ashary N, Modi D. Expression map of entry receptors and infectivity factors for pan-coronaviruses in preimplantation and implantation stage human embryos.
J Assist Reprod Genet 38: 1709-1720, 2021
[IF: 3.357]
 22. Dadachanji R, Patil A, Joshi B, Mukherjee S. Elucidating the impact of obesity on hormonal and metabolic perturbations in polycystic ovary syndrome phenotypes in Indian women.
PLoS One 16(2): e0246862, 2021
[IF: 3.752]
 23. Dadachanji R, Patil A, Mukherjee S. Investigating oxidized LDL receptor 1 (OLR1) polymorphisms as putative genetic markers for polycystic ovary syndrome.

Meta Gene 27: 100829, 2021

[IF: NA]

24. Dadachanji R, Sawant D, Patil A, Mukherjee S. Replication study of THADA rs13429458 variant with PCOS susceptibility and its related traits in Indian women.

Gynecol Endocrinol 37: 716-720, 2021

[IF: 2.277]

25. Damani M, Baxi K, Aranha C, Sawarkar S. Recent advances in herbal drug nanocarriers against cervical cancer.

Crit Rev Ther Drug Carrier Syst 38: 37-78, 2021

[IF: 4.889]

26. Deshpande SS, Nemani H, Balasinor NH. Diet-induced- and genetic-obesity differentially alters male germline histones.

Reproduction 162: 411-425, 2021

[IF: 3.923]

27. Dixit A, Nicole EJ, Ghule M, Battala M, Begum S, Yore J, Sagguriti N, Silverman J, Reed E, Benmarhnia T, Averbach S, Raj A. Male-Female concordance in reported involvement of women in contraceptive decision making and its association with modern contraceptive use among couples in rural Maharashtra, India.

Reproductive Health 18(1): 139, 2021

[IF: 3.355]

28. Durairajanayagam D, Singh D, Agarwal A, Henkel R. Causes and consequences of sperm mitochondrial dysfunction.

Andrologia 53(1): e13666, 2021

[IF: 2.532]

29. Ganie MA, Chowdhury S, Suri V, Joshi B, Bhattacharya PK, Agrawal S, Malhotra N, Sahay R, Rozati R, Jabbar PK, Sreenivas V, Sriwastva M, Wani IA, Singh S, Sharma RS. Evaluation of the prevalence, regional phenotypic variation, comorbidities, risk

factors, and variations in response to different therapeutic modalities among Indian Women: proposal for the Indian Council of Medical Research-Polycystic Ovary Syndrome (ICMR-PCOS) study.

JMIR Res Protoc 10(8): e23437, 2021

[IF: NA]

30. Gajbhiye RK, Mahajan NN, Kamath N, Bahirat S, Patokar G, Bhurke AV, Modi DN, Mahale SD. Clinical presentations, pregnancy complications, maternal outcomes in pregnant women with COVID-19 and tuberculosis: A retrospective cohort study.

Int J Gynecol Obstet 153: 176-179, 2021

[IF: 4.447]

31. Gajbhiye RK, Mahajan NN, Waghmare RB, Zala S, Chaaithanya IK, Kuppusamy P, Bhurke AV, Pious M, Surve S, Modi DN, Mahale SD and PregCovid Registry Network. Clinical characteristics, outcomes, & mortality in pregnant women with COVID-19 in Maharashtra, India: Results from PregCovid registry.

Indian J Med Res 153: 629-636, 2021

[IF: 5.274]

32. Gajbhiye RK, Montgomery G, Pai MV, Phukan P, Shekhar S, Padte K, DasMahapatra P, John BM, Shembekar C, Bhurke AV, Bagde N, Kulkarni K, Sardeshpande N, Humane A, Mahobia S, Shah M, Singh U, Srivastava A, Mishra G, Warty N, Chandra S, Mahale SD. Protocol for a case-control study investigating the clinical phenotypes and genetic regulation of endometriosis in Indian women: the ECGRI study.

BMJ Open 11: e050844, 2021

[IF: 3.006]

33. Gajbhiye RK, Sawant M S, Kuppusamy P, Surve S, Pasi A, Prusty RK, Mahale S D,

- Modi DN. Differential impact of COVID-19 in pregnant women from high-income countries and low-to middle-income countries: a systematic review and meta-analysis.
Int J Gynecol Obstet 155: 48-56, 2021
[IF: 4.447]
34. Gajbhiye RK, Tilve A, Kesarwani S, Srivastava S, Kore SJ, Patil K, Mahale SD, Mahajan NN. Increased rate of miscarriage during second wave of COVID-19 pandemic in India.
Ultrasound Obstet Gynecol 58: 946-949, 2021
[IF: 8.678]
35. Ganguly K, Kishore U, Madan T. Interplay between C-type lectin receptors and microRNAs in cellular homeostasis and immune response.
FEBS J 288: 4210-4229, 2021
[IF: 5.542]
36. Gogoi D, Biswas D, Borkakoty B, Dutta M. T cell responses in symptomatic moderate patients with pandemic 2009 H1N1 influenza A virus infection.
Acta Virol 65(3): 245-253, 2021
[IF: 1.82]
37. Gogoi D, Borkakoty B, Biswas D, Yadav K, Patel V. Characteristics of circulatory $\gamma\delta$ T cells in patients with symptomatic chronic hepatitis B infection.
Viral Immunol 34: 483-490, 2021
[IF: 2.175]
38. Gupta K, Modi D, Jain R, Dandekar P. A stable CHO K1 cell line for producing recombinant monoclonal antibody against TNF- α .
Mol Biotechnol 63: 9 828-839, 2021
[IF: 2.860]
39. Hasan AZ, Kumar MS, Prosperi C, Thangaraj JWV, R Sabarinathan, V Saravanakumar, Duraiswamy A, Kaduskar O, Bhatt V, Deshpande GRao, Padinjaremmattathil TU, Sapkal GN, Sangal L, Mehendale SM, Gupta N, Moss WJ, Hayford K, Murhekar MV, IMRVI group (Itta Krishna Chaaithanya). Implementing serosurveys in India: Experiences, lessons learned, and recommendations.
Am J Trop Med Hyg 105: 1608-1617, 2021
[IF: 3.707]
40. Hsieh MH, Beirag N, Murugaiah V, Chou YC, Kuo WS, Kao HF, Madan T, Kishore U, Wang JY. Human surfactant protein D binds spike protein and acts as an entry inhibitor of SARS-CoV-2 pseudotyped viral particles.
Front Immunol 12: 641360, 2021
[IF: 8.786]
41. Idicula-Thomas S, Gawde U, Jha P. Comparison of machine learning algorithms applied to symptoms to determine infectious causes of death in children: National survey of 18,000 verbal autopsies in the Million Death Study in India.
BMC Public Health 21: 1-11, 2021
[IF: 4.135]
42. Itta KC, Kulkarni R, Salvi N, Nahire KA, Kantela N, Patil S, Chauhan S. Sentinel surveillance of dengue virus infection in a tribal district of Maharashtra, India.
J Assoc Physicians India 69: 11-12, 2021
[IF: NA]
43. Jagtap D, Bhor V M, Bhowmick S, Kasarpalkar N, Sagvekar P, Kulkarni B, Pathak M, Chatterjee N, Dolas P, Palav H, Kaginkar S, Bhagat S, Munshi I, Parikh S, Agrawal S, Pawar C, Kaneria M, Mahale SD, Shastri J, Patel V. sMAdCAM: IL-6 ratio

- influences disease progression and anti-viral responses in SARS-CoV-2 infection.
Front Immunol 12: 619906, 2021
[IF: 8.786]
44. Jawade K, Sinha AY, Bhagat S, Bhowmick S, Chauhan B, Kaginkar S, Palav H, Kasarpalkar N, Devadiga P, Karandikar K, Agrawal S, Shastri J, Munne K, Bhor VM, Mahale SD, Bhowmik S, Jagtap D, Patel V. A novel ORF1a-based SARS-CoV-2 RT-PCR assay to resolve inconclusive samples.
Int J Infect Dis 106: 395-400, 2021
[IF: 12.074]
45. Joseph S, Mahale SD. Male Infertility Knowledgebase: decoding the genetic and disease landscape.
Database 2021: baab049, 2021
[IF: 4.462]
46. Joshi B, Girase B, Shetty S, Verma V, Acharya S, Deoraj P, Kulkarni R Begum S. Improving public health service delivery response to address contraceptive needs of socio-economically disadvantaged HIV positive people in Maharashtra, India.
Contracept Reprod Med 6(1): 14, 2021
[IF: NA]
47. Joshi B, Hasan G, Tyebkhan G, Udipi S, Patil A, Shah D, Patil M, Dalal R, Vaidya R. Knowledge and management practices about polycystic ovarian syndrome among health care providers in Mumbai, India: A cross-sectional study.
Indian Practitioner 74: 15-22, 2021
[IF: NA]
48. Joshi B, Moray KV, Sachin O, Chaurasia H, Begum S. Cost effectiveness of introducing etonorgestrel contraceptive implant into India's current family welfare programme.
Appl Health Econ Health Policy 19: 267-77, 2021
[IF: 3.686]
49. Joshi B, Shetty S, Moray K, Sachin O, Charasia H. Cost effectiveness of uterine balloon tamponade devices in managing atonic post-partum hemorrhage at public health facilities in India.
PLOS One 16: 1-23, 2021
[IF: 3.752]
50. Kaikini AA, Muke S, Peshattiwar V, Bagle S, Dighe V, Sathaye S. Ethyl ferulate, a lipophilic phenylpropanoid, prevents diabetes-associated renal injury in rats by amelioration of hyperglycemia-induced oxidative stress via activation of nuclear factor erythroid 2-related factor 2.
J Food Biochem 45: 4 e13607, 2021
[IF: 3.654]
51. Kamble PR, Patkar SR, Breed AA, Pathak BR. N-glycosylation status of Trop2 impacts its surface density, interaction with claudin-7 and exosomal release.
Arch Biochem Biophys 714: 109084, 2021
[IF: 4.114]
52. Kasarpalkar N, Bhowmick S, Patel V, Savardekar L, Agrawal S, Shastri J, Bhor V. Frequency of effector memory cells expressing integrin $\alpha 4\beta 7$ is associated with TGF- $\beta 1$ levels in therapy naïve HIV infected women with low CD4+ T cell count.
Front Immunol 12: 651122, 2021
[IF: 8.786]
53. Kashte S, Dhumal R, Chaudhary P, Sharma RK, Dighe V, Kadam S. Bone regeneration in critical-size calvarial defect using functional biocompatible osteoinductive herbal scaffolds and human umbilical cord Wharton's Jelly-derived mesenchymal stem cells.
Mat Tod Commun 26: 102049, 2021
[IF: 3.662]

54. Khambata K, Modi DN, Gupta SK. Immunoregulation in the testis and its implication in fertility and infections. *Explor Immunol* 1: 309-324, 2021
[IF: NA]
55. Khambata K, Raut S, Deshpande S, Mohan S, Sonawane S, Gaonkar R, Ansari Z, Datar M, Bansal V, Patil A, Warke H, Balasinor NH. DNA methylation defects in spermatozoa of male partners from couples experiencing recurrent pregnancy loss. *Hum Reprod* 36(1): 48-60, 2021
[IF: 6.353]
56. Khan SA, Kulkarni VR, Shah RS, Gokral JS, Meherji PK, Vadigoppula AD, Patil AD, Bhurke AV, Kokate PP, Barai RS, Surve SR, Modi DN, Mahale SD, Gajbhiye RK. Clinical presentations and semen characteristics of men attending the secondary referral infertility clinic at Mumbai, India. *J Human Reprod Sci* 14(4): 356-364, 2021
[IF: NA]
57. Kulkarni R, Begum S. A critical appraisal of NFHS-5 data on maternal health indicators: trends and implications for India. *Indian Practitioner* 74: 23-28, 2021
[IF: NA]
58. Kulkarni R, Kshirsagar H, Begum S, Patil, A, Chauhan S. Maternal near miss events in India. *Indian J Med Res* 154: 573-82, 2021
[IF: 5.274]
59. Kumar A, Dhillon A, Manjegowda MC, Singh N, Mary DJJ, Kumar S, Modi D, Limaye AM. Estrogen suppresses HOXB2 expression via ER α in breast cancer cells. *Gene* 794: 145746, 2021
[IF: 3.913]
60. Kumari A, Tripathi AH, Gautam P, Gahtori R, Pande A, Singh Y, Madan T, Upadhyay SK. Adhesins in the virulence of opportunistic fungal pathogens of human. *Mycology* 12: 296-324, 2021
[IF: NA]
61. Kumari S, Bhor V M. Association of cervicovaginal dysbiosis mediated HPV infection with cervical intraepithelial neoplasia. *Microb Pathog* 152: 104780, 2021
[IF: 3.848]
62. Kushawaha B, Yadav RS, Swain DK, Kumari P, Kumar A, Yadav B, Anand M, Yadav S, Singh D and Garg SK. Collapsed mitochondrial cristae in goat spermatozoa due to mercury result in lethality and compromised motility along with altered kinematic patterns. *Sci Rep* 11: 646, 2021
[IF: 4.996]
63. Madan T, Biswas B, Varghese PM, Subedi R, Pandit H, Idicula-Thomas S, Kundu I, Rooge S, Agarwal R, Tripathi DM, Kaur S, Gupta e, Gupta SK, Kishore U. A recombinant fragment of human surfactant protein D binds Spike protein and inhibits infectivity and replication of SARS-CoV-2 in clinical samples. *Am J Respir Cell Mol Biol* 65: 41-53, 2021
[IF: 7.748]
64. Mahajan N, Pophalkar M, Patil S, Yewale B, Chaaithanya IK, Mahale SD, Gajbhiye RK. Pregnancy outcomes and maternal complications during the second wave of coronavirus disease 2019 (COVID-19) in India. *Obstet Gynecol* 138: 660-662, 2021
[IF: 7.623]

65. Mahajan NN, Gaikwad C, Tayshete R, Saldanha C, Pednekar R, Mahale SD, Gajbhiye RK. Increased stillbirth rate during the second wave of COVID-19 pandemic in India.
Eur J Obstet Gynecol Reprod Biol 266: 191-192, 2021 [IF: 2.831]
66. Mahajan NN, Gajbhiye RK, Bahirat S, Lokhande PD, Mathe A, Rath S, Warty N, Mahajan KN, Srivastava V, Kuppusamy P, Mohite SC. Co-infection of malaria and early clearance of SARS-CoV-2 in healthcare workers.
J Med Virol 93: 2431-2438, 2021 [IF: 20.693]
67. Mahajan NN, Gajbhiye RK, Lokhande PD, Bahirat S, Modi D, Mathe AM, Bharmal R, Rath S, Mohite SC, Tilve A. Clinical presentation of cases with SARS-CoV-2 reinfection/ reactivation.
J Assoc Physicians India. 69: 16-18, 2021 [IF: NA]
68. Mahajan NN, Gajbhiye RK, Pednekar RR, Pophalkar MP, Kesarwani SN, Bhurke AV, Mahale SD. Delirium in a pregnant woman with SARS-CoV-2 infection in India.
Asian J Psychiatr 55: 102513, 2021 [IF: 13.89]
69. Mahajan NN, Pophalkar M, Patil S, Yewale B, Chaaithanya IK, Mahale SD, Gajbhiye RK. Pregnancy outcomes and maternal complications during the second wave of coronavirus disease 2019 (COVID-19) in India.
Obstet Gynecol 138: 660-662, 2021 [IF: 7.611]
70. Mahajan NN, Srivastava S, Chakor R, More P, Mahale SD, Gajbhiye RK. Neurological complications of COVID-19 and spontaneous abortion in a pregnant woman - A case report.
Eur J Obstet Gynecol Reprod Biol 263: 278-279, 2021 [IF: 2.831]
71. Malik S, Surve S, Wade P, Kondekar S, Sawant V, Shaikh M, Kuppusamy P, Bharmal R, Mahale SD, Modi DN, Gajbhiye RK. Clinical characteristics, management, and short term outcome of neonates born to mothers with COVID-19 in a tertiary care hospital in India.
J Tropical Pediatr 67:fma054, 2021 [IF: 1.794]
72. Midha IK, Kumar N, Kumar A, Madan T. Mega doses of retinol: A possible immunomodulation in Covid-19 illness in resource-limited settings.
Rev Med Virol 31 : 1-14, 2021 [IF: 11.043]
73. Mishra A, Ashary N, Sharma R, Modi D. Extracellular vesicles in embryo implantation and disorders of the endometrium.
Am J Reprod Immunol 85(2): e13360, 2021 [IF: 3.777]
74. Moray KV, Chaurasia H, Sachin O, Joshi B. A systematic review on clinical effectiveness, side-effect profile and meta-analysis on continuation rate of etonogestral contraceptive implant.
Reprod Health 18: 1-24, 2021 [IF: 3.355]
75. Mukherjee S, Kundu I, Askari M, Barai RS, Venkatesh KV, Idicula-Thomas S. Exploring the druggable proteome of Candida species through comprehensive computational analysis.
Genomics 113: 728-739, 2021 [IF: 4.31]

76. Munne K, Bhanothu V, Bhor V M, Patel V, Mahale SD, Pande S. Detection of SARS-CoV-2 infection by RT-PCR test: factors influencing interpretation of results. *Virusdisease* 32: 187-189, 2021
[IF: NA]
77. Munne K, Bhanothu V, Mayekar A, Birje S, Bhor V M, Patel V, Mahale SD, Pande SS. A retrospective analysis of COVID-19 diagnosis results obtained by rapid antigen tests and RT-PCR: Implications for disease management. *Indian J Med Microbiol* 39: 537-539, 2021
[IF: 1.347]
78. Munshi I, Khandvilkar A, Chavan SM, Sachdeva G, Mahale SD, Chaudhari UK. An overview of preclinical animal models for SARS-CoV-2 pathogenicity. *Indian J Med Res* 153: 17-25, 2021
[IF: 5.274]
79. Naigaonkar A, Dadachanji R, Hinduja I, Mukherjee S. Altered redox status may contribute to aberrant folliculogenesis and poor reproductive outcomes in women with polycystic ovary syndrome. *J Assist Reprod Genet* 38: 2609-2623, 2021
[IF: 3.357]
80. Nair S, Hatkar S, Patil A, Surve S, Joshi B, Balasubramanian N, Desai M. Age-related changes and reference intervals of RANKL, OPG, and bone turnover markers in Indian women. *Arch Osteoporos* 16(1): 146, 2021
[IF: 2.879]
81. Narayanasamy NS, Das A, Narayanasamy A, Prusty RK. Self-reported health status and its determinants among elderly widows in Puducherry, India. *Indian J Community Med* 46: 4 606-9, 2021
[IF: NA]
82. Pandey N, Maske P, Mote C, Dighe V. Exposure to Atrazine through gestation and lactation period led to impaired sexual maturation and subfertility in F1 male rats with congenital deformities in F2 progeny. *Food Chem Toxicol* 157: 112586, 2021
[IF: 5.572]
83. Patankar A, Gajbhiye RK, Surve S, Parte P. Epigenetic landscape of testis specific histone H2B variant and its influence on sperm function. *Clin Epigenet.* 13: 1-8, 2021
[IF: 7.259]
84. Patil K, Hinduja I, Mukherjee S. Alteration in angiogenic potential of granulosa-lutein cells and follicular fluid contributes to luteal defects in polycystic ovary syndrome. *Hum Reprod* 36: 1052-1064, 2021
[IF: 6.353]
85. Pednekar R, Gaikwad C, More P, Pophalkar M, Kesarwani S, Jnanananda B, Mahale SD, Gajbhiye RK, Mahajan NN. Impact of both the waves of COVID-19 pandemic on ectopic pregnancy in India. *J South Asian Feder Obs Gynae* 13: 403-406, 2021
[IF: NA]
86. Phanindranath R, Sudhakar DVS, Thangaraj K, Sharma Y. Conformational scanning of individual EF-hand motifs of calcium sensor protein centrin-1. *Biochem Biophys Res Commun* 570: 67-73, 2021
[IF: 3.322]
87. Prabhu VM, Padwal V, Velhal S, Salwe S, Nagar V, Patil P, Bandivdekar AH, Patel V. Vaginal epithelium transiently harbours HIV-1 facilitating transmission. *Front Cell Infect Microbiol* 11: 634647, 2021
[IF: 6.073]

88. Prabhudesai K S, Aathi M S, Dighe V, Idicula-Thomas S. A 5-mer peptide derived from hinge region of hFSHR can function as positive allosteric modulator *in vivo*.
Biochim Biophys Acta Biomembr 1863: 183492, 2021 [IF: 4.019]
89. Prabhudesai KS, Raje S, Desai K, Modi D, Dighe V, Contini A, Idicula-Thomas S. Central residues of FSH β (89-97) peptide are not critical for FSHR binding: Implications for peptidomimetic design.
Bioorg Med Chem Lett 15: 44 128132, 2021 [IF: 2.94]
90. Prosperi C, Kaduskar O, Bhatt V, Hasan AZ, Thangaraj JWV, Kumar MS, R Sabarinathan, Kumar S, Duraiswamy A, Deshpande GR, Thankappan UP, Chauhan SL, Kulkarni R, Bansal AK, Chaaithanya IK, Salvi NR, Sharma S, Moss WJ, Sangal L, Gupta N, Murherkar MV, Mehendale SM, Sapkal GN, Hayford K. Diagnostic accuracy of dried blood spots collected on HemaSpot HF devices compared to venous blood specimens to estimate measles and rubella seroprevalence.
mSphere 6(4): e0133020, 2021 [IF: NA]
91. Prusty R, Begum S. Missing men in family planning: understanding the socio-spatial differentials in male sterilization and male spacing methods of contraception in India.
J Biosoc Sci 1-15, 2021 [IF: 2.148]
92. Prusty R, Begum S, Patil A, Naik DD, Pimple S, Mishra G. Increasing breast cancer awareness and breast examination practices among women through health education and capacity building of primary healthcare providers: a pre-post intervention study in low socioeconomic area of Mumbai, India.
BMJ Open 11: 4 e045424, 2021 [IF: 3.006]
93. Raut S, Kumar AV, Deshpande S, Khambata K, Balasinor NH. Sex hormones regulate lipid metabolism in adult Sertoli cells: A genome-wide study of estrogen and androgen receptor binding sites.
J Steroid Biochem Mol Biol 211: 105898, 2021 [IF: 5.011]
94. Sane M, Dighe V, Patil R, Hassan PA, Gawali S, Patravale V. Bivalirudin and sirolimus co-eluting coronary stent: Potential strategy for the prevention of stent thrombosis and restenosis.
Int J Pharm 1: 600 120403, 2021 [IF: 6.510]
95. Shaikh N, Dadachanji R, Patil A, Mukherjee S. K121Q polymorphism in the ENPP1 gene shows association with obesity in Indian women with PCOS.
Polymorphism 7: 36-51, 2021 [IF: NA]
96. Sharma D, Bhartiya D. Stem cells in adult mice ovaries form germ cell nests, undergo meiosis, neo-oogenesis and follicle assembly on regular basis during estrus cycle.
Stem Cell Rev Rep 17: 1695–1711, 2021 [IF: 6.692]
97. Sharma M, Kundu I, Barai R. S, Bhaye S, Desai K, Pokar K, Idicula-Thomas S. Enrichment analyses of diseases and pathways associated with precocious puberty using PrecocityDB.
Sci Rep 11: 4203, 2021 [IF: 4.996]
98. Shende P, Gaikwad P, Gandhewar M, Ukey P, Bhide A, Patel V, Bhagat S, Bhor V, Mahale S, Gajbhiye R, Modi D. Persistence of SARS-CoV-2 in the first trimester placenta

- leading to transplacental transmission and fetal demise from an asymptomatic mother.
Hum Reprod 36: 899-906, 2021
[IF: 6.353]
99. Shetty SS, Moray KV, Chaurasia H, Joshi B. Cost of managing atonic postpartum haemorrhage with uterine balloon tamponade devices in public health settings of Maharashtra, India: an economic microcosting study.
BMJ Open 11: 1-10, 2021
[IF: 3.006]
100. Shishpal P, Patel V, Singh D, Bhor V M. pH stress mediated alteration in protein composition and reduction in cytotoxic potential of *Gardnerella vaginalis* membrane vesicles.
Front Microbiol 12: 723909, 2021
[IF: 6.064]
101. Shukla P, Singh KK. The mitochondrial landscape of ovarian cancer: Emerging insights.
Carcinogenesis 42: 663-661, 2021
[IF: 4.741]
102. Shukla P, Singh KK. Uncovering mitochondrial determinants of racial disparities in ovarian cancer.
Trends Cancer 7: 93-97, 2021
[IF: 19.161]
103. Singh I, Parte P. Heterogeneity in the epigenetic landscape of murine Testis Specific histone variants TH2A and TH2B sharing the same bi-directional promoter.
Front Cell Dev Biol 9: 755751, 2021
[IF: 6.081]
104. Sudhakar DVS, Shah R, Gajbhiye RK. Genetics of male infertility - present and future: A narrative review.
J Hum Reprod Sci 14: 3 217-227, 2021
[IF: NA]
105. Surve S, Begum S, Chauhan S, Khatkhatay MI, Joshi B. Determinants of vitamin D deficiency among under-five children in urban slums of Mumbai, India.
Indian Pediatr 58: 888-889, 2021
[IF: 3.839]
106. Surve S, Bhor V M, Naukariya K, Begum S, Munne K, Tipre P, Sutar N, Jaiswal A, Bhonde G, Chauhan S, Shah I. Discordance between TST and QFT-TBGold Plus for latent tuberculosis screening among under-five children: An interim analysis.
J Trop Pediatr 67: fma103, 2021
[IF: 1.794]
107. Surve S, Naukariya KR, Begum S, Shah I, Bhor V, Munne K, Jaiswal AA, Tipre PM, Sutar N, Dalvi RR, Tryambake VH, Kamat SS, Shikhare MK, Kamble RS, Chauhan SL. Impact of COVID-19 in implementing community research on Latent TB among pediatric population: challenges and way forward.
Indian J Comm Med 46: 760-71, 2021
[IF: NA]
108. Sutar J, Deshpande S, Mullick R, Hingankar N, Patel V, Bhattacharya J. Geospatial HIV-1 subtype C gp120 sequence diversity and its predicted impact on broadly neutralizing antibody sensitivity.
PLoS One 16: e0251969, 2021
[IF: 3.752]
109. Tahseen SS, Balasinor NH, Nishi K. Diverse role of endocannabinoid system in mammalian male reproduction.
Life Sci 286: 120035, 2021
[IF: 6.78]

110. Thakur G, Sathe G, Kundu I, Biswas B, Gautam P, Alkahtani S, Idicula-Thomas S, Sirdeshmukh R, Kishore U, Madan T. Membrane interactome of a recombinant fragment of human surfactant protein D reveals GRP78 as a novel binding partner in PC3, a metastatic prostate cancer cell line. *Front Immunol* 11: 600660, 2021

[IF: 8.786]

111. Tilve A, Mahajan NN, Pandey A, Jnanananda B, Gadekar S, Mahale SD, Gajbhiye RK. Impact of COVID-19 on pregnant women with rheumatic heart disease or peripartum cardiomyopathy. *Eur J Obstet Gynecol Reprod Biol* 258: 459-461, 2021

[IF: 2.831]

112. Tiwari A, Ashary N, Singh N, Sharma S, Modi D. Modulation of E-Cadherin and N-Cadherin by ovarian steroids and embryonic stimuli. *Tissue Cell* 73: 101670, 2021

[IF: 2.586]

113. Tyebkhan G, Joshi B, Vaidya R. ABHIYAAN PCOS, a consortium for multidisciplinary strategy for management. *Indian Practitioner* 74: 7 41-45, 2021

[IF: NA]

114. Varghese PM, Mukherjee S, Al-Mohanna FA, Saleh SM, Almajhdi FN, Beirag N, Alkahtani SH, Rajkumari R, Nal Rogier B, Sim RB, Idicula-Thomas S, Madan T, Murugaiah V, Kishore U. Human properdin released by infiltrating neutrophils can modulate Influenza A virus infection. *Front Immunol* 12: 747654, 2021

[IF: 8.786]

115. Verma, P, Parte P. Revisiting the characteristics of testicular germ cell lines GC-1(spg) and GC-2(spd)ts.

Mol Biotechnol 63: 941-952, 2021

[IF: 2.860]

116. Waghmare R, Gajbhiye RK, Mahajan NN, Modi D, Mukherjee S, Mahale SD. Universal screening identifies asymptomatic carriers of SARS-CoV-2 among pregnant women in India.

Eur J Obstet Gynecol Reprod Biol 256: 503-505, 2021

[IF: 2.831]

12.2 Books and Book Chapters

1. Balasinor N, Parte P, Singh D (eds). Translational epigenetics: Epigenetics and reproductive health, Vol. 21, Elsevier - Academic Press, 2021
2. Kale K, Singh I, Kishore U, Madan T. Collectins in regulation of feto-maternal cross-talk. *In: The collectin protein family and its multiple biological activities*, Springer Nature, Switzerland, pp. 185-206, 2021
3. Kishore U, Madan T, Sim RB (eds). The collectin protein family and its multiple biological activities. Springer Nature, Switzerland, pp. 1-244, 2021
4. Murugaiah V, Yasmin H, Pandit H, Ganguly K, Subedi R, Al-Mozaini M, Madan T, Kishore U. Innate immune response against HIV-1. *In: Microbial Pathogenesis*, Springer Nature, Switzerland pp. 23-58, 2021
5. Pande S. Clinical and Laboratory Approach-Chapter on genetics and genetic Counseling in male and female infertility. *In: Genetic counseling*, Jaypee publications, New Delhi, pp. 368-375, 2021
6. Rokade S, Kishore U, Madan T. Testicular immune privilege: a recently discovered

domain of collectins. *In: The collectin protein family and its multiple biological activities*, Springer Nature, Switzerland, pp. 165-184, 2021

7. **Shekhar BR, Das DK.** Induced pluripotent stem cells for modeling of Rett Syndrome. *In: iPSCs for modeling central nervous system disorders*, Elsevier - Academic Press, United Kingdom, pp. 117-216, 2021
8. **Singh I, Patankar AG, Parte P.** Chromatin remodeling of the male genome during spermiogenesis and embryo development. *In: Translational epigenetics: Epigenetics and reproductive health*, Vol. 21, Elsevier - Academic Press, pp. 47-67, 2021
9. **Thakur G, Mahajan L, Kaur A, Bulla R, Kishore U, Madan T.** Surfactant protein D in immune surveillance against cancer. *In: The collectin protein family and its multiple biological activities*, Springer Nature, Switzerland, pp. 147-164, 2021

12.3 Papers / Posters presented at Conference / Symposia

International

1. **10th Annual Meeting of International Society for Extracellular Vesicles**, Virtual, May 18-21, 2021
 - **Shishpal P, Patel V, Bhor V M.** Flow cytometry based approach to study the stress induced modulation of bacterial membrane vesicles.
2. **Keystone eSymposia on HIV Vaccines and HIV Pathogenesis and Cure 2021**, Silverthorne, Virtual, June 1-4, 2021
 - **Singh AK, Padwal V, Palav H, Velhal S, Nagar V, Patil P, Patel V.** Non-cytotoxic signatures dominate HIV specific T cell responses in viremic non-progression.
3. **The 39th Annual Meeting of the Obesity Society at Obesityweek, Virtual, November 1-5, 2021**
 - **Hansen BC, Chaudhari UK.** Determinants of anemia in aging obese nonhuman primates with diet and environment held constant.
4. **Virtual Keystone eSymposia - Global Priorities in Vaginal Health: Microbes, Mucosal Immunity and Interventions**, Virtual, November 10-12, 2021
 - **Aranha C, Pawar K.** Lactobacillus derived metabolites as biotherapeutics for cervical cancer.
 - **Madan T.** Distinct impact of TLR agonists on vaginal inflammation and HIV-1 infectivity: Host-protective modulation by human surfactant protein D.
5. **International Conference on Mechanistic and Therapeutic Approaches in Human and Animal Health**, Virtual, December 6-8, 2021
 - **Madan T.** Role of collectins in human health.
6. **International Conference on Reproductive Healthcare and the 32nd Annual Meeting of the Indian Society for the Study of Reproduction and Fertility-ISSRF 2022**, Virtual, February 11-13, 2022
 - **Arya D, Balasinor N, Pawar P, Gajbhiye R, Tandon D, Singh D.** Sperm mitochondrial functions and mitochondrial DNA methylation status in infertile men with clinical varicocele.
 - **Irani D, Balasinor N, Tandon D, Bansal, Patil A and Singh D.** Whole genome bisulfite sequencing of sperm reveals differentially methylated regions in male partners of idiopathic recurrent pregnancy loss cases.

- **Jondhale M, Sikchi A, Modi D, Mukherjee N.** Deciphering the role of trophoblast cells on breast epithelial cell phenotype: relevance in pregnancy associated breast cancer (PABC).
 - **Madan T.** Soluble Pattern Recognition Collectins are integral to human reproductive.
 - **Patil K, Joseph S, Shah J, Mukherjee S.** Identification of miRNAs regulating angiogenesis in women with polycystic ovary syndrome.
7. **Virtual Conference on Retroviruses and Opportunistic Infections (CROI) 2022, Virtual, February 12-24, 2022**
- **Kasarpalkar N J, Bhowmick S, Patel V, Savardekar L, Agrawal S , Shastri J , Bhor V. M.** Vaginal Dysbiosis associated modulation of peripheral immune cell subsets.
 - **Singh A K, Padwal V, Palav H, Velhal S, Nagar V, Patil P, Patel V.** Highly dampened HIV-specific cytolytic t cell

response define viremic non-progression.

National

8. **47th Annual Conference of the Indian Immunology Society, Virtual, December 18-19, 2021**
- **Madan T.** Innate immune sensing of coronavirus: A driver to pathogenesis or defense?
9. **38th National Conference on Reproduction and Endocrinology, SRBCE (Society for Reproductive Biology and Comparative Endocrinology), Mysore, December 28-30, 2021**
- **Biswas B.** Cross talk of Sertoli cells and N-glycosylation deficient germ cells - Impact on male fertility.
 - **Naigaonkar A, Mukherjee S, Hinduja I.** Unraveling the glucose metabolism dynamics in follicular environment of women with PCOS.

13. CAPACITY BUILDING

13.1 Conferences / Seminars/ Workshops / Training Programs conducted by Scientists

- **Balasinor NH.** Lecture workshop on reproductive health as a part of SERB Scientific Social Responsibility (SSR), December 22, 2021
- **Bhor V, Patel V, Pathak B.** DHR sponsored training on Applications of Technologies in Genomics for Clinical Research (ATGC), Virtual, May 3-28, 2021
- **Bhor V.** Training program on Good Clinical Practices (GCP) and Standard Operating Procedures (SOP) in clinical research, February 28 - March 1, 2022
- **Bhor V.** Lecture on 'Health, hygiene and immunity', Virtual, April 8, 2021
- **Chaudhari U.** International e-symposium on 'Pregnancy leaves its marks', September 24, 2021
- **Chaudhari U.** Talk on the occasion of World Health Day on 'Iron: The red-carpet nutrient for women's health', Virtual, April 7, 2021
- **Chaudhari U.** Lecture during World Immunization Week on 'COVID-19 Vaccines: Are these Magic Bullets?', Virtual, April 26, 2021
- **Gajbhiye R.** Virtual training of DBT Wellcome trust project staff carried out monthly
- **Gajbhiye R.** Virtual training of PregCovid Registry team at study sites regarding online data entry mistakes and quality controls carried out monthly
- **Gogoi D.** Application of flow-cytometry in health sciences, Virtual, November 1, 2021

- **Gupta SM.** Breast cancer awareness talk by Dr Jay Anam, Surgical Oncologist, October 22, 2021
- **Joshi B.** Prof PK Devi Memorial Oration by Dr Rajesh Kotecha, Virtual, January 27, 2022
- **Kaur P.** Webinar on 'Introduction to The Journal of Visualized Experiments (JoVE)', Virtual, December 21, 2021
- **Madan T, Gupta S.** World Immunology Day, Virtual, May 5, 2021
- **Madan T.** Lecture on 'TissueFAXS: in-situ flow cytometry in the context of a tissue section' by Dr Amit K Bhattacharya, TissueGnostics Asia Pacific Ltd., October 19, 2021
- **Modi D.** Frontier in clinical application of single cell genomics, Virtual, May 21, 2021
- **Pande S.** DHR training course on 'Medical Genetics', Virtual, July 5-30, 2021
- **Pande S.** Symposium on 'Principles and practice of prenatal diagnosis' Virtual, July 29, 2021
- **Thomas S.** DBT sponsored workshop on 'Orientation to online database and algorithms for biologists', Virtual, September 30 - October 1, 2021

13.2 Community Outreach Activities

- **Savardekar L.** Community based retinopathy screening camp for diabetes patients in collaboration with Dr Saroj Sahadev, Volk Optical Inc. USA, on the occasion of World Diabetes Day, Bone Health Clinic, Naigaon, November 30, 2021
- **Savardekar L.** Drawing and slogan competition on the occasion of

International Women's Day on March 8, 2022

- **Savardekar L.** Weekly IEC lecture series in collaboration with D B Kulkarni Vidyalaya, Naigaon, Kamgar Kalyan Mandal, Naigaon, Rehab Team of India at Bone Health Clinic, Naigaon, Virtual, March 2022
 - 'Take care of spine care for trouble free back' by Dr Ekta Singhal, Physiotherapist, Virtual, March 8, 2022
 - 'Knee exercises for strong and healthy joints' by Rehab Team of India, Virtual, March 15, 2022
 - Workplace posture related guidance by Rehab Team of India, Virtual, March 22, 2022
 - Sarcopenia: Building muscles by exercise for better quality of life by Dr Shiwani Redij, Virtual, March 29, 2022
- **Surve S.** A talk to celebrate Children's Day on 'Awareness and importance of healthy diet and physical activity among children in the era of virtual schooling', Child Health Clinic, November 17, 2021
- **Surve S.** Sensitization for mothers of school students on educating girls & boys about pubertal changes among children & menstruation, Family Welfare Clinic, March 8, 2022
- **Surve S.** Lecture on 'Cervical cancer awareness and screening', Family Welfare Clinic, March 8, 2022

13.3 Meetings / Conferences / Seminars / Workshops Attended

- **Aranha C.** Publishing Webinar on Tips from the Editors, Virtual, March 30, 2021
- **Aranha C.** Online Meeting on Pre and Probiotics: Current Trends and Future Scopes (PROBIO-07), January 18-19, 2021

- **Balasinor N.** 'Placental-endometrial dialogue and pregnancy', Centre for Trophoblast Research Annual Meeting, UK, Virtual, July 12, 2021
- **Balasinor N.** Paternal epigenetic factors in pregnancy outcome, International Conference on Reproductive Healthcare and 32nd Annual Meeting, Indian Society for the Study of Reproduction and Fertility, Virtual, February 11-13, 2022
- **Bhanothu V.** Workshop on Next Generation Sequencing Data Analysis for Clinical Diagnostics, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, October 25-29, 2021
- **Bhanothu V.** Workshop on Protein Purification, Crystallization, and Structure determination, Workshop, ACTREC, TMC Navi-Mumbai and Department of Botany, University of Kashmir, Srinagar, December 6, 2021
- **Bhor V.** 'Authorship and predatory journals', webinar series, 'Dissertation to Publication', T N Medical College and BYL Nair Hospital, Mumbai, Virtual, July 17, 2021
- **Bhor V.** 13th Annual Conference of TCS (The Cytometry Society): Virtual meeting, PGIMER, Chandigarh, Virtual, October 29, 2021
- **Dadachanji R.** 'Elucidating the phenotypic and genetic profile of PCOS in Indian women', 6th Annual Conference of The PCOS Society, Virtual, December 28-30, 2021
- **Gajbhiye R.** Endometriosis Panel discussion, The George Institute for Global Health, India, Virtual, December 2, 2021
- **Joshi B, Surve S, Tandon D.** Meeting with TB Unit of Dadra and Nagar Haveli team for Mega TB project, Virtual, December 26, 2021

- **Kulkarni R.** Sickle cell Disease: Global Perspective and Indian Scenario, webinar, organized by NIRTH, Jabalpur, June 19, 2021
- **Kulkarni R.** Clinical Trial Registry, India, webinar, organized by NIMS, New Delhi, August 24, 2021
- **Kulkarni R.** COVID Summit-A comprehensive webinar on COVID by experts: Current Scenario and Future preparedness, webinar organized by Maharashtra Medical Council, Mumbai, May 29-30, 2021
- **Kulkarni R.** Temporary Childbirth Migration (TCM), Panel Discussion, KEM Hospital Pune, Vadu Rural Health Program, KEM Hospital Research Centre, Pune, October 21, 2021
- **Kumari N.** NGS and Other Large Scale Biological Data Analysis, Shodhaka Life Sciences Pvt. Ltd., Bengaluru, Virtual, June 5-19, 2021
- **Kumari N.** Online Short-term Course on 'Biostatistics: A User's Perspective', IISER, Pune, January 8-16, 2022
- **Kumari N.** Virtual annual meeting on Placental-Endometrial Dialogue and Pregnancy, Centre for Trophoblast Research, University of Cambridge, UK, July 12-13, 2021
- **Kumari N.** Webinar on 'Impact of oxidative stress on male and female reproduction', ICAR-NDRI, Haryana, Virtual, July 1, 2021
- **Kumari N.** Webinar on 'Taming the beast of inflammation: COVID-19', Tata Memorial Centre and Mumbai Immunology Group, ACTREC, Navi Mumbai, April 29, 2021
- **Kuppusamy P.** Statistical methods in clinical trials, NIMS, Virtual, March 7-8, 2022
- **Kuppusamy P.** Training on Biostatistics: A user's perspective conducted by IISER-Pune, Virtual, January 8-16, 2022
- **Kuppusamy P.** Webinar on spreadsheet data management: simple, convenient yet powerful, NIOH, Virtual, March 9, 2022
- **Khambata K.** Paternal factors contributing to recurrent pregnancy loss: Investigating the sperm DNA methylation, European Gynecology and Obstetrics Congress, Virtual, September 20, 2021
- **Munne K.** 11th National Conference on One Health Initiative on the prevention and control of emerging and re-emerging zoonotic diseases, Virtual, June 6, 2021
- **Munne K.** The current scenario & future preparedness, Maharashtra Medical Council, Webinar on MMC COVID SUMMIT, Virtual, May 29, 2021
- **Munne K, Kamat S, Tryambake V.** Cervical cancer screening awareness activity for Akanksha School helper staff, October 23, 2021
- **Munne K, Kamat S, Tryambake V.** Health talk and cervical cancer awareness activity by F-South Health Post Staff with CDO Mrs. Shaila and Dr Avantika in Goutam Nagar, Dadar, January 20, 2022
- **Munne K, Trambake V, Kamat S, Dalvi R.** International Women's Day 2022 activity for mothers at Akanksha School, March 8, 2022
- **Munne K, Tryambake V, Kamat S.** Cervical cancer awareness activity conducted by F-South Health Post Staff with CHV, February 14, 2022
- **Munne K.** Regional webinar to accelerate progress towards elimination of cervical cancer as a public health

problem, Webinar, ICMR-NIRRH, Mumbai, September 15, 2021

- **Munne K.** WHO Regional webinar on transition to HPV DNA test as a primary screening test for cervical cancer elimination to achieve the 2030 interim targets, Webinar, Virtual, February 8, 2022
- **Pande S, Bhanothu V, Joseph S.** Workshop on Biotech PRIDE guidelines of DBT for deposition of sequencing data generated under the Mission project, CDFD and Indian Biological Data Center, Regional Center of Biotechnology, Hyderabad, March 2, 2022
- **Pande S, Bhanothu V.** A talk on building a resilient biomedical disaster response: learning from the COVID-19 pandemic, ICMR, Delhi & World Congress on Disaster Management, November 24, 2021
- **Pande S, Bhanothu V.** Webinar on 'Pathbreaking contribution of ICMR-NIV in fighting COVID-19', National Institute of Virology (NIV), Pune, April 1, 2021
- **Pande S, Bhanothu V.** Workshop on launch of the policy brief for India 2021, Workshop, ICMR, Delhi, November 24, 2021
- **Pande S.** Webinar on Covid 19 and human health, ICMR-NIOH, Ahmedabad, February 15, 2022
- **Patel V.** An Integrated approach towards delineating determinants of viral pathology and disease progression of SARS-CoV-2 infection, Centenary Year celebration of TN Medical College & BYL Nair hospital, Nair Hospital, Mumbai, August 12, 2021
- **Prusty R.** Demography and Population Studies (For MPH students), Lecture (14

sessions), Virtual, ICMR-RMRCB, July 24-August 13, 2021

- **Prusty R.** Quantitative research methodology for PhD students, Lecture (4 sessions), Virtual, NID Ahmedabad, July 19-22, 2021
- **Savardekar L, Mehta N.** 'Methods and Approaches for Health Research in Social Sciences' ICMR-NIRTH, Jabalpur, Virtual, June 26-27, 2021
- **Savardekar L, Mehta N.** Webinar on standardization, regulation and testing of the contraceptive devices, organized by Bureau of Indian Standards, Virtual, March 11, 2022
- **Savardekar L.** Randomized controlled trials organized by St. John's Medical College & Research Institute and McMaster University, Canada, Duke University, USA, Virtual, September 20-24, 2021
- **Savardekar L.** 'Reversal of lactation associated bone loss in women belonging to low socio-economic strata: A pilot study', World Breastfeeding Week celebrations conducted by AYUSH CoE, Interdisciplinary School of Health Sciences, Pune, August 7, 2021
- **Savardekar L.** 'Roles and responsibilities of the institute and investigators in clinical research' organized by Amrita School of Dentistry, Amrita Vishwa Vidyapeetham, Kerala, Virtual, March 9, 2022
- **Savardekar L.** World Diabetes Day CME organized by Department of Mind Body Medicine and Lifestyle Sciences, SRIHS, Chennai, Virtual, November 13, 2021
- **Savardekar L.** CME on UTIs and Treatment, MS Ramaiah University of Applied Sciences, Bangalore & Docplexus, Virtual, October 17, 2021
- **Singh D.** International Webinar on Impact of oxidative stress on male and

female reproduction, ICAR-National Dairy Research Institute, Karnal, Virtual, July 1, 2021

- **Singh D.** Virtual Symposium on 'Role of Epigenetics in Health: Challenges and Opportunities', BITS Pilani, Virtual, February 25-26, 2022
- **Surve S, Banerjee A.** Kisspeptin+ 2021, Virtual, April 19-21, 2021
- **Surve S, Joshi B.** Randomized control trial workshop, St. John Institute Bangalore, Virtual, September 20-24, 2021
- **Surve S, Munne K, Tryambake V, Kamat S.** A lecture on 'Breaking the bias educating girls and boys too about menstruation', Akanksha School, Abhyudaya Nagar, Akanksha School, Abhyudaya Nagar, March 8, 2021
- **Surve S.** A lecture on 'Child nutrition and obesity', Training to MSc Students of SVT College of Home Science, Virtual, October 13, 2021
- **Surve S.** A lecture on COVID 19 and children, Recreational Club for Women, Virtual, June 5, 2021
- **Surve S.** COVID 19 and Children, Maharashtra Medical Council, Virtual, June 4, 2021
- **Surve S.** MMC COVID SUMMIT - A Comprehensive Webinar on Covid by Experts: The current scenario & future preparedness, Virtual, May 30, 2021
- **Surve S.** Online training on Data software in Newborn sickle cell project, ICMR, New Delhi, Virtual, January 7, 2022
- **Surve S.** Training on ethical issues in research, ICMR-NIIRNCD, Jodhpur, in association with ICMR Bioethics Unit, NCDIR Bengaluru, June 30, 2021
- **Surve S.** Webinar on sickle cell disease - Global scenario and Indian perspectives,

ICMR-NIRTH, Jabalpur, Virtual, June 19, 2021

- **Tandon D.** Family planning: An online evidence-based course 2021, Geneva Foundation for Medical Education and Research (GFMER) works close collaboration with Department of Sexual and Reproductive Health & Research, World Health Organization, Geneva, Virtual, June 14 - July 10, 2021
- **Tandon D.** Global priorities in vaginal health: Microbes, mucosal immunity and interventions, part of the Keystone symposia Global health series supported by Bill & Melinda Gates Foundation, Virtual, November 10-12, 2021
- **Tandon D.** Guidelines for provision of Family Planning services during and post COVID pandemic, GOI, Family Planning Division, Virtual, September, 2021
- **Tandon D.** Lecture workshop on reproductive health for postgraduate students, introducing them to the institute's research activities, facilities available and services provided for Maharshi Dayanand College, Parel, ICMR-NIRRH, December 22, 2021
- **Thomas S.** Online biological resources developed by Biomedical Informatics Centre of ICMR-NIRRH, Health Informatics Summit, October 17, 2021
- **Thomas S, Kaur P.** ICMR Nodal Communication Officers Meet at NIREH, Bhopal, September 16-17, 2021

13.4 Invited Lectures

- **Bhor V.** Covid-19: Delta, Omicron & Beyond Online International Symposium, National Institute of Immunology, New Delhi, Virtual, February 23, 2022

- **Bhor V.** Exploring the Role of Integrin $\alpha 4\beta 7$ in HIV Infection, Virology - Parasitology Seminar, Institute of Virology, University of Marburg, Germany, Virtual, December 2, 2021
- **Bhor V.** Harnessing research infrastructure for response to COVID-19: Experience from ICMR-NIRRH, AICTE-ISTE sponsored online refresher / induction program on 'Leveraging academic researchers on developing diagnostic kits, vaccines and drug product for improved therapy management against deadly viruses: Lesson learnt from COVID-19', Tatysaheb Kore College of Pharmacy, Kolhapur, Virtual, December 18, 2021
- **Bhor V.** Immunopathogenesis of COVID-19 - Lessons learnt so far, Microbes 4-U Lecture Series, GN Khalsa College, Mumbai, Virtual, February 25, 2022
- **Biswas B.** Role for N-Glycans during spermatogenesis and male fertility, Invited Lecture, Department of Zoology, Guwahati University, November 3, 2021
- **Biswas B.** 'Cross talk of Sertoli cells and N-glycosylation deficient germ cells - Impact on male fertility', BRICS YSF Conclave, Virtual, September 13-16, 2021
- **Chaudhari U.** Animal models in Covid-19 Research and Vaccine Development, Invited talk One Day National Level Webinar on Insight and Challenges of Covid-19 Research, Treatment & Vaccine Development, Oriental Collage of Pharmacy, Navi Mumbai, Virtual, July 3, 2021
- **Chaudhari U.** COVID-19 Vaccines: Myths and Misconception and importance COVID-19 treatment on time, Invited talk by Social Networking Forum, Nashik, Social Networking Forum, Nashik, Virtual, May 1, 2021
- **Chaudhari U.** Diabetes Awareness and Prevention, Invited expert Panel-list by SHARP NGO webinar to celebrate World Diabetes Day, SHARP NGO, New Delhi, Virtual, November 14, 2021
- **Chaudhari U.** Food and Mood: How Nutrition Affects Mental Health?, An expert talk to celebrate World Food Day 16th October and World Mental Health Day 10th October, 2021, MES'S Pillani College of Education and Research, Virtual, October 30, 2021
- **Chaudhari U.** Longitudinal Trajectories of Metabolic Syndrome in Nonhuman Primate Models, Invited Talk National Online Training Programme, Lab Animals in Bio-Medical Research, Department of Animal Genetics and Breeding, MAFSU, Parbhani, Virtual, September 27, 2021
- **Chaudhari U.** National and International Guidelines for Harmonisation of Pre-Clinical Research, Invited talk online national workshop PHARMATECH-2021, Beyond Boundaries, Seth G.S. Medical College & K.E.M. Hospital, Mumbai, Virtual, August 6, 2021
- **Dighe V.** Evaluation of a novel combination herbal scaffold and its application in bone tissue engineering, XXVI Annual Convention of Indian Society for Veterinary Immunology and Biotechnology (ISVIB) and the International conference on Transforming Livestock Economy through Innovations in Immunology and Biotechnology, College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, July 16, 2021
- **Dighe V.** Good clinical practices & standard operating procedures in clinical research, Advanced GCP training & recent updates on regulatory

requirements, MGM Institute of Health Sciences, Navi Mumbai, July 16, 2021

- **Dighe V.** Targeted delivery of recombinant proteins, international e-training programme on 'Gene cloning - Advances and applications in veterinary sciences', ICAR-NAHEP-CAAST Project at Mumbai Veterinary College Mumbai, February 4, 2022
- **Dighe V.** Use of Lab Animals for Disease Model, Toxicity Testing and Stem Cell Research, National training program on, College of Veterinary and Animal Sciences, Parbhani, May 31, 2021
- **Gajbhiye R.** 'Updates on genetic risk factors in endometriosis', CME Endometriosis, AIIMS Raipur, Virtual, March 10, 2022
- **Gajbhiye R.** Clinical Phenotypes and genetic regulation of endometriosis in Indian women - ECGRI study, DBT WT India Alliance Annual Conclave, Virtual, October 25-28, 2021
- **Gajbhiye R.** Endometriosis and infertility related research, The Association of Maharashtra Obstetrics & Gynecology Society, Virtual, June 25, 2021
- **Gajbhiye R.** Impact of Covid-19 On Pregnant Women and their Newborns: Evidence from Pregcovid Registry, ISSRF Professor N R Moudgal Oration award lecture at the International Conference on Reproductive Healthcare and 32nd Annual Meeting of the Indian Society for the Study of Reproduction and Fertility, Virtual, February 12, 2022
- **Gajbhiye R.** Standard treatment protocols for clinical management of COVID-19 complicating pregnancy for the WHO South-East Asia Region, WHO CCR in Human Reproduction, AIIMS New Delhi and WHO-SEARO, Virtual, December 7-8, 2021
- **Madan T.** Reduced levels of surfactant protein D (SP-D) in serum and seminal plasma of Indian infertile males with infection / inflammation, ASRI annual meeting, Virtual, May 14-21, 2021
- **Modi D.** Epigenetic control in deciding to become a girl or a boy, role of epigenetics in health: challenges and opportunities, BITS-Pilani, Virtual, February 25-26, 2022
- **Modi D.** Genetic networks in RIF, Implantation failure and genetics, Indian Fertility Society, Virtual, August 6, 2021
- **Modi D.** Lhx2 drives meiosis in mammalian germ cells, Subhash Mukhopadhyay e-Symposium, ADMAS University, Virtual, January 13-15, 2022
- **Modi D.** Lhx2 drives the decision of the developing germ cells to enter in meiosis, Recent advances in reproductive health, CSIR-CDRI, March 30, 2022
- **Modi D.** Mosaicism - what we need to know, Reproductive genetics - how it influences ART practices, ACE 2021, Virtual, October 1-3, 2021
- **Modi D.** Mosaicism in human embryos: Implications for PGT-A, YUVA ISAR 2021, The Bengal Club, Kolkata, November 26-28, 2021
- **Modi D.** Science and sexuality, SB Centenary Talk Series: 2 - Transgender lives: Issues, challenges and future directions, SB college Kerala, Virtual, May 22, 2021
- **Modi D.** Starting out as a young investigator in India, Young Investigators Meet (YIM) 2021, Virtual, May 18, 2021
- **Mukherjee N.** 'Role of toll like receptors as therapeutic targets in Triple negative breast cancer', One-day symposium on the occasion of National Science Day Celebration, Kolkata, February 28, 2022

- **Mukherjee S.** 'Genetics of polycystic ovary syndrome', 1st International PCOS symposium, Kattankulathur, Virtual, January 22-23, 2022
- **Mukherjee S.** 'Unraveling pathophysiology of PCOS by multidisciplinary approach', Symposium of Reproduction, Amity University, Noida, Virtual, April 20, 2021
- **Munne K.** An awareness talk for tribal women on reproductive tract infections, screening of oral, cervical & breast cancers and non-communicable diseases at Dahanu beach organized by Kashtakari Sanghatana, Dahanu on March 7, 2022
- **Singh D.** Electron microscopy: Applications and advancements in the field, Accelerate Vigyan Karyashala DST-SERB sponsored workshop on Transmission Electron Microscope (TEM), ICAR-National Rice Research Institute, Cuttack, February 15-22, 2022

13.5 Participation in Task Force Meeting as Advisors/Faculty

- **Gajbhiye R.** Training of National Task Force on ICMR National Snakebite Project (INSP) on capacity building of health workers at ICMR-NIRRH, February 23-25, 2022

13.6 Inter-Institutional Collaborations

13.6.1 National Collaborations

- Ankur fertility centre, Dadar, Mumbai
- Amity University, Noida
- ACI Cumballa Hill Hospital
- Bai Jerbai Wadia Hospital for Children, Mumbai
- Bhabha Atomic Research Centre, Mumbai

- Brihanmumbai Municipal Corporation
- DY Patil Medical College, Pune
- Grant Medical College and JJ Group of Hospitals
- Guwahati Medical College, Guwahati, Assam
- Institute of Life Sciences, Bhubaneswar
- Indian Institute of Technology Bombay
- Indian Institute of Science, Bangalore
- Jaslok Hospital
- National Institute of Animal Biotechnology (NIAB), Hyderabad
- Kasturba Medical College, Manipal, Karnataka
- King George Medical University, Lucknow, UP
- Lokmanya Tilak Municipal General Hospital, Sion, Mumbai
- Mangeshkar's Clinic, Tardeo, Mumbai
- MGM Hospital, Kamothe, Navi Mumbai
- Municipal Corporation of Greater Mumbai, Mumbai
- National AIDS Control Organisation
- Norwojee Wadia Maternity Hospital, Mumbai
- Oasis Center for Reproductive Medicine, Hyderabad
- PD Hinduja Hospital, Mumbai
- Population Council, Delhi
- Regional Medical Research Centre, Bhubaneswar (RMRCBB)
- SATH, Medical College, Thiruvananthapuram, Kerala
- Seth G.S. Medical College and K.E.M. Hospital, Mumbai
- Spectrum Endoscopy Center, Kolkata, West Bengal
- TN Medical College and BYL Nair Hospital, Mumbai
- Tata Memorial Hospital, Mumbai

13.6.2 International Collaborations

- Albert Einstein College of Medicine,

New York

- Brunel University, London, UK
- School of Public Health, UQ, Australia
- University of South Florida, Tampa USA

13.7 Trainees

Twenty-four winter trainees were inducted in various departments namely molecular immunology & microbiology, bioinformatics, neuroendocrinology, infectious disease biology, innate immunity. These trainees were introduced to technological innovations in different fields and were trained in various scientific processes in ongoing projects.

13.8 ICMR National Snakebite Project (INSP) on Capacity Building of Health System on Prevention and Management of Snakebite Envenomation including its Complications *(Partly Funded by ICMR)*

Principal Investigator : **R Gajbhiye**

Principal Co-Investigator : H Bawaskar, Bawaskar Hospital and Research Centre, Mahad, District Raigad, Maharashtra

Project Associates : H Munshi Scientist C (Medical) M Gavhande, Technical Officer, Shahapur, Thane, G Bhad Technical Officer, Aheri, Gadchiroli, Bijaylaxmi Mohanty, Technical Officer, Khordha, Khordha, Odisha, J P Dash, Technical Officer, Kasipur, Rayagada

Collaborators : A Mahapatra, S Palo, ICMR- Regional Medical Research Centre Bhubaneswar (RMRCBB), Odisha
A Yadav, Directorate of Health Services, Maharashtra State
M V Bansode, Sub District Hospital, Shahapur
S Shambharkar, Former District Health Officer, Gadchiroli
K Madavi, Sub District Hospital, Aheri
K C Itta, R K Prusty

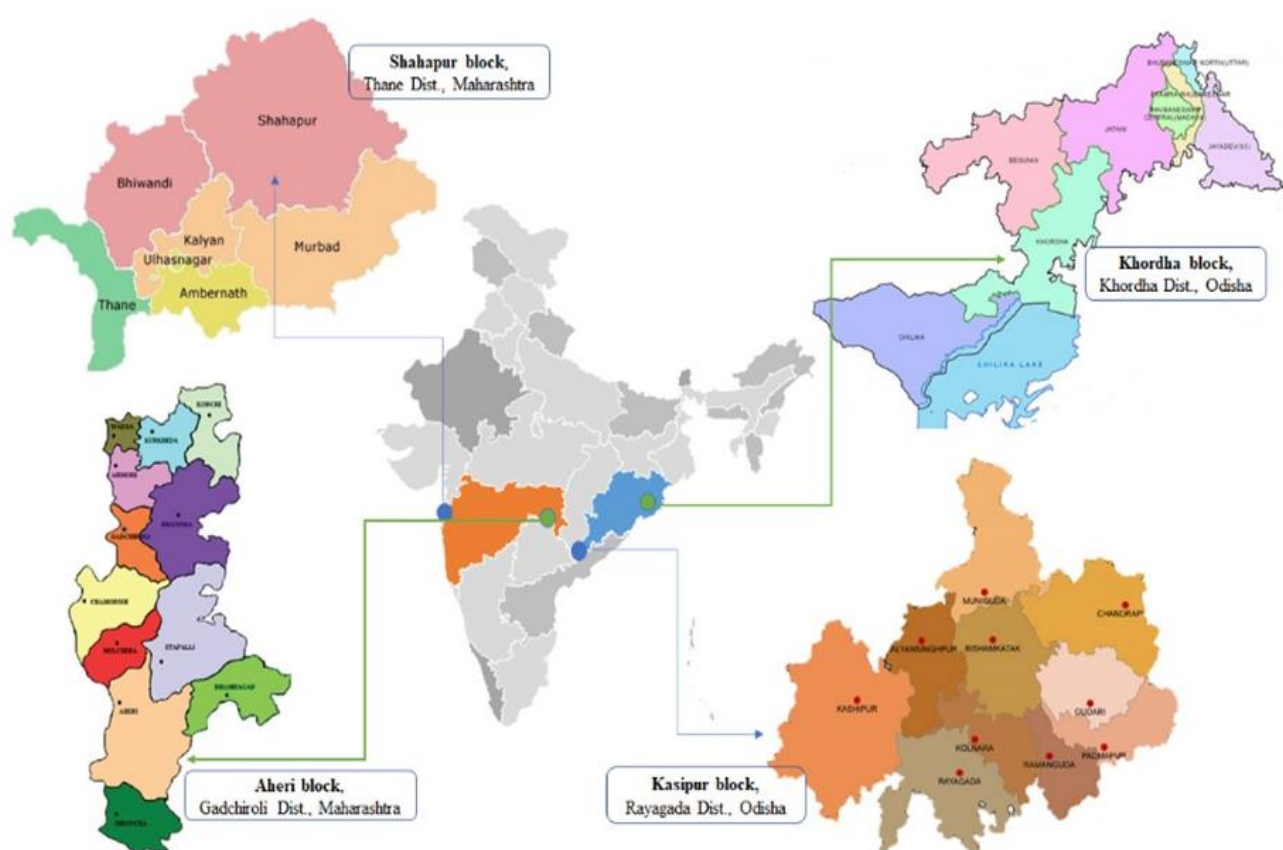
Project Coordinator : Smita Mahale

Duration : 2021-2023

Snakebite Envenomation is one of the Priority Research Areas for ICMR under the Hon'ble Prime Minister's Vision for New India 2022 and thereafter. Currently, the phase I of the Indian Council of Medical Research (ICMR) funded project entitled "ICMR National Snakebite Project on capacity building of health systems on prevention and management of snakebite envenomation including its complications" is being implemented in Maharashtra and Odisha. Study sites in Maharashtra include Shahapur block of Thane district and Aheri block of Gadchiroli district while study sites in Odisha include Khordha block in Khordha district and Kasipur block in Rayagada district (Table 31, Fig. 110).

Table 31: Study blocks with Scheduled Tribe population and female literacy rate

State	District	Study block	Scheduled Tribes	Tribal Population in Study Block (%)	Female Literacy (%)
Maharashtra	Thane	Shahapur	Katkari (PVTG), Thakar	35.7	58.4
	Gadchiroli	Aheri	Madiya (PVTG), Gond, Pardhan	49.8	55.7
Odisha	Khordha	Khordha	Kandha, Kolho, Santali, Munda, Saora (PVTG)	8.4	73.2
	Rayagada	Kasipur	Kandha, Jhodia, Kutia Khond (PVTG)	58.8	20.4

**Figure 110:** Geographical location of the study areas in Maharashtra and Odisha states in India

The project aims to i) increase the awareness and empower the community on prevention, first aid, and early transport of snakebite patients to the nearest health facility; ii) evaluate the healthcare providers regarding their knowledge and practices during management of snakebite envenomation (SBE) and understand the anti-venom distribution and utilization at public health facilities; iii)

empower the health system for management of SBE through the implementation of Standard Treatment Guidelines (STG) of the Government of India; and d) study the impact of the interventions on reducing SBE mortality and morbidity. In the preparatory phase of the project, retrospective data on snakebite cases (n=1449) reported at health facilities in study blocks during 2020-2021 has been collected.

Total 28 focus group discussions (14 male, 14 female) have been conducted in the study blocks on knowledge, perceptions and awareness about snakebite prevention, diagnosis and first aid. Total 188 medical officers and community health officers have been trained on prevention, diagnosis and

management of SBE by national experts including Dr Himmatrao Bawaskar, Dr Dayal Bandhu Mazumdar and Dr Sadanand Raut. Training was provided on endotracheal intubation and cardio-pulmonary resuscitation (Table 32).

Table 32: Blockwise data collection in the preparatory phase of the study

State	District	Study block	Retrospective Data Cases	FGDs Conducted	Medical Officers Trained
Maharashtra	Thane	Shahapur	900	8	66
	Gadchiroli	Aheri	279	8	59
Odisha	Khordha	Khordha	200	6	23
	Rayagada	Kasipur	70	6	40
Total			1449	28	188

Tribal residential schools' officials (n=60) and forest department officials (n=20) were also trained on snakebite prevention and first aid. Pre-training and post-training test of medical officers and community health officers was conducted for assessing the impact of training. Post-training prospective data collection on snakebite management from public health facilities is currently ongoing. A comparative analysis of retrospective and prospective data would aid in evaluating the impact of the interventions. Facility surveys to assess the preparedness of public health facilities for snakebite management are also ongoing. Gap

analysis would help in strengthening the public health facilities to provide adequate snakebite management. Interviews of peripheral healthcare workers including ASHAs, ANMs and MPWs on snakebite prevention and first aid are ongoing. Their knowledge gaps would be filled by medical officers who have attended the training by national experts. Treatment aids including snakebite management flowchart and snake identification charts for health facilities along with IEC material for community awareness and an informative project website are in the process of development.

14. HONORS AND AWARDS

14.1 Awards

- **Arya D.** Best poster award for 'Sperm mitochondrial functions and mitochondrial DNA methylation status in infertile men with clinical varicocele' at International Conference on Reproductive Healthcare & 32nd Annual Meeting of the Indian Society for the Study of Reproduction and Fertility (ISSRF), February 11-13, 2022.
 - **Bane K.** ICMR-NIRRH Foundation Day Award 2022, February 21, 2022.
 - **Biswas B.** Selected as a speaker in the highly prestigious BRICS YSF Conclave, September 13-16, 2021.
 - **Gajbhiye R.** Awarded Professor N R Moudgal Oration Award at the ISSRF, February 12, 2022.
 - **Gupta T.** Dr (Mrs.) Mridula Kamboj Memorial Oration Award 2022, for her 'Soluble pattern recognition collectins are integral to human reproductive health' at the ISSRF, February 12, 2022.
- Kaushik A.** Prof NR Moudgal Young Scientist Award at the ISSRF, February 12, 2022.
- **Nair S.** Extra-ordinary Entry Prize for e-poster 'Investigation of imprinted gene methylation in spermatozoa of preeclampsia cases' at Academic e-carnival, Association of Clinical Biochemists and Microbiologists, Nagpur, October 2-3, 2021
 - **Patil K.** Dr Shanta Rao Award 2022 for Best Research Paper, February 21, 2022.

- **Patil K.** Best Poster Award for 'Identification of miRNAs regulating angiogenesis in women with polycystic ovary syndrome' at the ISSRF, February 12, 2022.
- **Singh P.** Dr (Mrs) Mridula Kamboj Young Scientist Award at the ISSRF, February 12, 2022.

14.2 PhD Degrees Awarded

1. Sukeshani Salve

Thesis title: Deciphering the effects and mechanism of Action of butylparaben on fertility

Research Guide: Dr Vainav Patel, (Late) Dr Atmaram Bandivekar

2. Rucha Lalingkar

Thesis title: Study of links between bone health and cardiovascular status in women: a molecular biology approach

Research Guide: Dr Ikram Khatkhatay

3. Kavita Kale

Thesis title: Evaluation of factors affecting innate immunity in Women with pre-eclampsia

Research Guide: Dr Taruna Gupta

4. Priyanka Wagh

Thesis title: Immune Response genes: variants and their association with cervical cancer

Research Guide: Dr Jayanti Mania Pramanik

5. Nikhil Bardeskar

Thesis title: Variants of human leukocyte antigen and their possible association with HIV transmission

Research Guide: Dr Jayanti Mania Pramanik

6. Jyoti Prakash Sutar

Thesis title: Association of viral variation in blood and semen with transmission and pathogenesis of HIV

Research Guide: Dr Vainav Patel, (Late)
Dr Atmaram Bandivekar

7. Rinku Pramanik

Thesis title: Identification of vaginal Lactobacilli from Indian women and delineating their functional properties

Research Guide: Dr Clara Aranha

8. Sharvari Deshpande

Thesis title: Investigation on obesity induced epigenetic changes in male germline

Research Guide: Dr Nafisa Balasinor

15. MEMBERS OF ADVISORY COMMITTEES

15.1 Members of Scientific Advisory Committee

Dr Neerja Bhatla

Chairperson

Professor, Obstetrics and Gynaecology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi

Dr Reeta Rasaily (ICMR Representative)

Scientist 'G' and Head, Division of RBMCH & Nutrition, Chief Nodal Officer-North East Region, Indian Council of Medical Research V Ramalingaswami Bhawan, Ansari Nagar, New Delhi

Prof Vanita Suri

Head, Department of Obstetrics & Gynaecology, Postgraduate Institute of Medical Education and Research, Madhya Marg, Sector 12, Chandigarh

Dr T Sundararaman

30, Second Cross Street, Thiru Nagar, Moolakulam, Puducherry

Dr Subeer S Majumdar

Distinguished Professor, National Institute of Animal Biotechnology, Opposite Journalist Colony, Near Gowlidoddy, Gopanpally, Hyderabad

Dr B K Thelma

Department of Neurology, Paras Hospitals, Haryana

Dr Ashutosh Halder

Professor & Head, Reproductive Genetics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi

Prof Sidharth Ramji

Director & Professor, Head of Neonatology, Maulana Azad Medical College & Lok Nayak Hospital, New Delhi 110002

Dr Sanjay Mehendale

Director Research, PD Hinduja Hospital and Medical Research Center, 8-12, SVS Rd, Mahim West, Mahim, Mumbai

Dr Smita Mahale

ICMR Emeritus Scientist, ICMR-NIRRH, Parel, Mumbai

Dr Manisha Madkaikar (Special Invitee)

Director, ICMR - National Institute of Immunohaematology, 13th floor, New Multistoreyed Building, KEM Hospital Campus, Parel, Mumbai

Dr Sumita Ghosh

Additional Commissioner, In-Charge (Child Health, RBSK, AH, CAC & AD), Ministry of Health and Family Welfare, Nirman Bhawan (Room No. 525 A), New Delhi

Dr Teja Ram

Additional Commissioner, I/c, Family Planning and Maternal Health, Ministry of Health and Family Welfare, 519-A, Nirman Bhawan, New Delhi

Dr Mangala Gomare

Ex Health Officer, Brihan Mumbai Mahanagapalika, F/S Ward Office Building, 3rd Floor, Dr B Ambedkar Marg, Parel, Mumbai

Dr Geetanjali Sachdeva (Member Secretary)

Director, ICMR-NIRRH, Parel, Mumbai

15.2 Members of ICMR-NIRRH Ethics Committee for Clinical Studies

Dr Padmavathy S Menon

Chairperson (till January 29, 2022)

Ex-Professor and Head, Department of Endocrinology,
Seth GS Medical College & KEM Hospital,
Parel, Mumbai

Prof Shubhada Chiplunkar

Chairperson (from January 30, 2022)

Former Director ACTREC-TMC,
Principal Investigator, Chiplunkar Lab,
ACTREC, Tata Memorial Centre,
Kharghar, Navi Mumbai

Dr Beena Joshi

Member Secretary (till January 29, 2022)

Scientist-F, Department of Operational Research,
ICMR-NIRRH, Parel, Mumbai

Dr Vikrant Bhor

Member Secretary (from January 30, 2022)

Scientist D, Molecular Immunology and Microbiology,
ICMR-NIRRH, Parel, Mumbai

Dr Suchitra Surve

Joint Member Secretary (from January 30, 2022)

Scientist C, Child Health Department,
ICMR-NIRRH, Parel, Mumbai

Dr Yogeshwar S Nandanwar

Professor, Obstetrics and Gynaecology,
DY Patil Medical College, Nerul,
Navi Mumbai

Dr Sandeep Bavdekar

Ex-Professor & Head, Paediatrics Department,
BYL Nair Charitable Hospital &
TN Medical College, Mumbai

Dr Sivakami Muthusamy

Professor, School of Health Systems Studies,
Tata Institute of Social Sciences,
Deonar, Mumbai

Dr Ketaki Kulkarni

Assistant Professor,
Nowrosjee Wadia Maternity Hospital,
Parel, Mumbai

Advocate Ajay Shinde

Gulisatan, 3rd floor, CAT Bar Association,
Ghanshyam Talwatkar Road,
Fort, Mumbai

Dr Bhakti Pathak

Scientist-E, Cellular and Structural Biology,
ICMR-NIRRH, Parel, Mumbai

Mrs Vibhavari Kuberkar

A-504, Gagagniri Park,
Samtanagar, near Cadbury factory, Thane West

Mrs Minakshi Bansode

Flat No. 13, Orchids Complex,
Madhumalati CHS, Majiwada, Thane West

Mrs Sudha Sathaye (from February 14, 2022)

C/9/8 Sukumar society, Dayaldas road,
Vileparle (East), Mumbai

Dr Shirish Joshi

Alternate Member - Pharmacology

Professor Additional,
Department of Pharmacology & Therapeutics,
Seth GS Medical College & KEM Hospital,
Parel, Mumbai

Dr Nayreen Daruwalla

Alternate Member- Social Scientist (Relieved from
February 23, 2022)

Director, Program on Prevention of Violence
against Women and Children, Society for
Nutrition, Education & Health Action,
Mumbai

Advocate Meghana Shirke

Alternate Member- Legal Expert (from February 14, 2022)

Room No. 81, 3rd floor, Mahavir Sukh Building,
Dr Ambedkar Road, Dadar (East),
Mumbai

15.3 Members of Institutional Animal Ethics Committee**Dr Smita D Mahale**

Chairperson

Emeritus Scientist,
ICMR-NIRRH, Parel, Mumbai

Dr Nafisa Balasinor

Member Secretary

Scientist-F, Neuro-endocrinology Department
ICMR-NIRRH, Parel, Mumbai

Dr Akaram Tanaji Bagal

Chief Nominee

Flat No. 504, 'D' Wing, Nea Society,
Near Green Zone/ TUV Nord,
Baner-Sus Road, Sus, Pune

Dr Dilip Rewa Patil

Scientist

National Institute of Virology,
20-A Dr Ambedkar Road, Pune

Ms Nisha Subramanian Kunju

Treasurer, PRO - Cum - Administrator,
2 Shiv Shakti, Patkar Compound,
Gamdevi Road, Bhandup (West),
Mumbai

Dr SM Metkari

Experimental Animal Facility,
ICMR-NIRRH, Parel, Mumbai

Dr Sadhana Sathaye

Associate Professor,

Department of Pharmaceutical Sciences and
Technology,
Institute of Chemical Technology,
Matunga, Mumbai

Mr Shashikant D Ahire

Tata Memorial Centre, Cancer Research
Institute,
Advanced Centre for Treatment Research and
Education in Cancer (ACTREC),
Sec- 22, Kharghar, Navi Mumbai

Dr Uddhav Chaudhari

Scientist-E, Cell Physiology and Pathology,
ICMR-NIRRH, Parel, Mumbai

15.4 Members of Institutional Committee for Stem Cell Research (ICSCR)**Dr Aparna Khanna**

Chairperson

Ex- Dean -Research (Science & Technology) &
Ex-Director, Amity Institute of Biotechnology
(AIB), Mumbai

Dr Manisha Madkaikar

Co-Chairperson

Director & Scientist G,
ICMR-National Institute of
Immunohaematology,
Honorary Assistant Professor, Department of
Haematology,
KEM Hospital, Mumbai

Dr Dhanashree Jagtap

Member Secretary

Scientist-D, Cellular and Structural Biology
Division,
ICMR-NIRRH, Parel, Mumbai

Dr Abhijit Majumdar

Associate Professor, Department of Chemical
Engineering,
IIT Bombay

Dr Sanjeev Waghmare

Scientific Officer F

ACTREC, Tata Memorial Centre, Navi
Mumbai

Dr Pradip Chaudhari

Scientific Officer G, ACTREC

Tata Memorial Centre, Navi Mumbai

Dr Bipin Kulkarni

Scientist D, Department of Hemostasis and
Thrombosis,

ICMR- National Institute of
Immunohaematology, Mumbai

Dr Nilesh Gawade

Assistant Professor, Centre for Public Health,

School of Health Systems Studies,

Tata Institute of Social Sciences, Mumbai

Dr Smitha Sasidharan Nair

Assistant Professor,

Centre for Health and Mental Health School of
Social Work,

Tata Institute of Social Sciences, Mumbai

Adv Shraddha Kulkarni

Flat No. 201, Techno Heights CHS Ltd.,

Plot No. 155A, Sir Bhalchandra Road,

Hindu colony, Dadar, Mumbai

Mrs Sapphire D'Penha

101 Tahir Manor, 77 Bazar Road,

Bandra West, Mumbai

16. EXTRAMURALLY FUNDED PROJECTS

Sr. No.	Title	PI	Funding agency	Start year	End year
1.	Role of Kisspeptin mediated signaling in onset of puberty	Antara Banerjee	SERB- (Women Excellence Award)	2021	2024
2.	Delineation of the role of isoforms of kisspeptin in mammalian reproduction	Antara Banerjee	SERB-Start up grant	2022	2024
3.	Role of complex N glycans and functional significance of Basigin in the testicular germ cells for spermatogenesis	Barnali Biswas	DST-INSPIRE	2018	2023
4.	Crosstalk of Sertoli cells and N-Glycosylation deficient germ cells- Impact on Male fertility	Barnali Biswas	DBT	2019	2022
5.	Health system analysis and evaluations of the barriers to availability, utilization and readiness of FP and contraceptive services in COVID-19 affected areas.	Beena Joshi (Co-investigator)	WHO	2022	2022
6.	Evaluation of prevalence, regional phenotypic variation, comorbidities, risk factors and the variations in response to different therapeutic modalities among Indian women with polycystic ovary syndrome (PCOS): A multicenter study across India	Beena Joshi	ICMR	2018	2022
7.	Implementation research to explore operational feasibility acceptability and cost-effectiveness of using IV Ferric Carboxy Maltose (FCM) in management of Iron Deficiency Anemia (IDA) among pregnant women through sub-district health system in Maharashtra	Beena Joshi	DHR	2021	2023
8.	HTA Resource Hub	Beena Joshi	DHR HTAIn	2018	2026
9.	HTA for strengthening prong 2 interventions of PPTCT	Beena Joshi	DHR HTAIn	2021	2022
10.	Development of a multi-strain probiotic formulation effective against reproductive tract infections	Clara Aranha	ICMR	2013	2023
11.	In-vitro evaluation of vaginal Lactobacilli isolated from Indian women for potential anticancer effects and elucidating its possible mechanism of action	Clara Aranha	DHR	2018	2022
12.	Deciphering the immunomodulatory roles of Homeobox A 10 in the mouse	Deepak Modi	DBT	2021	2024

	endometrium during embryo implantation				
13.	Delineating the role of human β -microseminoprotein in male reproduction	Dhanashree Jagtap	ICMR	2022	2025
	Therapeutic potential of Epigallocatechin-3-gallate (EGCG) for improving sperm quality, fertility and pregnancy outcomes in a murine model of endocrine disruption	Dipty Singh	ICMR	2022	2025
14.	Investigating the contribution of DNA damage, repair and demethylation in pathogenesis of endometriosis	Geetanjali Sachdeva	DBT	2019	2022
15.	Uterine alarmins and their relevance in implantation	Geetanjali Sachdeva	SERB	2020	2023
16.	In vitro and In vivo functions of cell surface estrogen receptor in the context of prostate cancer	Geetanjali Sachdeva	DAE	2018	2021
17.	Eutopic endometrial cell repertoire in women presenting different subtypes of endometriosis and its association with endometrial receptivity	Geetanjali Sachdeva	ICMR	2022	2025
18.	Primary screening of high risk HPV DNA by a low cost molecular HPV test for early detection of cervical precancers and cancers among women in urban and rural community of Maharashtra	Kiran Munne	DHR	2019	2023
19.	Improving treatment literacy and adherence among People Living with HIV (PLHIV) through innovative strategies.	Kiran Munne	NACO	2020	2022
20.	Osteoporosis screening and intervention programme using AYUSH approach: A community based programme in Maharashtra state – Public Health Initiatives study	Kuldip Raj Kohli Co-PI: Lalita Savardekar	Ministry of AYUSH, GOI	2017	2021
21.	Study of drug utilization pattern among elderly patients	Lalita Savardekar	ICMR	2020	2022
22.	Bone exercise for life: An intervention model on bone health and quality of life among menopausal women	Lalita Savardekar	DHR	2016	2021
23.	Omics of placental exosomes in early onset preeclampsia: an approach towards identifying predictive biomarkers	Nafisa Balasinor	DBT	2017	2023

24.	Molecular mechanism involved in prolactin and dopamine signaling in male reproduction	Nafisa Balasinor	SERB	2020	2023
25.	Aberrant paternal imprinting: A risk factor for preeclampsia	Nafisa Balasinor	LTMT	2016	2023
26.	Elucidating the role of mucosal homing cell adhesion molecules in eliciting B cell associated anti-HIV and anti-SARS-CoV-2 immune responses	Nandini Kasarpalkar Mentors: Vikrant Bhor and Vainav Patel	ICMR-IAVI	2022	2023
27.	Role of Toll-like receptors and TLR agonists in modulating response to chemotherapy in TNBC patients	Nupur Mukherjee	DBT-BioCARE	2019	2022
28.	Immune and microbiome correlates of TB reactivation in PLHIV and a NHP model	Nupur Mukherjee	Indo-US Joint Program on HIV/AIDS & STI Prevention Research Collaboration Awards	2020	2023
29.	Study of maternally inherited mitochondrial DNA variants in women with polycystic ovarian syndrome	Pallavi Shukla	ICMR	2021	2024
30.	Evaluation of immunogenicity and protective efficacy of bivalent vaccine candidate against Shigella and Salmonella using food grade bacteria Lactococcus lactis	Preeti Desai Co-PI: Vainav Patel	DST SERB	2018	2021
31.	Functional significance of Testis Specific Histone H2B variant (TH2B) in sperm and early embryonic development	Priyanka Parte	Women and Child Health Program - DBT	2021	2024
32.	Design and development of a microfluidic chip for sperm selection/sorting based on chemotaxis	Priyanka Parte	Biomedical Engineering Medical Devices & Diagnostics-DBT	2021	2023
33.	Maternal Near Miss Review (MNM-R) and corrective measures at selected tertiary hospitals in India	Ragini Kulkarni	ICMR	2018	2020
34.	Investigating in-vitro and in-vivo potential of rfhSP-D to inhibit prostate cancer metastasis and mechanistic involvement of GRP78	Rambhadur Subedi Co-PI: Taruna Madan	ICMR-RA	2021	2024
35.	Assessing the coagulation and fibrinolytic system as contributors of thrombotic state in PCOS	Roshan Dadachanji	ICMR-PDF	2021	2023

		Co-PI: Srabani Mukherjee			
36.	Identification of circulating microRNA signatures as diagnostic markers for early stage and metastatic breast cancer	Sadhana Gupta	ICMR	2021	2024
37.	Increasing access to postpartum contraception by linking family planning and infant vaccination services	Shahina Begum	NIH, USA	2022	2024
38.	Comprehensive genetic evaluation of fetus in antenatally detected abnormal pregnancies with fetal malformations: Outcomes, benefits and limitations- a pilot study	Shailesh Pande	DHR	2021	2024
39.	DBT-Mission program on pediatric rare genetic disorders	Shailesh Pande	DBT	2022	2027
40.	Building and analysing gene network for Polycystic ovary syndrome (PCOS)	Shaini Jospeh	DST-WOSA	2019	2022
41.	Exploring 5-hydroxymethylcytosine (5hmC) marks as epigenetic drivers in PCOS pathophysiology	Srabani Mukherjee	DHR	2021	2024
42.	Unraveling pathogenetic mechanisms of PCOS by whole exome sequencing	Srabani Mukherjee	SERB	2020	2023
43.	Archiving the work of Dr Subhas Mukherjee: The architect of India's first test tube baby	Srabani Mukherjee	INSA	2019	2022
44.	Integrated analyses of genomic scale metabolic models and omics profiles to capture the host-pathogen-environment interplay of Candida sp	Susan Thomas	SERB	2021	2024
45.	Establishment of Bioinformatics and Computational Biology Centre (Centre for advanced research in bioinformatics and computational biology for woman and child health)	Susan Thomas	DBT	2021	2026
46.	Identification of enriched biochemical networks and polypharmacological targets in metabolic syndrome	Susan Thomas	SERB (STAR)	2020	2023
47.	Identification of differentially expressed genes in lean and obese PCOS and validation using rat animal models	Susan Thomas	DHR	2019	2023
48.	Machine learning algorithms trained on voice to predict psychological distress and postpartum depression	Susan Thomas	ICMR	2022	2024
49.	Longitudinal cohort study to evaluate the effect of various contraception	Deepti Tandon	ICMR	2019	2022

	methods on the composition and diversity of the vaginal microbiota				
50.	Evaluating the inflammatory, microbiome profile and co-infections in women diagnosed with treatment failure, relapse or recurrent bacterial vaginosis - a prospective analytical study	Deepti Tandon	ICMR	2022	2025
51.	Investigating therapeutic potential of a recombinant fragment of human surfactant protein D against SARS-CoV-2: In vitro and animal models	Taruna Madan	BIRAC-PACE	2021	2022
52.	Evaluation of therapeutic potential of PVF-VEGF in trophoblast differentiation and placental spiral artery remodeling in murine models of Preeclampsia	Taruna Madan	DBT	2021	2024
53.	Identification of salivary biomarkers for early prediction of gestational diabetes.	Uddhav Chaudhari	DHR	2018	2021
54.	Role of Receptor for Advanced Glycation End Product (RAGE) and High Mobility Group B1 (HMGB1) protein in endometrial repair	Uddhav Chaudhari	SERB	2019	2022
55.	Developing broadly neutralizing monoclonal antibody (bnAb) mediated prevention and treatment strategy by assessing their effectiveness in neutralizing HIV-1 Subtype C circulating in India across different regions and distinct risk groups	Vainav Patel	India Alliance (DBT Welcome) Team Science Grant	2020	2025
56.	Identification and evaluation of novel metabolites with potential of prenatal diagnosis of fetal congenital heart diseases	Venkanna Bhanothu	DST-SERB	2022	2025
57.	Molecular profiling of common clinical phenotypes associated with congenital hypothyroidism (CH)	Venkanna Bhanothu	ICMR	2022	2025
58.	Sertoli and leydig cell homing peptides as molecular steering for testicular targeted drug delivery	Vikas Dighe	DHR	2021	2024
59.	Exploring the therapeutic potential of peptides targeting lysophosphatidic acid (LPA) receptors in ovarian cancer	Vikas Dighe	DBT	2021	2024
60.	Evaluation of synergistic impact of oral Nano-curcumin and Alpha-Linolenic Acid on maternal and fetal health in rat model of Preeclampsia	Vikas Dighe	ICMR	2021	2024

61.	Evaluation of drugs-cytochrome P450 enzyme interaction through fluorometric high throughput screening assays	Vikas Dighe	CCRAS	2021	2023
62.	Intranasal mucosal vaccine for COVID-19	Vikrant Bhor	DBT-BIRAC	2020	2023
63.	Longitudinal cohort study of lactating women to assess impact of SARS-CoV-2 exposure and vaccination on systemic and vertically transferred SARS-CoV-2 specific immunity in the mother-infant dyad	Vikrant Bhor	ICMR	2022	2024

AYUSH	<i>Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy</i>
BioCARE	<i>Biotechnology Career Advancement & Re-orientation Programme</i>
BIRAC	<i>Biotechnology Industry Research Assistance Council</i>
CCRAS	<i>Central Council for Research in Ayurvedic Sciences</i>
DAE	<i>Department of Atomic Energy</i>
DBT	<i>Department of Biotechnology</i>
DHR	<i>Department of Health Research</i>
DST	<i>Department of Science and Technology</i>
HTAIn	<i>Health Technology Assessment in India</i>
IAVI	<i>International AIDS Vaccine Initiative</i>
ICMR	<i>Indian Council for Medical Research</i>
INSA	<i>Indian National Science Academy</i>
INSPIRE	<i>Innovation in Science Pursuit for Inspired Research</i>
LTMT	<i>Lady Tata Memorial Trust</i>
NACO	<i>National AIDS Control Organization</i>
NIH	<i>National Institutes of Health</i>
PACE	<i>Promoting Academic Research Conversion to Enterprise</i>
PDF	<i>Post-Doctoral Fellowship</i>
SERB	<i>Science and Engineering Research Board</i>
STAR	<i>Science and Technology Award for Research</i>
WHO	<i>World Health Organization</i>
WOSA	<i>Women Scientists Scheme-A</i>

17. STAFF AND STUDENTS

DIRECTOR'S OFFICE

Dr. Geetanjali Sachdeva, *Director*
Mr. M. Chabukswar, *Personal Assistant*
Mr. K. N. Kadam, *Laboratory Attendant-2*

BIOMEDICAL INFORMATICS

Dr. Susan Thomas, *Scientist 'E'*
Mr. R. S. Barai, *Technical Officer*
Mr. N. B. Shelar, *Laboratory Assistant*
Ms. Kaushiki S. Prabhudesai, *Ph. D. student*
Ms. Shuvechha Mukherjee, *Ph. D. student*
Ms. Indra Kundu, *Ph. D. student*
Ms. Kshitija S. Rahate, *Ph. D. student*

BIOSTATISTICS

Dr. Shahina Begum, *Scientist 'E'*
Dr. R. K. Prusty, *Scientist 'B'*
Dr. D. D. Naik, *Sr. Technical Officer-3**

CELL PHYSIOLOGY AND PATHOLOGY

Dr. Geetanjali Sachdeva, *Scientist 'G'*

Dr. U. K. Chaudhari, *Scientist 'E'*
Dr. R. R. Katkam, *Sr. Technical Officer-3*
Ms. Sushma Gadkar, *Sr. Technical Officer-2***
Mr. J. S. Lokhande, *Technician 'C'****
Mr. B. P. Mayekar, *Laboratory Assistant*
Ms. Sushma Gadkar, *Ph. D. student*
Ms. Kashmira V. Bhusane, *Ph. D. student*
Ms. Rithika Rajendran, *Ph. D. student*
Ms. Junita Desouza, *Ph. D. student*
Ms. Itti Munshi, *Ph. D. student*
Mr. M. I. F. J. Shaikh, *Ph. D. student*
Mr. A. Khandvilkar, *Ph. D. student*
Mr. A. Hussain, *Ph. D. student*
Mr. G. R. Paswan, *Ph. D. student*

* Retired on 30.09.2021

** Transferred from NIREH on 21.01.2022

*** Retired on 31.03.2022

CELLULAR AND STRUCTURAL BIOLOGY

Dr. Bhakti Pathak, *Scientist 'F'*
Dr. Dhanashree Jagtap, *Scientist 'D'*
Dr. Antara Banerjee, *Scientist 'B'*
Ms. Ananya Breed, *Sr. Technical Officer-1*
Mr. B. J. Kulkarni, *Technical Assistant*
Mr. R. G. Rane, *Laboratory Assistant*
Mr. J. M. Rabhadiya, *Laboratory Assistant*
Ms. Pradnya R. Kamble, *Ph. D. student*
Ms. Vaidehi Miya, *Ph. D. student*
Ms. Apoorva Pawar, *Ph. D. student*
Ms. Meghali Borkotoky, *Ph. D. student*

CLINICAL RESEARCH

Dr. Lalita Savardekar, *Scientist 'F'*
Ms. Neera Mehta, *Technical Officer*
Ms. Devyani Rathod, *Nursing Orderly*
Mr. K. Y. Chavan, *Laboratory Attendant-2*

CLINICAL RESEARCH LABORATORY

Dr. R. Gajbhiye, *Scientist 'D'*
Dr. P. Kuppusamy, *Scientist B*
Mr. A. Vadigopulla, *Sr. Technical Officer-3*
Ms. Shagufta A. Khan, *Sr. Technical Officer-2*
Ms. Vaishali Chalke, *Laboratory Assistant*

GAMETE IMMUNOBIOLOGY

Dr. Priyanka Parte, *Scientist 'F'*
Dr. Kushaan Khambata, *Scientist 'B'*
Ms. Smita Yevate, *Technical Officer*
Mr. M. J. Marchande, *Technician 'C'**
Mr. M. T. More, *Laboratory Assistant*
Mr. D. G. Gaikwad, *Laboratory Assistant*
Ms. Veena Dalvi, *Ph. D. student*

* Retired on 31.08.2021

Ms. Pratibha Verma, *Ph. D. student*

Mr. A. Patankar, *Ph. D. student*

Ms. Isha Singh, *Ph. D. student*

Ms. Durva Panchal, *Ph. D. student*

GENETIC RESEARCH CENTER

Dr. S. Pande, *Scientist 'D'*

Dr. V. Bhanothu, *Scientist 'B'*

Ms. Shaini Marina Joseph, *Scientist 'C'*

Dr. D. V. S. Sudhakar, *Scientist 'C'*

Ms. Seema Kadam, *Sr. Technical Officer-2*

Mr. H. M. Gawde, *Sr. Technical Officer-2*

Ms. Neha Minde, *Sr. Technical Officer-2*

Ms. Shiny Babu, *Sr. Technical Officer-2*

Mr. K. Mali, *Laboratory Assistant*

INFECTIOUS DISEASES BIOLOGY

Dr. R. Gajbhiye (*Additional Charge*), *Scientist 'D'*

Dr. Sadhana Gupta, *Scientist 'C'*

Mr. A. K. Tembhurne, *Ph. D. student*

Mr. P. Kumar, *Ph. D. student*

Ms. Samruddhi Ranmale, *Ph. D. student*

Ms. Puja Kumari, *Ph. D. student*

INNATE IMMUNITY

Dr. Taruna M. Gupta, *Scientist 'F'*

Mr. R. D. Shinde, *Laboratory Assistant*

Ms. Aishwarya Rao, *Ph. D. student*

Ms. Kasturi Ganguly, *Ph. D. student*

Ms. Hajra Gupta, *Ph. D. student*

MOLECULAR AND CELLULAR BIOLOGY

Dr. D. N. Modi, *Scientist 'F'*

Dr. Nupur Mukherjee, *Scientist 'C'*

Ms. Sarika Ahire, *Technical Officer **

Mr. S. G. Sakpal, *Laboratory Assistant*

Mr. A. Dixit, *Ph. D. student*

Ms. Anuradha Mishra, *Ph. D. student*

Ms. Neha Singh, *Ph. D. student*

Ms. Nancy S. Achary, *Ph. D. student*

Ms. Richa R. Sharma, *Ph. D. student*

Mr. A. Bhide, *Ph. D. student*

Ms. Mamta S Sawant, *Ph. D. student*

MOLECULAR ENDOCRINOLOGY

Dr. Srabani Mukherjee, *Scientist 'F'*

Dr. Pallavi Shukla, *Scientist 'D'*

Ms. Sushma Khavle, *Sr. Technical Officer-1*

Ms. Gayatri Shinde, *Sr. Technical Officer-1*

Ms. Nanda Joshi, *Technical Officer*

Mr. P. P. More, *Technician 'C'*

Mr. V. M. Khedekar, *Laboratory Assistant*

Ms. Krutika M. Patil, *Ph. D. student*

Mr. A. Naigaonkar, *Ph. D. student*

Ms. Snehal Bhingardev, *Ph. D. student*

MOLECULAR IMMUNOLOGY AND MICROBIOLOGY

Dr. V. Bhor, *Scientist 'E'*

Dr. K. C. Itta, *Scientist 'C'*

Dr. Clara Aranha, *Sr. Technical Officer-3*

Ms. Gauri Bhonde, *Sr. Technical Officer-1*

Mr. S. D'souza, *Laboratory Assistant*

Ms. Nandini J. Kasarpalkar, *Ph. D. student*

Ms. Parul S. Bedi, *Ph. D. student*

Ms. Kalyani A. Karandikar, *Ph. D. student*

Mr. P. Devadiga, *Ph. D. student*

Ms. Jyoti S. Batgire, *Ph. D. student*

NEUROENDOCRINOLOGY

Dr. Nafisa H. Balasinor, *Scientist 'G'*

Dr. Dipti Singh, *Scientist 'D'*

Dr. Kumari Nishi, *Scientist 'B'*

Ms. Vaishali Nakhawa, *Sr. Technical Officer-2*

Ms. Shobha Sonawane, *Sr. Technical Officer-1*

Ms. Reshma Gaonkar, *Technical Officer*

Ms. Zakiya Ansari, *Technical Assistant**

Mr. M. G. Pawar, *Technician 'C'*

Mr. S. Mandavkar, *Technician 'C'*

Mr. D. B. Shelar, *Technician 'C'*

Mr. V. V. Chavan, *Technician 'C'*

Ms. Sweta Mohan, *Ph. D. student*

Ms. Mamata V. Datar, *Ph. D. student*

Ms. Sandhya G. Nair, *Ph. D. student*

Ms. Sanketa Raut, *Ph. D. student*

Ms. Delna Irani, *Ph. D. student*

* Transferred from NIREH on 31.10.2021

* Transferred from NIREH on 27.01.2022

Ms. Deepashika Arya, *Ph. D student*
 Ms. Anushruti Singh, *Ph. D student*

OPERATIONAL RESEARCH

Dr. S. L. Chauhan, *Scientist 'G'*
 Dr. Beena Joshi, *Scientist 'F'*
 Dr. Ragini Kulkarni, *Scientist 'E'*
 Dr. G. Srimathi, *Scientist 'B'**
 Dr. M. K. Bhavya, *Scientist 'B'**
 Mr. I. S. Mashal, *Technician (2)*
 Mr. P. S. Sanap, *Technician A*
 Smt. M. S. Kumre, *Laboratory Assistant*

PRECLINICAL REPRODUCTIVE AND GENETIC TOXICOLOGY

Dr. V. D. Dighe, *Scientist 'E'*
 Mr. S. V. Jadhav, *Sr. Technical Officer-2*
 Ms. Shilpa C. Kerkar, *Sr. Technical Officer-1*
 Mr. P. S. Salunkhe, *Sr. Technician-1*
 Mr. N. B. Shelar, *Laboratory Assistant*
 Ms. Yugandhara Jirwankar, *Ph. D. student*
 Ms. Bhavana Bhat, *Ph. D. student*
 Mr. A. Tiwari, *Ph. D. student*

REPRODUCTIVE ENDOCRINOLOGY AND INFERTILITY

Dr. S. L. Chauhan, *Scientist 'G'*
 Dr. Anushree Patil, *Scientist 'E'*
 Dr. Suchitra Surve, *Scientist 'C'*
 Dr. Kiran Munne, *Scientist 'B'*
 Dr. Deepti Tandon, *Scientist 'B'*
 Ms. Pratibha Kokate, *Sr. Technical Officer-2*
 Ms. Leena Tendulkar, *Sr. Technical Officer-2*
 Ms. Varsha Tryambake, *Sr. Technical Officer-2*
 Ms. Sunita Kale, *Sr. Technical Officer-2*
 Ms. Shobha Banage, *Sr. Technical Officer-2*
 Ms. Rachna Dalvi, *Sr. Technical Officer-2*
 Ms. Sunita Kharat, *Sr. Technician-1*
 Ms. Anamika Akula, *Sr. Technician-1*
 Ms. Sharmila Kamat, *Technician A*
 Ms. Sunita Kendre, *Laboratory Assistant*

* Appointed on 18.10.2021

** Appointed on 14.03.2022

Ms. Akshaya A. Rathod, *Nursing Orderly*
 Ms. Shalini S. Lambade, *Nursing Orderly*
 Ms. Sarita Bhange, *Nursing Orderly*

STEM CELL BIOLOGY

Dr. Deepa Bhartiya, *Scientist 'G'**
 Dr. D. K. Das, *Scientist 'E'*
 Dr. Shyla Ravindran, *Sr. Technical Officer-1*
 Ms. Sandhya A, *Technical Officer*
 Mr. S. Gondhali, *Laboratory Assistant*
 Ms. Ankita Kaushik, *Ph. D. student*
 Ms. Diksha Sharma, *Ph. D. student*
 Ms. Pushpa Singh, *Ph. D. student*
 Mr. B. R. Shekhar, *Ph. D. student*
 Ms. Debolina Saha, *Ph. D. student*
 Ms. Aishwarya Nithyandan, *Ph. D. student*
 Ms. Mousumi Bal, *Ph. D. student*

VIRAL IMMUNOPATHOGENESIS

Dr. V. Patel, *Scientist E*
 Dr. D. Gogoi, *Scientist 'B'*
 Ms. Varsha Padwal, *Sr. Technical Officer-3*
 Ms. Shilpa M. Velhal, *Sr. Technical Officer-2*
 Ms. Jacintha Pereira, *Sr. Technical Officer-2***
 Mr. S. T. Bhagat, *Technical Officer*
 Mr. R. B. Kadam, *Sr. Technician-3****
 Mr. G. A. Shinde, *Laboratory Assistant*
 Mr. A. K. Singh, *Ph. D. student*
 Ms. Snehal Kaginkar, *Ph. D. student*
 Ms. Harsha Palav, *Ph. D. student*
 Ms. Shilpa Bhowmick, *Ph. D. student*

EXPERIMENTAL ANIMAL FACILITY

Dr. D. K. Das, *Scientist 'E'*
 Dr. S. M. Metkari, *Sr. Technical Officer-3*
 Mr. H. V. Koli, *Sr. Technician-3*****
 Mr. S. C. Gondhalekar, *Sr. Technician-1*
 Mr. H. Raut, *UDC*

* Retired on 30.07.2021

** Retired on 30.06.2021

*** Retired on 30.06.2021

**** Retired on 30.04.2021

Mr. P. G. Tawade, *Laboratory Assistant*
Mr. K. Naik, *Laboratory Assistant*
Mr. A. S. Hatle, *Laboratory Assistant*
Mr. S. D. Ghadigaonkar, *Laboratory Assistant*
Mr. Y. Joshi, *Laboratory Assistant*
Mr. G. C. Patil, *Laboratory Attendant-2*
Mr. P. R. Chavan, *Laboratory Attendant-2*
Mr. R. S. Sandis, *Laboratory Attendant-2*
Mr. V. V. Pawar, *Laboratory Attendant-2*
Mr. S. B. Bavdane, *Laboratory Attendant-2*
Mr. K. V. Kadam, *MTS*
Mr. P. K. Shingare, *MTS*
Mr. S. S. Chavan, *MTS*
Mr. S. S. Kadam, *MTS*
Mr. B. Koli, *MTS*
Mr. M. S. Qureshi, *MTS*
Mr. R. S. Merchande, *MTS*
Mr. Y. Shinde, *MTS*
Mr. M. V. Mali, *MTS*

CONFOCAL FACILITY

Dr. Nafisa H. Balasinor, *Scientist 'F'*
Ms. Shobha Sonawane, *Sr. Technical Officer-1*
Ms. Reshma Gaonkar, *Technical Assistant*

DNA SEQUENCING FACILITY

Dr. Srabani Mukherjee, *Scientist 'F'*
Ms. Nanda Joshi, *Technical Assistant*

ELECTRON MICROSCOPY

Dr. Dipty Singh, *Scientist 'C'*
Dr. Kumari Nishi, *Scientist 'B'*
Ms. Vaishali Nakhwa, *Sr. Technical Officer-2*
Mr. M. G. Pawar, *Technician 'C'*

ETHICS COMMITTEE

Dr. Vikrant M. Bhor, *Scientist 'E'*
Ms. Vaishali Bhogate, *Sr. Technical Officer-1**
Ms. Zakiya Ansari, *Technical Assistant***
Mr. A. H. Hankare, *Laboratory Assistant*

* Transferred from NIREH on 27.09.2021

** Transferred from NIREH on 27.01.2022

FLOW CYTOMETRY

Dr. Srabani Mukherjee, *Scientist 'F'*
Ms. Sushma Khavale, *Sr. Technical Officer-1*
Ms. Gayatri Shinde, *Sr. Technical Officer-1*

ACCOUNTS

Mr. S. S. Surve, *Accounts Officer**
Ms. Supriya Lad, *Section Officer*
Mr. M. K. Kanukuntla, *Office Assistant*
Mr. V. M. Guram, *Sr. Technician-1*
Mr. S. Gaikwad, *UDC*
Ms. Swara Zagde, *UDC*
Mr. M. Gawit, *UDC*

ADMINISTRATION

Mr. A. S. Gaikwad, *Sr. Administrative Officer***
Mr. S. D. Mate, *Administrative Officer*
Mr. A. R. Magade, *Section Officer****
Mr. D. D. Salunkhe, *Section Officer*****
Mr. P. K. Chavan, *Section Officer*
Mr. A. Sarnaik, *Office Assistant*
Mr. S. A. Sangelkar, *Office Assistant*
Mr. K. Pawar, *UDC*

ESTABLISHMENT

Ms. Sunayna L. Barde, *Sr. Private Secretary*
Ms. Kranti S. Patankar, *Personal Assistant*
Mr. S. Sasi kumar, *Personal Assistant*
Ms. Harsha Kurup, *UDC*

ICMR INTERNATIONAL HOSTEL AND STAFF QUARTERS

Ms. Vasanthi Rajan, *Sr. Technical Officer-3*
Mr. G. R. Devadiga, *Sr. Technician-3*

INSTRUMENTATION RESEARCH & MAINTENANCE / WORKSHOP

Dr. Susan Thomas, *Scientist 'E'*
Mr. V. D. Koli, *Sr. Technical Officer-2*
Mr. J. Patharwat, *Sr. Technical Officer-1*

* Retired on 31.12.2021

** Transferred from NIV on 23.12.2021

*** Retired on 31.12.2021

**** Transferred to NIIH on 13.08.2021

Mr. K. R. Sukumar, *Technical Assistant**
 Mr. V. G. Rane, *Sr. Technician-2*
 Mr. A. Anglekar, *MTS*

LIBRARY AND INFORMATION CENTER

Dr Prabhjeet Kaur, *Library & Information Officer*
 Ms. Simmy Saji, *Sr. Technical Officer-1*
 Ms. Priya Menon, *Technical Officer*
 Mr. V. L. Shinde, *Technician-2*
 Mr. S. A. Gavvas, *Laboratory Assistant*

PAY BILL SECTION

Mr. K. T. Solanki, *Section Officer*
 Mr. V. M. Satav, *Office Assistant*
 Mr. H. V. Jadhav, *Office Assistant*
 Mr. S. T. Chorage, *UDC*

PROJECT CELL

Mr. K. T. Solanki, *Section Officer*
 Mr. S. S. Sawant, *UDC*
 Mr. I. M. Jagirdar, *UDC*
 Mr. M. M. Shinde, *UDC*

SECURITY AND MAINTENANCE

Mr. J. D. Lobo, *Sr. Technician-3*
 Ms. Swaruparani Karunakaran, *Office Assistant*
 Mr. G. Mestry, *Sr. Technician-3***

* *Transferred from NIREH on 14.02.2022*

** *VRS on 31.08.2021*

Mr. N. S. Bhilare, *Sr. Technician-2*
 Mr. G. P. Narayan, *Sr. Technician-2*
 Mr. S. L. Shrivastava, *Sr. Technician-1*
 Mr. S. S. Subramanian, *Technician-2*
 Mr. S. K. Jadhav, *Technician-2*
 Mr. M. Palande, *Laboratory Assistant*
 Mr. A. D. Bhandwalkar, *Laboratory Attendant-2*
 Mr. A. Y. Lokhande, *Laboratory Assistant*
 Mr. S. F. Cardoza, *Laboratory Assistant*
 Mr. S. Y. Urankar, *Laboratory Assistant*
 Mr. R. Naik, *Laboratory Assistant*
 Mr. K. B. Pawar, *Laboratory Assistant*
 Ms. K. M. Rabhadiya, *Laboratory Assistant**

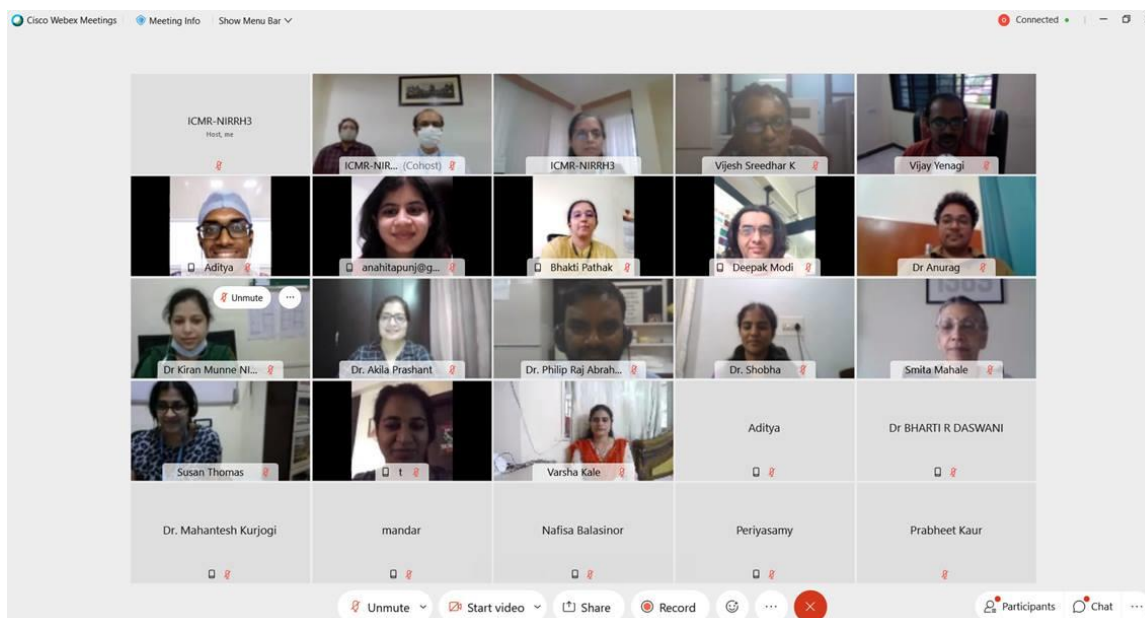
STORES

Mr. R. D. Bhandwalkar, *Administrative Officer***
 Ms. Akansha A. Dalvi, *Section Officer*
 Mr. G. M. Darpe, *Office Assistant*
 Mr. K. Rama Rao, *Office Assistant*
 Ms. Mamta Jadhav, *UDC*
 Mr. S. Petkar, *UDC*
 Mr. K. Keni, *Laboratory Assistant*
 Mr. N. P. Bavdane, *Laboratory Attendant-2*

* *Retired on 30.06.2021*

** *Retired on 31.01.2022*

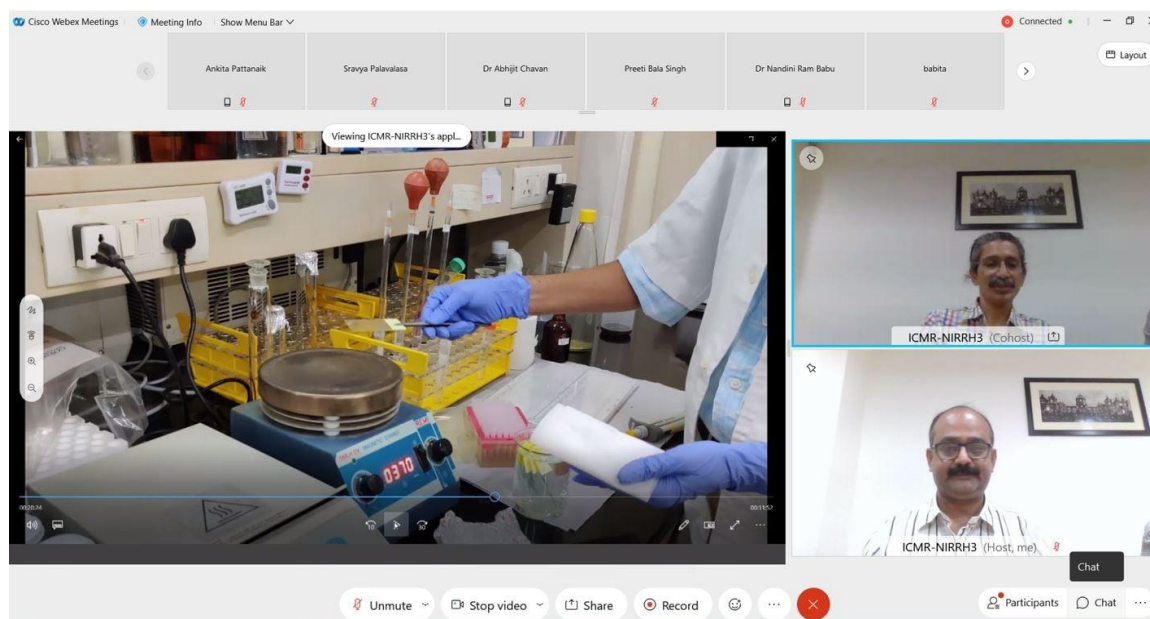
18. ACITIVITIES DURING THE YEAR 2021-2022



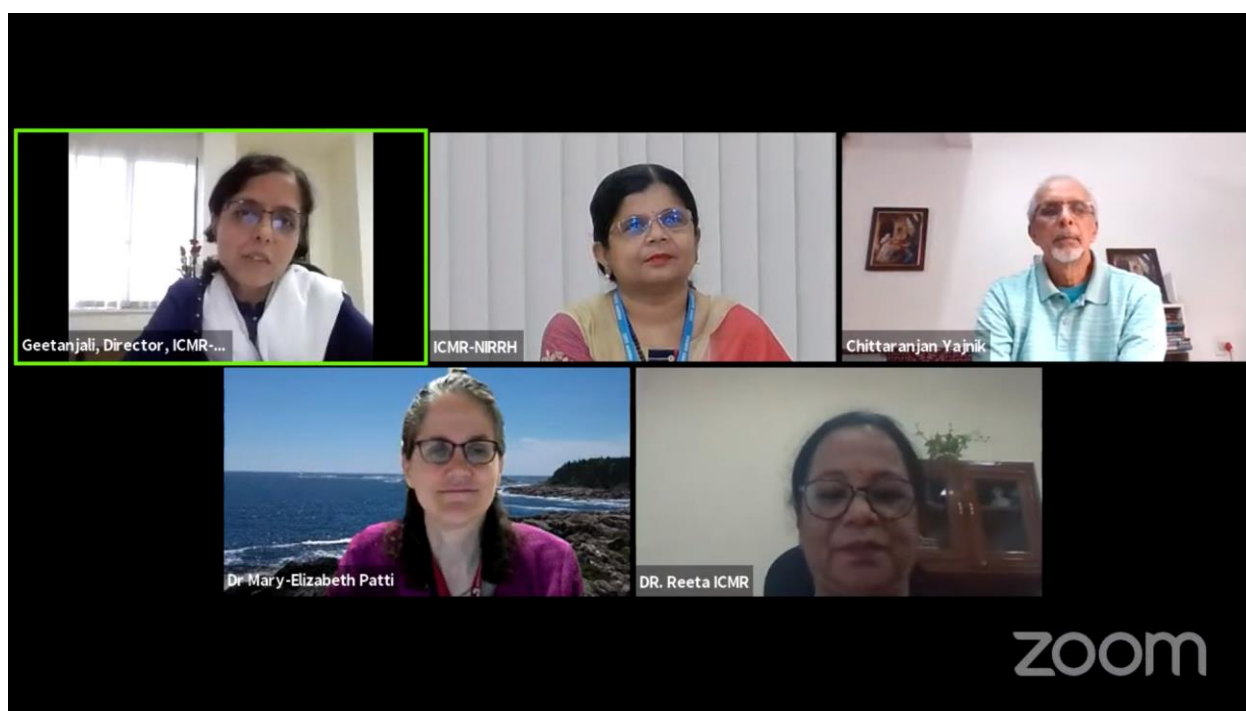
DHR sponsored training course on Applications of Technologies in Genomics for Clinical Research (ATGC) held virtually from May 3-28, 2021.



Our staff and students pledged not to smoke/consume/use any tobacco products and motivate others for the same on World No Tobacco Day on May 31, 2021.



DHR sponsored training course on Medical Genetics held virtually from July 5–30, 2021.



An International E-Symposium on Pregnancy Leaves its Marks was held on September 24, 2021.



Staff and students participated in the Fit India Freedom Run 2.0 flagged off by Dr Geetanjali Sachdeva, Director ICMR-NIRRH, on September 28, 2021.



Interactive activity session with games was organized for school children along with an awareness lecture for their mothers at our Child Health Clinic on November 17, 2021.



Technology to diagnose the cause of male infertility (Y chromosome microdeletions), handed over to the industry partner APS Lifetech on February 17, 2022.



Union Minister of Health and Family Welfare Dr Mansukh Mandaviya along with Union Minister of State for Health and Family Welfare Dr Bharati Pawar visited the high throughput COVID-19 testing facility at the institute on February 19, 2022.



Visit of Union Minister of Health and Family Welfare Dr Mansukh Mandaviya along with Union Minister of State for Health and Family Welfare Dr Bharati Pawar to the institute and its laboratories on February 19, 2022.



Satyanarayana pooja and aarti were conducted to celebrate our 52nd Foundation Day on February 21, 2022.



DST STUTI sponsored awareness program and tour of the R&D equipment/facilities was arranged for students from Kendriya Vidyalaya, Colaba, on February 23, 2022.



Training workshop on ICMR - National Snakebite Project (INSP) capacity building of health systems on prevention and management of snakebite envenomation was held on February 23, 2022.



National Science Day was celebrated with an Inter-College Quiz on the theme 'Physics meets Biology' on February 28, 2022.



An awareness talk on reproductive tract infections, screening of oral, cervical and breast cancers and non-communicable diseases was given to more than 500 tribal women at an event organized by Kashtakari Sanghatana at Dahanu beach on March 7, 2022.



Drawing and slogan writing competitions on the theme 'Break The Bias' was organized for students of Dr DB Kulkarni Vidyalaya, Naigaon on March 7, 2022.



Mothers of students at Akanksha School were sensitized on educating girls and boys about pubertal changes among children and menstruation on March 8, 2022.