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INDIAN COUNCIL OF MEDICAL RESEARCH | NATIONAL INSTITUTE FOR RESEARCH IN REPRODUCTIVE AND CHILD HEALTH

वार्षिक प्रतिवेदन
Annual Report
2022-2023

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

ICMR - National Institute for Research
in Reproductive and Child Health

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From the Desk of the Director...

We began the year 2023 by paying tributes to our Founder Director, Dr Shanta S Rao. The year is Dr Rao's birth centenary year. The institute is a manifestation of her dreams and aspirations. To celebrate her legacy, we organized International Conference on Kaleidoscopic Insights into Reproductive and Child Health to provide a platform for researchers and stakeholders to interact and share their perspectives on current reproductive and child health issues. The conference was a roaring success where many eminent researchers working in the area of reproductive and child health participated and presented their work.

Prof (Dr) Rajiv Bahl visited the institute on November 18, 2022, after taking over the reins as the Director-General, ICMR for reviewing the ongoing research programs at the institute. He emphasized the need to pursue implementation research for effective delivery of various health care schemes with a larger goal of alleviating many morbidities associated with reproductive and child health in India. Institute's scientists with expertise in public health have been gearing up for this responsibility. They have been enthusiastically responding to various initiatives based on implementation research undertaken by the Council in recent months.

During the year, we continued with our programs on research in polycystic ovary syndrome (PCOS), endometriosis, reproductive cancers, RTI/STIs/HIV and other infections in areas of women's health. In the area of child health research, programs on CMV infection and latent tuberculosis were continued. We have initiated studies on genetic abnormalities, congenital defects and precocious puberty. A population-based birth defect surveillance has been initiated at MRHRU Dahanu in seven rural blocks of Palghar district, Maharashta to estimate prevalence of birth defects in linkage with Rashtriya Bal Swasthya Karyakram (RBSK) Programme. A community-based study in urban slums in collaboration aims to screen children in 5-12 years age group at risk for latent tuberculosis infection (LTBI) to estimate the prevalence is also underway. Another study on neonatal screening aims to identify infants with sickle cell disease, for whom early diagnosis, parental education, prophylactic penicillin and comprehensive medical care markedly reduce morbidity and mortality.

A longitudinal cohort study provided important insights into the impact of different contraceptive methods on the vaginal microbiota. Another study revealed therapeutic and prophylactic potential of a recombinant fragment of human SP-D (rfhSP-D) in the hamster and transgenic mice model of COVID-19.

Various studies pertaining to maternal health are also being carried out at the institute, which comprise areas like high-risk pregnancy factors, pre-conception care, recurrent pregnancy losses, iron deficiency in pregnancy, pre-term births, preeclampsia etc. Initial data indicate the cost-effectiveness of Inj FCM in treating Iron deficiency anemia in pregnancy. We also hope that placental growth factor (P_LGF) would prove to be a useful marker to predict preterm deliveries with small-for-gestational age (SGA) infants. Effect of paternal epigenetic factors on pregnancy outcomes is also being studied.

Our studies on reproductive tract cancers encompass pathophysiological mechanisms underlying prostate, breast, ovarian, and cervical cancers. We learnt that effective implementation of non-communicable diseases and common cancer screening in tribal regions is feasible through the health and wellness centers and National Programme for Prevention and Control of Cancer, Diabetes, Cardiovascular diseases and Stroke (NPCDCS). Community involvement with health education, engaging ASHAs with incentives and upgrading infrastructure as well as trained manpower are keys for successful implementation.

The institute has been entrusted with the responsibility of establishing Model Rural Health Research Unit (MRHRU) at Vani, Nashik, to address local health burden through research. Under ICMR National Taskforce Snakebite project, a future road map for the national program on prevention and control of snakebite in India was provided to Niti Ayog. IEC materials on snakebite, training manual for healthcare workers, and snakebite treatment flow chart for Medical Officers were prepared and circulated in various regional languages for dissemination in different states. We are happy to share that the National Health Mission has directed the

States to procure and use three new Point of Care Sickle Cell diagnostic tests after their evaluation for cost-effectiveness through HTA study conducted at the institute.

Human resource development continues to be our mission. This year we organized two DHR sponsored training courses, each of 4-week duration, on Pediatric Medical Genetics and Omics in Biomedical Research and Clinical Practice for clinicians and researchers from medical colleges across India. We continued disseminating our research observations on different platforms and published 91 research articles in 2022 in peer-reviewed journals.

I want to express my gratitude towards Secretary, Department of Health Research, Government of India and Director General, ICMR, for his encouragement and support. I am also thankful to Sr Deputy Director General (Administration), ICMR; Senior Financial Advisor, ICMR; Head and Staff of the Reproductive, Child Health & Nutrition Division, ICMR for their unwavering support and cooperation. I thank Dr Neerja Bhatla, chairperson and other members of the Scientific Advisory Committee and also experts of the Ethics Committee for Human Studies, Institutional Animal Ethics Committee, Institutional Committee for Stem Cell Research and Scientific Review Committee for providing their valuable time, critiques, suggestions and guidance. We truly appreciate their engagement with our research.

On a more personal note, I thank our scientific, technical, administrative, security, housekeeping staff and students for dedicating themselves towards the growth of the institute. It is because of their continuous efforts and enthusiasm that the institute has been able to tread the path of excellence. I look forward to their continued support in the years to come.

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

PCOS is prevalent condition in women of reproductive age, characterized by hyperandrogenemia, skewed gonadotropins, and other reproductive and metabolic anomalies. Genome wide DNA methylation analysis of granulosa cells (GC) revealed differential methylation of several miRNAs coding genes in women with PCOS, suggesting that epigenetic dysregulation of miRNAs may be crucial for follicular development and may contribute to ovarian defects in PCOS. miR-10b and miR-127 genes were hyper methylated and their expression was reduced while miR-182 and miR-140 were found to be hypo-methylated with increased expression. The target gene of miR-10b AMOTL2 was upregulated in GCs of women with PCOS. This indicates that methylation of miRNA genes could potentially alter their expression and also expression of their target genes.

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

Granulosa Cells (GCs) uptake and metabolize glucose and supply energy substrates to oocyte as occyte has limited capacity to utilize glucose. This metabolic cooperation is regulated by gonadotropins and insulin. Many women with PCOS are infertile and opt for IVF, however IVF outcome is poor which can be attributed to poor oocyte quality; and altered metabolism may contribute towards this. We studied basal and PON1 (multifactorial antioxidant) stimulated glucose uptake in GCs and found it to be lower in PCOS women. The transcript and protein levels of GLUT4, a factor responsible for insulin mediated glucose uptake, were found low in the GCs from PCOS women. Levels of advanced glycation end products were higher in GCs of PCOS which can affect the function of GC.

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

PCOS women have been reported to have a high thromboembolic risk characterized by an imbalance of the thrombogenic and antithrombogenic components of the coagulation system. An increased risk of cardiovascular disease may be due to altered levels of coagulation and fibrinolysis markers, leaning towards a hypercoaguable prothrombotic state in PCOS as compared to controls. This study was undertaken to assess factors regulating the equilibrium between coagulation and fibrinolysis in plasma and follicular fluid in PCOS. No major difference was observed in clotting assays however fibrinolytic factors were found to be altered at both systemic and ovarian levels in women with PCOS compared to controls. This may affect clot dissolution in PCOS.

1.4 Unraveling Pathogenetic Mechanisms of PCOS by Whole Exome Sequencing

High throughput studies have been performed globally to trace the genetic cause of PCOS. However, conclusive genetic markers for PCOS are yet to be established. As ethnicity shapes disease propensity, high throughput genetic analyses carried out for other populations do not necessarily reflect genetic susceptibility of Indian women to PCOS. Therefore, Whole Exome Sequencing (WES) study has been undertaken to determine an association of genotypes with PCOS-related phenotypes and identify the best candidate genes for determining predisposition to PCOS. Our analysis revealed association of known genes (known and new variants) and novel genes with PCOS susceptibility. Both polymorphic and rare variants identified in the study, influence important pathways in PCOS pathophysiology including gonadotropin imbalance, steroidogenesis, insulin resistance, oxidative stress, lipid metabolism, oocyte development. Some of these genes are reported to be involved in the regulation of transcriptional and epigenetic mechanisms. Some of the identified genes play role in sex determination and infertility. In-depth analysis is ongoing.

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

Role of mitochondria in the etiopathophysiology of polycystic ovary syndrome (PCOS), a common cause of infertility is not understood fully. The study revealed that mitochondrial DNA (mtDNA) copy number was significantly reduced in women with PCOS who were insulin resistant (PCOS-IR) compared to women with PCOS who were insulin sensitive (PCOS-IS) and non-PCOS-IR group. Furthermore, mtDNA copy number in PCOS-IS was significantly reduced compared to non-PCOS IS. This implies that mitochondrial dysfunction in women with PCOS plays a crucial role in its pathophysiology, more importantly in insulin resistant women. Comparison of mtDNA copy number between frank PCOS and normoandrogenic PCOS phenotypes identified that frank phenotype showed reduced mtDNA copy number compared to normoandrogenic PCOS phenotype though the change was not significant.

1.6 Study of Maternally Inherited Mitochondrial DNA Variants in Women with Polycystic Ovarian Syndrome

Around 20%-40% of women with PCOS have an affected mother or sister with PCOS. As mitochondrial DNA (mtDNA) is exclusively maternally inherited, mtDNA variants may contribute to missing heredity of PCOS and may be implicated in the pathogenesis of PCOS. The study groups included PCOS mother daughter pairs as following; Group I: PCOS Daughters- PCOS Mothers, Group II: PCOS Daughters- Non-PCOS Mothers, Group III: Non-PCOS daughters- Non-PCOS mothers. The study revealed significant mtDNA copy number alterations in PCOS daughters with non-PCOS mothers compared to non-PCOS daughters with non-PCOS mothers. No significant difference was found in mtDNA copy number when compared among other groups. The mtDNA variant sequencing analysis in mother-daughter pairs is also ongoing. Data from increased sample size is needed to reach a definite conclusion

1.7 Implementation of Multidisciplinary Intervention Model (MDIM) to Improve Reproductive and Health Outcomes in a Cohort of Adolescent and Infertile Women with Polycystic Ovary Syndrome (PCOS)

ICMR-NIRRCH has established a multidisciplinary model of care for women with PCOS is one of its kind in India and a unique platform for services and research in PCOS. The Multidisciplinary PCOS Clinic provides holistic care under one roof for lifestyle management (with diet and Yoga), cosmetic concerns, and psychological issues, reproductive and metabolic co-morbidities. The clinic data of 447 women with PCOS was analyzed. Hypothyroidism (TSH>2.5µIU/ml) was present in 56.6%, hyperprolactinemia (s. prolactin >25ng/ml) in 6.9%, raised 17(OH)P >2.5ng/ml in 6.5% women, biochemical hyperandrogenemia with FAI (>4.59%) in 41.8% women. Hyperinsulinemia (HOMA-IR >2.42) in 61.1%, metabolic syndrome as per Indian Consensus criteria in 27.6%, Non Alcoholic Fatty Liver (NAFL) in 24.3% of women. Acne in 57%, hirsutism in 50% anxiety in 54.1% and depression in 34.1% of women with PCOS. All women were treated with a multidisciplinary approach, which improved in most of the parameters.

1.8 Uterine Alarmins and their Relevance in Implantation

The present study was undertaken to determine whether alterations in the level of HMGB1 during embryo implantation alters the uterine immune profile in rats. In the reporting year, the frequency of uterine macrophages was assessed. A reduction in the frequency of activated M2 macrophages (CD163+CD86+MHCII+) was observed in the endometrium of HMGB1-treated animals. Furthermore, we observed a decrease in the number of DBA-lectin+ uterine NK cells in the HMGB1 treated rats. These results suggest that an excess of HMGB1 in the uterine microenvironment during the preimplantation period leads to altered immune profile may contribute to implantation failure. Studies are being performed to determine the levels of HMGB1 and S00A8 in the uterine fluid of women with idiopathic Recurrent Implantation Failure (iRIF) and assess the frequency of macrophages in the endometrial tissues of women with iRIF. These studies will help in identifying the causes of implantation failure in women with iRIF.

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

Endometrial repair during menstruation is a highly dynamic process that occurs simultaneously during active shedding of endometrium under highly inflammatory conditions. Endometrial repair during menstruation is hormone independent process. Impairment in the endometrium repair leads to heavy menstrual bleeding or Menorrhagia. The aim of this study is to understand the mechanism of endometrial repair and whether molecules released from shedding endometrium orchestrate with inflammatory cells for efficient repair of endometrium. In an ovariectomized rat, decidualized endometrium initiated breakdown at 8-24 hours post-progesterone withdrawal and complete repair of endometrium by the re-epithelization takes place in 48 hours. The expression and release of damage associated molecules (HMGB1) was increased at 8-24 hours post-progesterone withdrawal. The expression of RAGE (the receptor for HMGB1) was also increased at 8-24 hours post-progesterone withdrawal. Inhibition of HMGB1 release and RAGE signaling by HMGB1 antagonist, glycyrrhizin and FPS-ZM1 delayed the endometrial repair at 48 hours. Study is ongoing to elucidate the role of HMGB1-RAGE axis modulation in endometrial repair.

1.10 Studies to Evaluate the Effect of Metformin on Endometrial Functions

Metformin, primarily an anti-diabetic drug, is reported to improve pregnancy outcomes in women with metabolic reproductive disorders such as PCOS. This study primarily aimed to analyze the direct effects of metformin on endometrial functions. Previously we had shown that therapeutic concentrations of metformin positively regulate *in vitro* proliferation of endometrial epithelial cells. In the reporting year, we investigated the effects of metformin in an *in vivo* rat model of thin endometrium. Rat model of thin endometrium was developed by ethanol perfusion into uterine horns. Oral administration of metformin to thin endometrium rats led to the restoration of endometrial thickness. Histological analyses revealed increased endometrial thickness in rats treated with 0.1 and 1% concentrations of metformin. This was further confirmed by immunohistochemical localization of a cell proliferation marker (PCNA protein). However, thickness restored by metformin treatment did not improve the embryo implantation and pregnancy in thin endometrium rat model. Further studies are underway to determine the effects of metformin on endometrial angiogenesis and vascularization.

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

Endometriosis is a condition in which eutopic endometrium like cells are found outside the uterus. Several reports suggest higher level of inflammatory and oxidative factors in the peritoneal fluid from women with endometriosis. However, data on DNA damage in the eutopic endometrium from women with endometriosis are scanty. Our previous study reported higher DNA damage response (DDR) in the eutopic endometrium from women with endometrioma (Stage III-IV) compared to eutopic endometrium from fertile women (EUC). The present study was initiated to explore the DDR in the ectopic endometrium from women with endometriosis (EUE). Endometrial stromal cells ($CD10^+$) were isolated from the proliferative phase EUE and ectopic endometrium (ECE; Stage IV Endometrioma) from women with endometriosis. RNA isolated from these sorted cells were utilized to access the DDR status in these women. DDR genes such as DDIT3, RAD51B, MBD4 and XPC etc showed significantly higher expression in the ECE compared with EUE. Higher expression of MBD4 (Methyl CpG Binding Domain 4) was found in the EUE compared to EUC. Studies are being conducted to identify the genomic targets of MBD4.

1.12 Clinical Phenotypes and Genetic Regulation of Endometriosis in Indian Women (ECGRI)

ECGRI is the first large-scale, case-control study covering the Indian population from east/northeast, north, central, west, and southern zones. Clinical data from the 540 endometriosis cases and 540 hospital controls recruited from 5 geographical zones and 14 centers were analyzed. Significant differences were observed in the proportion of endometriosis lesion types between government and private hospitals, and between regions, especially in the North and Central zones. The risk of endometriosis was highest in the women who were underweight (BMI $\leq$ 18.5). Period irregularity, longer menstrual bleeding, heavy menstrual bleeding, and pelvic pain were associated with endometriosis. Lower pregnancy, conception rate, and infertility were significantly associated with endometriosis. Congenital anomalies of the uterus, cervix, and vagina, thyroid disorders, PCOS, hypertension, fibroids, and cancer were all significantly higher in endometriosis cases. As a

part of endometriosis awareness month, nationwide events were organized for educating the healthcare workers, and community about endometriosis symptoms.

2. MALE INFERTILITY AND ASSOCIATED REPRODUCTIVE DISORDERS

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

We previously reported lower pTSH2B levels in sperm of asthenozoospermic men. The present study addressed whether the low levels of pTSH2B reflected a reduction in the total TSH2B levels or its phosphoform. Towards this, histone retention, relative levels of total H2B, TSH2B and pTSH2B were estimated in sperm of fertile and infertile men. *H2BC1* polymorphisms were investigated. *H2BC1* gene sequencing identified two rare variants and 4 SNPs. Minor allele frequency of 5'UTR variant rs4711096 was significantly lower in infertile men. Rare non-synonymous variant rs368672899 (p.Ser5Pro) was detected in one oligoasthenoteratozoospermic individual. MS analysis revealed a phosphate group at this site of TSH2B protein in sperm of fertile men. Our study reveals a defect in replacement of somatic histones with testis-specific variants in infertile men. Chromatin compaction positively correlated with sperm motility, suggesting its utility in diagnostic semen analysis. Study indicates an essential role for TSH2B in meiosis and its phosphorylation in sperm motility, respectively.

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

Estrogenic endocrine disruptors are reported to cause adverse effects on male fertility. The present project aimed to elucidate changes in the sperm DNA methylome 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 revealed large-scale changes in sperm DNA methylation patterns after treatment with ERα (PPT)- and ERβ (DPN) agonists. Gene ontology and enrichment map analysis of differentially expressed methylated genes revealed an effect on a large network of developmental processes. For PPT, these differentially methylated genes (DMGs) were involved in transcriptional regulation; while for DPN, these DMGs were involved in cellular localisation and transport. Several of the DMGs were also found to be differentially methylated in clinical conditions of male infertility, hinting at a possible role of estrogen in dysregulation of sperm DNA methylome. To conclude, activation of ER signalling can alter the sperm DNA methylome, critically affecting male fertility and embryogenesis.

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

Varicocele is one of the risk factors associated with male infertility. Elevated oxidative stress has been implicated as a key factor in varicocele induced male infertility. High oxidative stress during spermatogenesis may affect the sperm functions and lead to aberrant epigenetic modifications. Whole Genome Bisulfite Sequencing (WGBS) of sperm genomic DNA was carried out to identify differentially methylated CpG (DMC) sites in varicocele men compared to control. A total of 6414 DMCs with highest enrichment in intergenic regions were obtained, out of these DMCs; 3730 were hyper-methylated and 2684 were hypo-methylated. WGBS analysis identified 1484 differentially methylated genes, which were further used for pathway analysis using KEGG and DAVID tools. DMCs within genes relevant to spermatogenesis, sperm function and sperm mitochondria regulation will be further selected to validate their methylation levels by pyrosequencing. This study highlights the altered methylation landscape in the sperm of varicocele men.

2.4 Transgenerational Effects of Paternal Hypertension on Fertility and Pregnancy Outcome: An Epigenetic Approach

Hypertension, a lifestyle disorder, is increasing in men of reproductive age. It is known also to affect the reproductive health. The Nω-Nitro-L-arginine-methyl ester (L-NAME)-induced hypertension is a well-established pharmacological model of hypertension. To develop L-NAME induced hypertension male Wistar rat model for fertility studies toxicity assessment of L-NAME was done. Doses of 20 mg/kg bw and 10 mg/kg bw for three weeks were found least toxic and suitable for the development of hypertension. The hypertension

was retained even after two months of the termination of L-NAME treatment. The fertility of this male hypertension animal model is being assessed.

2.5 Studying Sperm Chemotactic Behaviour using a Microfluidic Chip

We have identified N-Formyl-L-Aspartate (NFA) as a sperm chemoattractant. This year we investigated the putative mechanism/s by which NFA may mediate chemotaxis. The responses of sperm to gradient concentrations of NFA and β-2 adrenergic receptor (β-2-AR) specific antagonist (ICI-118,551) were evaluated using the microfluidics device-based chemotaxis assay developed by us. NFA exhibited a bell-shaped dose-response curve typical of chemotaxis; β-2-AR localization was detected on sperm head and mid-piece region of the flagella. Inhibition of sperm chemotaxis by ICI-118,551 confirms that sperm respond to NFA via β-2-AR. NFA increased intracellular Ca^{2+} but decreased cAMP in capacitating sperm. However, NFA *per se* did not induce capacitation as seen from the lack of effect on tyrosine phosphorylation, and membrane potential of uncapacitated sperm. Acrosome structure was not altered by NFA. Our data thus provides evidence indicating that NFA induces sperm chemotaxis, which is mediated through the β-2-AR on sperm possibly via the non-canonical signaling.

2.6 Deciphering the Role of PSP94 and CRISP Family Proteins in Ion Channel Modulation

Mammalian CRISPs or Cysteine RIch Secretory Proteins are members of the CAP superfamily that tend to show an expression bias in the reproductive tract. Functional analysis of knockouts in mice suggests their role in fertility. CRISPs have been implicated in ion channel modulation; however, direct evidence for their association remains lacking. Interactome analysis of rodent CRISP4, an epididymal CRISP, in caudal spermatozoa revealed PMCA4 as a novel binding partner. PMCA4 is the principal channel responsible for calcium extrusion and interaction with CRISP family proteins suggests probable implications in calcium homeostasis during sperm maturation and fertilization. Further experiments revealed that it interacts with multiple CRISPs via the N-terminal CAP domain which is suggestive of functional compensation in PMCA4 mediated signaling.

2.7 Pathway Analysis of Genome Wide Association Studies (GWAS) Data Associated with Male Infertility

To decipher the genetic basis of male infertility, an Identify Candidate Causal SNPs and Pathway (ICSN Pathway) analysis was pursued using a genome-wide association study (GWAS) dataset, and NCBI-PubMed search, which included 632 SNPs in GWAS and 451 SNPs from PubMed server. The ICSN Pathway analysis produced 3 hypothetical biological mechanisms associated with male infertility: (1) rs8084 and rs7192 → HLA-DRA → inflammatory pathways and cell adhesion; (2) rs7550231 and rs2234167 → TNFRSF14→ TNF Receptor Superfamily Member 14→ T lymphocyte proliferation and activation; (3) rs1105879 and rs2070959→UGT1A6→UDP glucuronosyltransferase family 1 member A6→Metabolism of Xenobiotics, androgen, estrogen, retinol and carbohydrates. The analysis suggests that genetic contribution to male infertility operates through multiple genes affecting common inflammatory diseases and metabolism of gonadal steroid hormones.

2.8 Role of Complex N Glycans and Functional Significance of Basigin (Glycoprotein Possessing N Glycans) in the Testicular Germ Cells for Spermatogenesis

N-linked glycosylation is a prime post-translational modification that plays important role in sustaining spermatogenesis and male fertility. Cell-surface proteins isolated from wild type CHO cells, and two CHO mutants with N-glycosylation defect, Lec1 (Mgat1 deficient) and Lec4 (Mgat5 deficient) cells, were analyzed by High Resolution LC/MS. The GTPase activating protein (IQGAP-1) and IGF-1R protein were significantly reduced in Lec1 cells. The docking study provided the evidence that IQ domain of IQGAP1 directly binds to the kinase domain of IGF-1R plausibly causing the downregulation of ERK signalling observed in Lec1 cells.

2.9 Cross talk of Sertoli cells and N-glycosylation-Defective Germ Cells-Impact on Male Fertility

Previously it was reported that spermatogonia with a conditional knock out of a key glycosyltransferase gene, known as MGAT1 (alpha-1, 3-mannosyl-glycoprotein2-beta-N acetylglucosaminyltransferase) causes arrest

of spermatogenesis in spermatid stage, multinucleated cells and male infertility in mice. Transmission electron-microscopy revealed that MGAT1-knockout testis have multiple abnormalities in the haploid germ cells. Most seminiferous tubules in the mutant testis were devoid of late spermatids, whereas some contained few late spermatids with multiple condensed nuclei sharing a common cytoplasm and lack of proper orientation within the epithelium. Observed abnormality in elongated spermatids with round acrosome head and irregular nuclear shapes, were akin to the testicular disorders in human. Proteome analysis of spermatids extracted from MGAT1 knock out testis revealed alterations in the CABS1, KLHL10 and PGK2, potentially involved in the spermiogenesis process.

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

This study is primarily aimed at identification and characterization of genetic causes associated with MMAF in infertile men from India. A total of 15 infertile men with asthenoteratozoospermia phenotype were recruited and semen analysis was performed. Based on the semen parameters and inclusion criteria of multiple MMAF, six infertile men were further selected for the study. Semen analysis was performed as per the WHO criteria (WHO manual, 2010) and semen samples that showed multiple flagellar defects (coiled, bent, short and absent flagella) were further processed for ultrastructure imaging using electron microscope. Flagellar ultrastructure imaging was performed for three samples and remaining three samples are currently being processed for imaging. Currently, we are recruiting study participants only from NIRRCH andrology clinic. We are planning to include a couple of centers to meet the sample numbers (n=100) required for the study.

3. MICROBES AND REPRODUCTIVE HEALTH

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

This prospective study investigated the impact of different contraceptive methods on the vaginal milieu of 40 randomly assigned women in Mumbai. Four groups were studied: copper T 380 A IUD user, condom users, Depot medroxyprogesterone injectable users, and non-users. Nugent score, vaginal cytokine profile, and microbiome analysis were used to evaluate the vaginal milieu at baseline, 90 days, and 180 days. Lactobacillus iners was the most prevalent species (43.56%), while *Gardnerella vaginalis* was the most abundant anaerobic species (7.83%). Barrier method users showed an improvement in the vaginal milieu with no changes in Nugent score and a significant decrease in *Gardnerella vaginalis* abundance at 180 days (p=0.0391). Copper T users showed a slight shift towards intermediate flora and increase in pro-inflammatory cytokine at 90-180 days. DMPA users initially had dysbiosis but improved over 180 days. Non-users had increased dysbiosis at 180 days.

3.2 Immune Correlates of HCMV Congenital Transmission in Pregnant Women with Bad Obstetric History and Pregnancy Complications

HCMV infection is associated with bad obstetric history (BOH) and adverse pregnancy outcomes (APO). We characterized antiviral humoral profiles, systemic and virus specific cellular immune responses concurrently in pregnant women (n=67) with complications including BOH and associated these signatures with pregnancy outcomes. Systemic and HCMV specific (pp65) cellular immune responses were evaluated. Seropositivity was determined for other TORCH pathogens (n=33) on samples with recorded pregnancy outcomes. We demonstrated the utility of an integrated screening approach for antenatal HCMV infection in the context of BOH, where infection is associated with systemic and virus specific cellular immune dysfunction as well as APO.

3.3 Isolation and Characterization of Biosurfactants Produced by Vaginal *Lactobacillus salivarius* and *Lactobacillus reuteri*

During the current year, characterization of biosurfactants from vaginal isolates *Lactobacillus reuteri* and *Lactobacillus salivarius* and investigation of antimicrobial, biofilm disrupting and anti-proliferative activities

of the biosurfactants were undertaken. Cell-bound and excreted biosurfactant production was observed in both Lactobacillus isolates. However, the surface activity was greater in cell-bound biosurfactants. The cell-bound biosurfactants from *L. salivarius* had greater inhibitory activity against urogenital bacteria (*E. coli, S. aureus, P. 0aeroginosa*) than *L reuteri* and no activity against fungal pathogens. Both Lactobacillus species inhibited biofilm formation and adhesion of *G. vaginalis*. These results suggest further exploration of biosurfactants of indigenous vaginal lactobacilli as biotherapeutics for reproductive tract infections. Further characterization is in progress to reveal the specific composition of the natural biosurfactants.

3.4 Molecular Characterization and Biochemical Properties of Vaginal *Lactobacillus salivarius*

Lactobacillus salivarius is a part of the indigenous microbiota of the vaginal and gastrointestinal tract (GIT) and oral cavity of humans. There is no data on the genomic variability of the species for identifying strain-specific properties. Eighteen Strains of *Lactobacillus salivarius* from a collection of vaginal lactobacillus isolates were explored to evaluate the probiotic utility of diverse *L. salivarius* strains by phenotyping and genotyping. The genomic diversity of *L. salivarius* isolates were characterized by genetic fingerprinting using random amplified polymorphic DNA (RAPD) and Multilocus Sequence Typing (MLST). Genetic fingerprinting of the strains by RAPD identified five different strains among the 18 isolates. Seven different housekeeping genes were used to identify different genotypes among the strains. Different phenotypic traits of *Lactobacillus* strains such as lactic acid and hydrogen peroxide production, inhibitory potential towards urogenital pathogens, effects on vaginal epithelial cells, biofilm formation, and adherence to vaginal cells are being investigated.

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

A study was conducted on 341 sexually active women with lower genital tract infections reporting to the Municipal STD clinic & ART Centre. *Mycoplasma genitalium* was detected in 6.7% of cases, with the highest detection rate in endocervical swab samples 26% (n=6) followed by urine sample 17.3% (n=4). *Neisseria gonorrhoea* and *Chlamydia trachomatis* were detected in 2.3% of cases. The most common coinfection with *Mycoplasma genitalium* was HCV. The Uniplex and Multiplex kits had a disparity in *Mycoplasma genitalium* detection Kit with 47.8% in concordance for endocervical swab sample among both kits. Tobacco use was a significant risk factor for *Mycoplasma genitalium* infection. Positive test of cure was seen in 66.6% of women after three months. Studies on antimicrobial resistance in collected samples will be undertaken.

3.6 Longitudinal Analysis of Integrin $\alpha_4\beta_7$ Expressing T Lymphocytes in People Living with HIV

Intestinal $CD4^+$ T lymphocytes are depleted during HIV infection, irrespective of the route of exposure. Detection of integrin $\alpha_4\beta_7$, the gut homing marker, on circulating T cells would provide an estimate of activated, exhausted and memory subset cells trafficking between the systemic circulation and the gut. Longitudinal analysis of antiretroviral therapy (ART) naïve HIV+ve individuals (n=14) recruited at Integrated Counselling and Testing Centres (ICTC) and ART centres in Mumbai was undertaken. A trend of a decrease in the frequency of integrin $\alpha_4\beta_7$ expressing $CD8^+$ T cells along with a decrease in the frequency of integrin $\alpha_4\beta_7$ expressing activated $CD8^+$ T lymphocytes ($CD38^+$ and $HLADR^+$) was observed after 6 month of ART therapy. Conversely, an increase in integrin β_7^+ effector memory T cells was observed and suggests that these cells are likely to be infected and become latent HIV reservoirs. The altered proportion of integrin $\alpha_4\beta_7$ expressing T cell subsets following ART warrants further exploration for its suitability as a potential maker for disease progression and response to treatment.

3.7 Gut Dysbiosis is accompanied by Immune Dysregulation in HCMV Infected Infants with Neonatal Cholestasis

The interplay of active HCMV infection with gut dysbiosis in the immunopathology of neonatal cholestasis remains unexplored. In this study, gut microbiome (GM) and immune profiles were investigated in a cohort of HCMV infected cholestatic infants (IgM-positive, n=21; IgM-negative, n= 25) compared to healthy infants (n=10). HCMV infected IgM-positive individuals exhibited increased clinical severity in terms of liver dysfunction, altered $CD4^+$: $CD8^+$ ratio, and elevated Granzyme B levels in cellular immune subsets. GM

analysis revealed distinct and differential diversity and composition within infected groups aligned with clinical severity reflected through the increased abundance of Gammaproteobacteria, reduced Bifidobacteria, and a unique microbiome signature mapping to the HCMV infected IgM-negative group. These findings suggest that a synergistic effect of gut dysbiosis and immune dysregulation may influence disease severity in cholestatic infants with active HCMV infection.

3.8 Improving Treatment Literacy and Adherence among People Living with HIV (PLHIV) through Innovative Strategies

Total 280 PLHIV participants and 10 healthcare provider participants from Bhopal and Jabalpur ART centers have been recruited in the study. Both the groups have been exposed to the different interventions designed for them for the duration of 5 months. Mean score of post-intervention questionnaire for PLHIV participants has significantly increased from 9.14 at baseline to 11.90, suggesting improvement in their knowledge after being exposed to different interventions. Tenofovir blood levels measured by liquid chromatography mass spectrometry (LC-MS) were significantly associated with viral load. Pre and post intervention assessment of the PLHIV study participants showed that knowledge score, ART adherence, CD4 count and viral load had improved post-intervention and found statistically significant for knowledge score, adherence and CD4 count. Healthcare providers had good mean score of pre-intervention questionnaire (8.7) which increased post-intervention to 9.4.

3.9 $CAMP_{R4}$: A Database of Natural and Synthetic Antimicrobial Peptides

Antimicrobial peptides are gaining popularity as anti-infective agents due to their broad range activity and limited resistance. The Biomedical Informatics Centre at the institute has developed the Collection of AntiMicrobial Peptide ($CAMP_{R4}$) database that provides manually curated information on natural and synthetic antimicrobial peptides (AMPs) such as sequence, structure, protein definition, accession numbers, activity, source organism, target organisms, protein family descriptions, N and C terminal modifications and links to AMP related databases for the benefit of users (Fig. 1). It also provides family specific signatures for natural AMPs. A highlight of this release is that there are ML-based algorithms for the prediction and rational design of natural as well as synthetic AMPs. CAMP is freely accessible at http://camp.bicnirrh.res.in/.

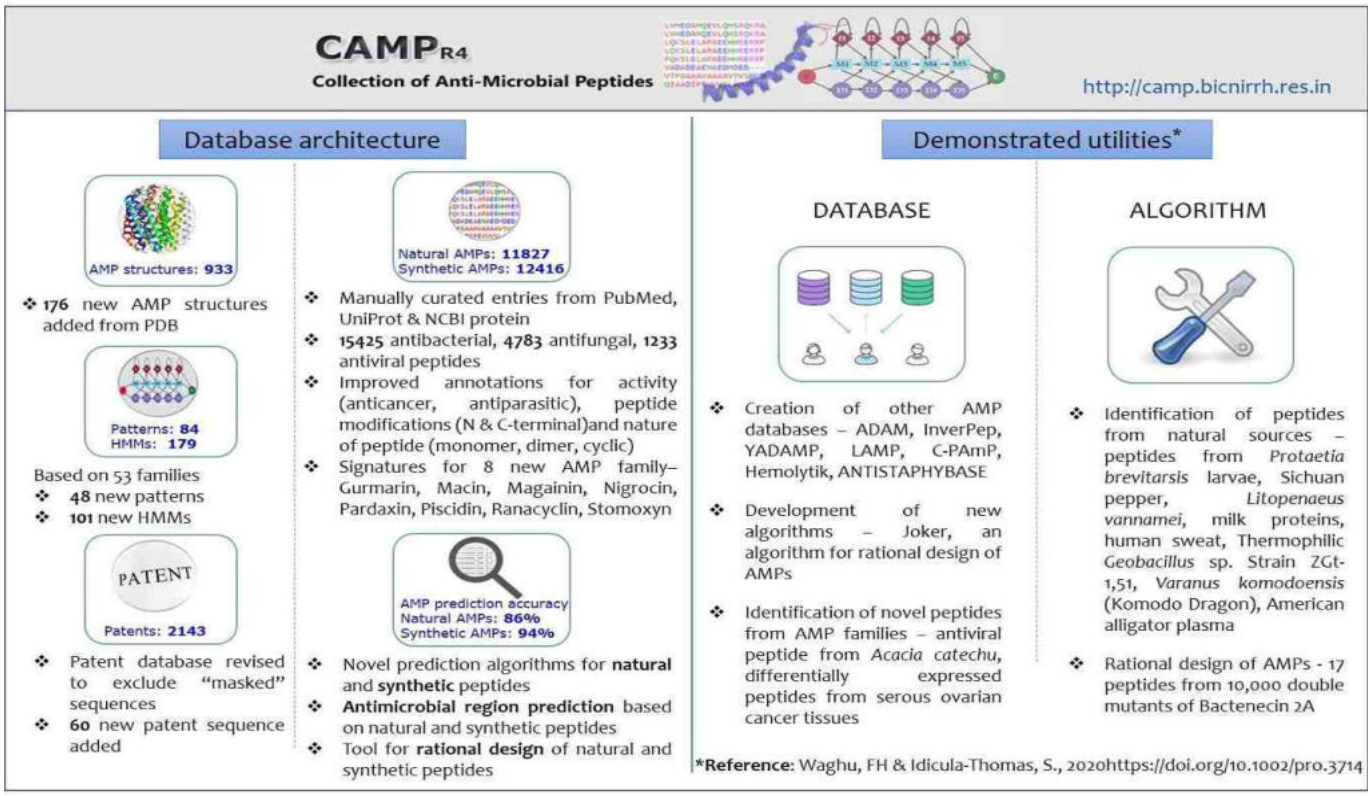


Figure 1: Overview of $CAMP_{R4}$ database.

4. MATERNAL HEALTH

4.1 Factors Associated with Increase in the Prevalence of Hypertension among Women between NFHS (2015-16) and NFHS (2019-21)

The objective of the study was to examine the drivers of the increase in hypertension among women in childbearing age (15-49 years) in India during 2015-16 and 2019-21 using large-scale NFHS data. During 2016-21, hypertension prevalence among women in reproductive age nearly doubled (from 11% to 21%). The increase in hypertension prevalence was higher among older women than younger women. There was a 2.6 percentage point increase in the prevalence of overweight women between the two surveys, which explained 17% of the increase in hypertension prevalence.

4.2 Factors Associated with Intimate Partner Violence: Insights from National Family Health Survey

The present study comprehensively investigated the initiation of intimate partner violence (IPV) defined as any physical or sexual violence after marriage. The study used data from National Family Health Survey (2019-21). Around one third (29%) of the ever married women had experienced any physical or sexual violence and the mean duration of initiation of IPV was found to be 3.3 years (SD: 3.94) in India. Around 90% of women had experienced IPV within the first 7 years of marriage. IPV prevalence was significantly more in rural (30.7%) than in urban (23.8%) areas. However, IPV within first year of marriage was experienced by 13.7% and 18.2% of women from rural and urban areas respectively.

4.3 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

The study is being carried out in MRHRU Dahanu at two secondary care public hospitals. Eligible pregnant women are randomized to either FCM or IV Iron Sucrose group. They are followed up after 4 weeks and 6 weeks post-partum to assess an improvement in hematological parameters and pregnancy outcomes. Systematic reviews have been done to (i) assess effectiveness of IV FCM on IDA in pregnancy and (ii) effectiveness of available oral iron preparations in India. Out of Pocket expenditure and Quality of life data are being collected from the enrolled participants. Out of estimated 284 participants, 184 participants were enrolled until date. Preliminary analysis reveals that FCM improves serum ferritin levels better than IV Iron Sucrose. There were no major related side effects observed in the FCM group. Minor side effects were observed in IV Iron Sucrose group.

4.4 Effect of Maternal Gestational Micronutrient Deficiency on Offspring's Fertility and its underlying Epigenetic Mechanisms in Germline

This study aims to unravel the effects of maternal methyl donor deficiency (Vitamin B12, Folic acid and methionine) during gestation on offspring's development. The pregnant female OCT4-GFP mice were fed control chow diet (CCD), 40% methyl donor deficient diet (MDD) and 60% MDD from gestation day (GD) 5 to GD20. F1 offspring were fed CCD after weaning. Developmental and molecular effects were studied on gestation day (GD) 13, GD18, post-natal day (PND) 22 and PND60. Teratological defects were observed in pups with twisted tail in 40% MDD and abnormally elongated teeth in 60% MDD as compared to control. Significant alterations in testes histopathology were observed at GD13, GD18, PND22 and PND60. Quantitative histomorphometry analysis suggested an increase in germ cells sloughing percentage and testicular atrophy percentage at PND60. Homocysteine levels were elevated in 40% MDD and reduced in 60% MDD. Global DNA methylation levels in fetal gonads were also altered in both the MDD groups. The present observations suggest that gestational methyl donor deficiency has a detrimental effect on fetal development.

4.5 Addressing Challenges for Effective Implementation of Dakshata Programme to Improve Quality of Institutional Deliveries in Tribal Blocks of Maharashtra: An Implementation Research

In order to strengthen the quality of intra-partum and immediate post-partum care, the Government of India launched the Dakshata programme in 2015. There is a paucity of data on the facilitators and barriers faced by the health system providers in implementing the Dakshata programme in tribal areas. The objective of the present study was to understand these factors in a tribal district of Palghar in Maharashtra at four high delivery load public health facilities. As per the study findings, prioritizing training of staff, adherence to safe childbirth checklist, development of obstetric ICUs, availability of human resources and emergency laboratory investigations and promotion of coherence between various levels of healthcare can aid the providers in delivering quality maternal care. These findings are relevant for policymakers to strengthen the implementation of the Dakshata program in the underserved tribal areas.

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

The project aimed to evaluate possible association of paternal exposure to endocrine disruptors (EDCs) and sperm epigenetic modifications in idiopathic recurrent pregnancy loss (iRPL) cases. Sperm DNA fragmentation and serum testosterone levels were higher whereas, no significant difference in the serum 5-methylcytosine (5-mC) and estradiol levels were observed in the iRPL group. A strong significant negative correlation of sperm DNA fragmentation index (DFI) and 5-mC in control group was observed but no significant correlation was observed in iRPL group. This could be due to other underlying DNA methylation alterations in genomic regions probably unsusceptible to fragmentation in this group. A non-significant negative correlation of serum testosterone levels and sperm DFI was observed in both groups indicating a possible protective function of testosterone in lowering chances of sperm DFI. No other significant correlation trends were observed. Further, EDCs exposure assessment is underway.

4.7 Investigating the Role of Endocannabinoid System in First Trimester Chorionic Villi of Women Experiencing Recurrent Spontaneous Abortions

Recurrent spontaneous abortion (RSA) is identified by two or more consecutive miscarriages before 20 weeks of gestation that affects about 1-2% of females of reproductive age. In the past few years, various studies on the endocannabinoid system (ECS) have demonstrated its importance during pregnancy. This study is being conducted to understand the role and mechanism of ECS in chorionic villi. Immunohistochemical studies on the expression of cannabinoid receptors CB1, CB2 and metabolic enzymes FAAH and NAPE-PLD was done on three chorionic villi tissue sections of each recurrent spontaneous abortion and medical termination of pregnancy. The CB1 was found to be expressed in both cytotrophoblasts and syncytiotrophoblasts as well as extraembryonic mesoderm of chorionic villi. The expression of CB1 in syncytiotrophoblats was found to be higher in RSA cases as compared to controls. The CB2 expression was observed only in syncytiotrophoblasts. The RSA villi had a higher CB2 expression as compared to control. The expression of FAAH was limited to syncytiotrophoblasts. Interestingly, a low FAAH immunopositivity was observed in RSA villi as compared to control. NAPE-PLD was observed in cytotrophoblasts, syncytiotrophoblasts and extraembryonic mesoderm. NAPE-PLD was found to be higher in controls as compared to RSA cases. The NAPE-PLD was found to be localized in nuclei of few syncytiotrophoblasts.

4.8 Developing an Immunochromatography Based Strip Test for Analysing PlGF Concentration for Prediction of Risk for Developing Preeclampsia

Low levels of placental growth factor (P_LGF) in circulation are associated with preeclampsia and delivery of small-for-gestational age infants. P_LGF levels in blood correlate with its level in the urine. We have determined P_LGF levels in random urine samples of healthy pregnant (HP) women in third trimester (at 28-32 weeks of

gestation) by ELISA. There was wide variation seen in urinary P_LGF levels (0-388pg/ml) of HP. Low urinary P_LGF levels (< 10^{th} percentile: 3.242 pg/ml) were found to be associated with preterm delivery as compared to high P_LGF (<37 week; 37.5% versus 6.8%, *p* value 0.028, Odds ratio 8.16). Interestingly, all the preterm deliveries in the low urinary P_LGF group resulted in low birth weight infants. Our data strongly suggests low urinary P_LGF in early third trimester to be indicative of preterm deliveries with low birth weight infants.

4.9 Omics of Placental Exosomes in Early Onset Preeclampsia: An Approach towards Identifying Predictive Biomarkers (Phase 2)

Early Onset Preeclampsia (EOPE) is a disorder of defective placentation associated with high feto-maternal mortality. Omics analysis of the isolated term placental exosomes from EOPE women (n=45) in a prospective cohort of 958 pregnant women revealed several novel factors and many previously known factors associated with the pathogenesis of EOPE in other populations. miRNA transcriptomics revealed 16 dysregulated miRNAs associated with the metabolic pathways and allograft rejection. 355 differentially methylated CpGs, majorly of mitochondrial origin, were associated with the oxidative stress, decreased trophoblast differentiation, and invasion. 23 differentially expressed proteins were majorly involved in coagulation cascade, complement system and cholesterol metabolism.

4.10 To Study the Prevalence and Risk Factors of Gestational Diabetes Mellitus: Hospital Based Retrospective Study

Gestational Diabetes Mellitus (GDM) is major public health concern and prevalence of GDM reported between 1.7% to 14% across the India. There is no consensus among health care professionals on the diagnosis and treatment of GDM. The aim of this study is to determine the prevalence, onset or diagnosis of GDM during gestation, management of GDM and maternal and neonatal outcomes. Retrospective data of 3 years from January 2017 to December 2019 were collected from Nowrosjee Wadia Maternity Hospital, Parel, Mumbai. Out of the total n=14915 total delivery records, n=1097 (7.3%) pregnancies were affected by GDM. One year (2019) data analysis of GDM pregnancies (n=332) showed 40% women were diagnosed with GDM before 23rd weeks of gestation (early diagnosis) and 60% diagnosed after 24th weeks of diagnosis (late diagnosis). Overweight (BMI >23-<24.9) and obesity (BMI>25) were reported in approximately 85% of GDM women. Further study is ongoing to understand the maternal and neonatal outcomes in early diagnosed versus late diagnosed GDM pregnant women.

4.11 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

Scant Indian community-based studies on the association of Osteoporosis and depression among middle & low-income urban menopausal women necessitated this research. A cross-sectional study design was used wherein 100 menopausal women with osteoporosis between the age group 50-75 years were selected. Patient Health Questionnaire-9 was used for screening depressive symptoms while WHO-QOL-BREF 26 used for assessing quality of life. Osteoporosis was detected by iDXA scans done at Bone Health Clinic, ICMR-NIRRCH. The mean age (yrs) in women with and without depression was 60.86 ± 5.82 SD. and 62.35 ± 5.96 SD respectively. PHQ-9 scores revealed 17% mild, 4% moderate, and only 1% moderately severe depressive symptoms. A negative correlation was seen between the physical domain of QOL and depressive symptoms among the women. Regular screening of depressive symptoms is needed in primary care setting for an early diagnosis & treatment as depression impacts the QOL. Holistic approach for treating elderly population & large-scale studies are needed to validate the link between Depression and Osteoporosis.

5. CHILD HEALTH

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

Anomalies detected in the fetus is a major reason for most couples opting for elective abortions. Most of the congenital anomalies detected in early gestation are known to have an underlying genetic etiology. Assessing Products of Conception (POC) for genetic anomalies both at the chromosomal and molecular level will aid in effective genetic counseling and management of these couples in future pregnancies. Forty-five POC samples were evaluated to delineate the genetic cause for the observed fetal anomalies. Pathogenic genetic defects were identified in approximately 30% of Products of Conception. The interim results of this study suggest genetic defects to be one of the major causes for fetal anomalies and advocate for a need to evaluate the POCs for genetic defects to enable appropriate counseling and management in future pregnancies.

5.2 Molecular Profiling of Common Clinical Phenotypes Associated with Congenital Hypothyroidism (CH)

This project focuses on identifying pathogenic genetic variants in children with Thyroid dyshormonogenesis (TDH) presenting different clinical phenotypes. Blood samples from 15 index cases (Age: 0-12 yrs) and their parents and 2 cases and their fathers were collected. 11 of the 17 children with TDH were females and 6 were males. The samples are being processed for whole exome sequencing. Exome sequencing for two samples has been carried out and results are being analyzed. This study may aid in developing a cost-effective molecular diagnostic panel, which will be disseminated to clinicians for early detection of CH.

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

The study was initiated in MRHRU Dahanu in November 2020. Currently data is being collected from public healthcare facilities and communities through ASHAs. From April 2022 till date a total of 149 birth defects were reported through facilities (112) and ASHAs (37). Blockwise distribution of birth defects reveals highest BDs in Jawhar (35), followed by Palghar (32), Dahanu (31), Vikramgadh (20), Wada (13), Mokhada (10) and Talasari (07). Commonly occurring major BDs were cleft lip and or palate (32), clubfoot (29) & CHD (18). Among other major defects found were hydrocephalous (8), NTD (8), gastrochisis and omphalocele (4), imperforate anus (2), multiple defects i.e. more than one defect including one major defect (11) and miscellaneous (37).

5.4 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

Sickle cell disease is an important public health problem in India with highest prevalence amongst the tribal ethnic groups. The study was initiated in MRHRU Dahanu in December 2019. During the reporting year, out of total 3632 deliveries at SDH Dahanu and Kasa, 2165 newborns were screened for sickle cell Disease. Amongst them, 15 babies were sickle cell homozygous (Sickle cell Disease) and 201 babies were sickle cell heterozygous (Sickle cell trait). Ninety-three babies had other haemoglobinopathies of undetermined significance. Iron profile was evaluated for babies with lower haemoglobin. It revealed that during early stages of Iron deficiency anaemia (IDA), an elevation of total iron binding capacity (TIBC) occurs before the decrease of the serum iron suggesting possibility of a compensatory mechanism mobilizing tissue iron to maintain normal erythropoiesis. Thus, raised TIBC with normal serum iron in presence of low hemoglobin may be interpreted as early IDA indicating need of iron therapy in SCD babies.

5.5 Community Based Screening and Management of Latent TB among Under-Five Children from Urban Slums in Mumbai: Phase 2 - Screening TB Contacts of Index Case in Age Group of 5-12 Years

Latent tuberculosis infection (LTBI) management is an integral part of WHO's End TB Strategy. Being a systematic approach, this study would assist policy makers to determine the actual burden of LTBI among children, especially in endemic areas like urban slums. This study also highlights general receptiveness of testing and adherence to treatment of LTBI among parents. The study was initiated in Sept 2019 and phase I (recruitment of 369 under-five children) was completed in July 2022. Throughout the reporting year, 49 eligible children (5-12 Years, Phase 2) were screened for LTBI by TST and IGRA. Our findings from Phase 2 showed that the prevalence by IGRA was 34.6% whereas prevalence by TST turned out to be 44.8%. In Phase 1 prevalence by IGRA was 12.46% and prevalence by TST was 21.40%. LTBI positives were ruled out for active TB by GeneXpert and Chest Xray. Treatment was given as per NTEP guidelines.

5.6 Role of Kisspeptin Mediated Signaling in Onset of Puberty

The signaling of the neuropeptide, kisspeptin-10 through its cognate G-protein coupled receptor, the kisspeptin receptor (KISS1R), heralds the onset of puberty. Consequently, mutations in this hormone-receptor pair lead to pubertal disorders. Activating mutations in kisspeptin-10 or KISS1R are implicated in idiopathic central precocious puberty. Whole exome sequencing was carried out in a girl with central precocious puberty who exhibited high circulating levels of kisspeptin. She was found to harbor the nonsynonymous variant p.Leu364His in KISS1R in a homozygous state. Further, functional studies were carried out to determine the effect of this substitution on the receptor function. Results revealed that an augmented signaling response through H364 KISS1R was associated with the pathophysiology of idiopathic central precocious puberty.

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

This project was initiated in March 2021 at Child Health Clinic, ICMR-NIRRCH and B. J. Wadia Hospital for Children. At the end of 2nd year of the project, recruitment of 40 controls (normal healthy girls -6 to 9 years) is completed. Out of 50 probable cases of Idiopathic Central Precocious Puberty (ICPP) and incomplete variants of PP, 35 girls were recruited as per the inclusion criteria in the reporting year. Total 18 cases are classified as ICPP and 11 as incomplete PP so far. The interim analysis revealed a higher level of Kisspeptin in ICPP and incomplete PP cases as compared to controls, but there is no difference among ICPP and incomplete PP. Comparison of pre-pubertal levels of these biomarkers with other ethnic population suggests that the circulating levels of these biomarkers may vary in Indian populations and needs further validation in large numbers.

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

Oxidative phosphorylation (OXPHOS) disorders also known as mitochondrial disorders is an important cause of neurometabolic crisis. Mitochondrial disorders are basically having a defect in oxidative phosphorylation pathway (OXPHOS). The OXPHOS disorders in Indian population remain largely unexplored and there is limited data. In this project, 30 controls and 8 patients samples were collected. Muscle biopsy were collected only from 2 patients and blood samples have been collected from the rest. Biochemical analysis of two patients samples have been reported earlier. Further, genetic analysis of all the patients have been done either with clinical exome or mitochondrial sequencing. A novel heterozygous nonsense variation p.Trp261Ter in Polymerase Gamma (POLG) gene has been identified in one patient with Leigh syndrome. This variation has been found to be pathogenic. Another heterozygous missense variation p.Leu304Arg in POLG gene has been identified in the same patient. Further, both the genetic variations have been validated with Sanger sequencing.

6. NATIONAL CENTER FOR PRECLINICAL REPRODUCTIVE AND GENETIC TOXICOLOGY

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

Triclosan (TCS), an antimicrobial agent, is widely used in consumer products. The present study investigated the effect of TCS on testis and prostate physiology in adult Wistar rats. TCS being an endocrine disruptor decreased testosterone and increased the levels of estrogen in treated animals. A significant decrease in sperm count, motility, and daily sperm production suggests an effect on testicular steroidogenesis. Histological analysis of testes from treated animals revealed alterations like sloughing of germ cells, degenerative changes of germ layers in few seminiferous tubules. Inter-papillary convolutions and multifocal functional hyperplasia of glandular epithelium with increased cellular layers of epithelium forming fingerlike projections in the lumen, which are the signs of prostate hyperplasia, were observed. TCS produces reactive oxygen species (ROS), leading to oxidative stress. Inceased levels of Malondialdehyde and decrease in superoxide dismutase and catalase levels were observed in the treatment groups compared to the vehicle control group.

6.2 Identification and Characterization of Sertoli and Leydig Cell Homing Peptides

Homing peptides can be an excellent vehicle for targeted drug delivery to the desired organ. To identify peptides for the targeted drug delivery to the testis, an attempt was made to select Sertoli Cell Homing Peptides (SCHPs) and Leydig Cell Homing Peptides (LCHPs) using the phage display peptide library. *In vivo* targeting efficacy of these selected SCHP1, SCHP2, LCHP1, and LCHP2 was assessed by Cy5.5 tagged synthetic peptides, CTP (control peptide), and Cy5.5 free dye were used as controls. *In vivo* bio-distribution pattern of these peptides differed from the CTP and Cy5.5 dye. All four peptides accumulated more in the testis compared to the controls. LCHP1 and LCHP2 were more promising compared with SCHP1 and SCHP2 as their testis uptake was significantly higher compared to other organs.

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

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 human CYP isoforms CYP1A2, CYP2C9, CYP2D6, and CYP3A4. In an in-silico study, Dhatri Lauha, Parthadyarishta, Amrita Guggulu, Sukumara Ghrita, and Gandharvahasta formulations showed higher interaction scores and interactions with key residues similar to respective inhibitors in all four crystal structures of human cytochrome P450 isozymes. In a fluorometric assay, the water and methanolic extracts of Ayush GG, Laksha Guggulu, and Ayush AD formulations showed no inhibitory activity against the selected CYPs isoforms (CYP1A2, CYP2C9, CYP2D6, and CYP3A4) indicating that it has the least interaction potential. Hence, the consumption of Ayush GG, Laksha Guggulu, and Ayush AD formulations may be regarded safe.

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

Pre-eclampsia (PE) is pregnancy-induced hypertension associated with increased oxidative stress and proteinuria. The present study aims to investigate the synergistic impact of ALA and Nanocurcumin in a Lipopolysaccharide (LPS) induced PE rat model. In the reporting year, the reproductive toxicity of Curcumin, Nano-curcumin and ALA and Nano-curcumin+ALA was undertaken in female Wistar rats. Animals were sacrificed at Gestation day (GD) 20 to assess preimplantation (PIL) and post-implantation losses (POL). There was no significant PIL and POL observed in the treated group compared with the control group. Hematological and biochemical parameters showed no significant change compared to the control. F1 offspring did not show any adverse effect on sexual maturation and growth. F1 male pups and females were mated with naïve male and female Wistar rats, to assess their fertility. No significant effect was observed in normotensive pregnancy with exposure to nano curcumin alone and in combination with ALA.

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

Lysophosphatidic acids (LPA) - LPA receptor (LPAR) interaction activates the signaling pathways leading to cell proliferation, invasion, and metastasis in ovarian cancer. The present study aims to explore the therapeutic potential of the peptides targeting LPAR3 in ovarian cancer. Recombinant LPAR3 (rLPAR3) was used as bait for *in vitro* panning by using a phage display library. In round 1 and 3 unique peptide count was 6177 and 1418 respectively suggesting that the peptide diversity decreased from round 1 to round 3. This suggests the enrichment of the peptide sequences in round 3 compared to round 1. 707 peptide sequences were common to both rounds of *in vitro* panning suggesting the enrichment of rLPAR3 binding peptides. XQMXXXYXQSXX and XHXAXXLGXVXX are the top two peptides of high frequency will be studied for their specificity to rLPAR3 *in vitro* and *in vivo*.

7. REPRODUCTIVE CANCERS

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

Estrogens mediate their physiological effects through estrogen receptors (ERs) via genomic and non-genomic signaling. Genomic signaling by estradiol is well studied in different cell types, however the role of non-genomic estrogenic signaling especially in prostate is not well researched. Non-genomic signaling is believed to be initiated at extranuclear sites and leads to rapid signaling events wherein different downstream secondary messenger are activated. In addition to conventional ERs (ERα/β), GPR30, a G-protein coupled receptor has been reported to bind to estrogen and is known to be localized onto the plasma membrane. GPR30, on activation with G1 agonist is reported to inhibit cell growth as well as migration in prostate cancer (PCa) cell lines. Thus, GPR30 has been proposed as a suitable therapeutic candidate. We previously demonstrated that activation of membrane ERs using cell-impermeable estradiol led to epithelial to mesenchymal transition (EMT). However, it remains to be established whether non-genomic estradiol signaling induced EMT is mediated through GPR30 or conventional ERs. The present study was undertaken to assess the role of GPR30 in non-genomic estradiol signaling induced EMT. As compared to control clones wherein EMT was observed in response to cell impermeable estradiol, GPR30 silenced clones failed to show a significant change in EMT. This was suggestive of the role of GPR30 in regulating EMT in PCa cells.GPR30 expression at the protein and transcript was assessed in the dorsoventral prostates of Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) mice. With disease progression, a significant loss of GPR30 protein expression was found. On the other hand, a significant increase was found at the HGPIN (High Grade Prostatic Intraepithelial Neoplasia) stage compared to age matched controls. A similar trend was observed for GPR30 transcripts at the HGPIN stage. TRAMP mice are being treated with different doses of G1 to deduce the role of GPR30 signaling in PCa pathogenesis. It is envisaged that these investigations may uncover the potential of GPER agonists as therapeutics against PCa.

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

Retinoic acid-inducible gene- I (RIG-I)-like receptors (RLRs), are cytosolic sensors of double-stranded and single-stranded exogenous RNAs, and induce type I interferons and other pro-inflammatory signaling. In a transgenic murine-model of prostate cancer (TRAMP), expression of two RLRs (RIG-I, MDA-5) and their common adaptor (MAVS) was significantly increased during the early stage but was significantly downregulated during the late stage. Importantly, local and transient intra-tumoral treatment with RIG-I agonist significantly inhibited tumor growth and induced tumor apoptosis with ecto-calreticulin expression in a subcutaneous syngeneic mice-model of prostate cancer.

7.3 Investigating the *In Vitro* and *In Vivo* Potential of rfhSP-D to inhibit Prostate Cancer Metastasis and Mechanistic Involvement of GRP78

An apoptotic and immunomodulatory role of SP-D in prostate cancer was reported by the laboratory for the first time leading to the investigations of its impact on metastasis. Treatment with a recombinant fragment of human SP-D (rfhSP-D) significantly reduced cell migration and invasion of prostate cancer cells with concomitant downregulated expression of N-cadherin, MMP9, and VEGF, and upregulated expression of E-cadherin along with GRP78 and Calreticulin, indicators of cell stress. The androgen-responsive LNCaP cells were more sensitive to rfhSP-D than the androgen-resistant PC3 cells. Basigin (induces proteases) and MCT1 (associated with lactic acid metabolism) were discovered as novel binding partners of rfhSP-D PC3 cells, suggestive of multiple anti-metastatic mechanisms. *In vivo* experiments using TRAMP mice showed that rfhSP-D treatment increased cell death and an increased number of circulatory NK cells, MDSCs, and inflammatory monocytes in the tumor, thus, converting an immunologically 'cold tumor' to 'hot tumor' with high responsiveness to therapy with immune checkpoint inhibitors.

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

The aim was to identify differentially expressed miRNAs associated with breast cancer in Indian women. The breast tissues and blood samples of 57 breast cancer patients and blood samples of 24 healthy controls have been collected. The data of 20 patient and 10 control samples was evaluated. The results indicated eleven significantly downregulated microRNAs and seven significantly upregulated miRNAs in breast cancer patients compared to controls. KEGG pathway and Gene ontology analysis revealed association of these miRNAs with cancer related pathways- Wnt, p53, PI3-Akt, Hippo and VEGF pathways and key genes- beta catenin, GSK3b, PTEN, YAP1, EGFR, CCNB1. The validation of identified miRNAs and associated genes is under progress. The enrollment of patients is ongoing.

7.5 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

Cervical cancer is the commonest cancer cause of death among women in developing countries. This study was initiated in urban (Abhyudaya Nagar) and rural (Dahanu) study sites in 2019. As of February 2023, total 1544 women (1024 from urban and 520 from rural/tribal study site) have been screened by low cost molecular careHPV test, PAP smear and Visual inspection with acetic acid. HPV positivity by care HPV was 5.76% and by HC2 was 4.72%. Around 83 (5.37) women were positive for visual inspection with acetic acid. Total 7 women had Low-grade Squamous Intraepithelial Lesion (LSIL), 3 had High Grade Squamous Intraepithelial Lesion (HSIL), 3 had Atypical Squamous Cells-HSIL cannot be excluded (ASC-H) and 12 had ASCUS. Two of them were treated by Loop Electrosurgical Excision Procedure (LEEP) at Tata Memorial Hospital, Mumbai. Two of the recruited women underwent cryotherapy. All the screen positive women were referred to TMH for colposcopy.

7.7 Mechanistic Insights of FSHR Activation Mechanisms through Small Molecule Modulators

FSHR is an important molecule for fertility regulation and targeted chemotherapy of reproductive cancers. Both agonists and antagonists of FSHR have clinical utility. Hence, it is important to have a clear understanding of the binding sites of its modulators and the activation mechanism. Using docking and MD simulations, the modulator binding sites and accompanying structural changes in FSHR were delineated. The agonist interacting residues Ile522, Ala595, Ile602 and Val604 of FSHR were found to be conserved in LHCGR (a close homolog of FSHR) and participate in interaction with its agonist Org43553. Distinctly prominent domain motions and conformational changes in transmembrane helices 3, 4 and 6 for agonist bound FSHR structure were observed. These structural changes have also been reported for LHCGR and few GPCR members suggesting an important and well conserved mechanism of GPHR activation that can be exploited for design of novel modulators (Fig. 1).

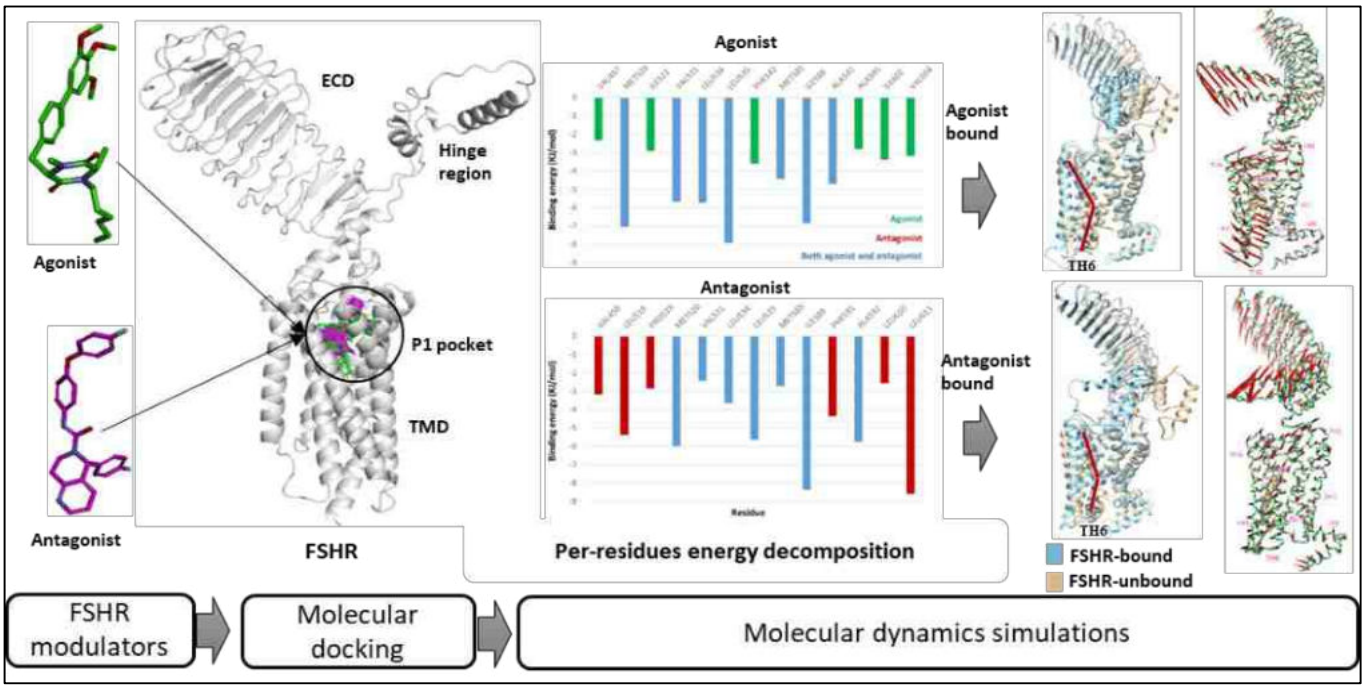


Figure 1: Graphical summary of the work done.

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

Tribal women were screened for NCDs and common cancers (n=503) (VIA and HPV co-testing along with common RTI testing), clinical breast examination and oral cavity examination. 5.8% (n=29) women tested positive with visual inspection using acetic acid (VIA) and 6.6% (n=33) tested positive for HPV by CARE HPV test. On oral cavity examination, 0.4% (n=2) had precancerous and 3.2% (n=16) had benign lesions. In NCDs screening 21.3% (n=107) women were found to be hypertensive and in 2.0% (n=10) were diabetics, 79.1% (n=398) of the women were found anemic, 5.9% (n= 30) were tested positive for Candida and bacterial vaginosis was found in 42.1% (n=212).

Awareness and health seeking behavior (Fig. 2) for common cancers /NCDS needs to be improved through health education activities involving the ASHA workers. ASHA workers need incentives for travel and financial remuneration. Health care facilities need to be upgraded in terms of infrastructure, equipment and trained manpower (Fig. 3) to implement screening services.

Figure 1: Awareness activity conducted at Sub Center and Anganwadi at Sarawali, Dahanu

Figure 2: Training session of ANMs on acetic acid preparation at SDH, Dahanu

8. HEALTH TECHNOLOGY ASSESSMENT AND DRUG DISCOVERY

8.1 HTA for Strengthening Prong 2 Interventions of PPTCT Program at Public Health Facilities through Provision of Linked HIV and Family Planning Services

The study addressed the policy question of whether strengthening Prong 2 interventions of the PPTCT program through the provision of linked HIV-FP services to prevent unintended pregnancies among women living with HIV (WLHIV) in public health care settings is cost-effective. A modeling exercise among a hypothetical cohort of 782107 WLHIV in the reproductive age group over a time horizon of 31 years who were provided linked HIV and Family Planning (FP) services with emphasis on use of dual methods of contraception was undertaken. It demonstrated that use of dual methods through provision of linked services is a cost effective intervention which could prevent an unintended pregnancy at an incremental cost of INR 11,435. About seventy-two thousand unintended pregnancies, seventeen thousand unintended livebirths, forty-one thousand abortions, and 8722 maternal deaths could be averted by considering HIV and FP linkage. Considering the rate of mother to child transmission to be 5%, about 6880 infants born HIV positive could be averted by considering HIV and FP linkage intervention. About 1533 intended infant diagnosed (mother on ART) with 1061 intended infant diagnosed (mother not on ART) could be averted by considering HIV and FP linkage.

8.2 Cost Effectiveness of Point of Care Sickle Screening Tests

A rapid Health Technology Assessment analysis was undertaken along with HTAIN DHR and PGI HTA Resource Hub Chandigarh to answer the policy question using a Decision Tree Analytical Model. Evidence on clinical effectiveness of various POC tests available in India, prevalence of the disease in various age groups and geographical locations and health system costs for screening of the disease were collated from published sources. Mathematical modelling, threshold, probabilistic sensitivity and budget impact analysis were conducted and endorsed by stakeholders. According to the model, cost per individual screened using the POC tests Hemotype SC and Sickle Scan was INR 250.17 and for solubility test followed by HPLC as a confirmatory test is INR 53.32. ICER per case detected suggests that if Hemotype SC Kit can be procured below INR 100 it will become cost effective. Similarly, if Sickle SCAN Kit can be procured below INR 110, it will become cost-effective. The HTA analysis resulted in influencing the policy such that Ministry could negotiate the cost per test to be Rs.100 and issued a letter to the Principal Secretaries of 17 states advising them to use these tests at negotiated price and complete screening of all population aged 0-40 years by 2026.

8.3 Integrated Analyses of Genomic Scale Metabolic Models and Omics Profiles to Capture the Host-Pathogen-Environment Interplay of *Candida* sp.

The study aims to evaluate host-pathogen interactions of *Candida* spp. in environments that replicate host niches using multi-omics data and genome-scale metabolic models. The study findings will reveal novel therapeutic options for treating *Candida* infections. An *in-vitro* model of host-pathogen interactions in the vaginal milieu has been generated. The human metabolic model RECON3D and *C. albicans* metabolic model iRV781 were used to develop context-specific models for host and pathogen by overlaying publicly available transcriptomic data from GEO database. *In silico* gene deletion analysis in *Candida* was performed under infection state with OKF6/TERT-2 and HUVEC cells to identify essential genes. Genes belonging to amino acid metabolism, cofactor and vitamin metabolism were found to be critical for growth of Candida and could be explored as drug targets.

8.4 Identification of Enriched Biochemical Networks and Polypharmacological Targets in Metabolic Syndrome

An online webserver, GeDiPNet, with curated gene-disease association data was created and used for identification of polypharmacological targets for Metabolic Syndrome (MetS) and its components. Many of the identified targets are currently being used in management of components of MetS, thereby confirming the utility of the algorithm. In addition to the known targets, few novel targets have been identified by the

algorithm such as *DECR1*, involved in fatty acid metabolism and inflammation, and *HPGDS*, involved in renal programming (Fig. 1).

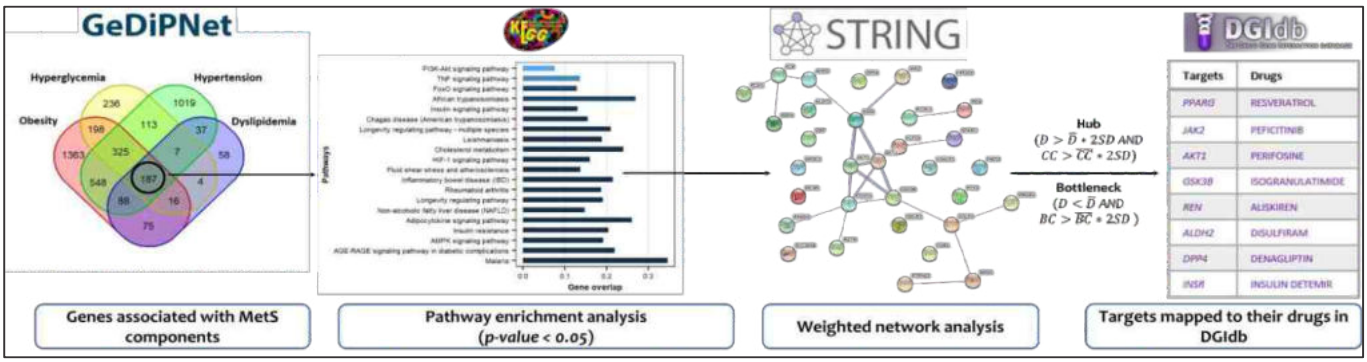


Figure 1: GeDiPNet algorithm for identification of polypharmacological targets for metabolic syndrome.

9. COVID-19 RESEARCH

9.1 Health Systems Analysis and Evaluations of the Barriers to Availability, Utilization and Readiness of Family Planning and Contraceptive Services in COVID-19 Affected Areas in Maharashtra, India

Women attending both urban and rural public health care facilities in Thane district of Maharashtra and health care providers participated in in-depth interviews and focus group discussions. Preliminary analysis reveals that the ministry had issued a circular stating family planning (FP) services under essential service category in the very first month of the lockdown. State instructed districts to procure additional contraceptive commodities. However, FP services could not be prioritized due to COVID. Tubal ligations were not conducted and home based distribution of oral pills and condoms were facilitated through ASHAs along with COVID surveys. PPIUCD were provided. The stock of injectable contraceptives was insufficient. Beneficiaries had no information on services provided at these facilities. Condoms were procured from private pharmacies and few refrained from sexual contact due to fear of COVID. During the first wave, a significant drop in contraceptive beneficiaries is noted which slowly picked up after the second wave. E-consultations as recommended by central ministry were not followed due to the lack of manpower. Additional budget was provided for COVID related expenses and there was no budget restrictions for FP commodities. E-meetings and monitoring of FP services continued in the state. FAQs on the effect of COVID infection on the eligibility of contraceptive users was developed by the Ministry. However, State health care providers were not aware about this.

9.2 National Registry of Pregnant Women with COVID-19 in India (PregCovid Registry)

The PregCovid registry data from five study sites in Maharashtra was analysed to understand the outcomes in neonates of mothers with COVID-19 during the first and second waves of the COVID-19 pandemic. The frequency of preterm births was higher in the second wave (15.0%, 111/742) compared to the first wave (7.8%, 139/1782). The proportion of neonates requiring NICU admission was significantly higher in the second wave (19.0%, 141/742) as compared to that in the first wave (14.8%, 264/1782). Analysis of 2058 pregnant and postpartum women with COVID-19 admitted during three wave periods at BYL Nair Hospital, Mumbai showed decreased severity of COVID-19, reduced maternal mortality, and morbidity in the third wave compared with the first wave and second wave of COVID-19. PregCovid registry data showed eight times higher risk of early onset preeclampsia (PE) in women with moderate to severe COVID-19 compared to asymptomatic group.

9.3 Investigating Therapeutic Potential of a Recombinant Fragment of Human Surfactant Protein D against SARS-CoV-2: *In Vitro* and Animal Models

Previous studies showed that a recombinant fragment of human SP-D (rfhSP-D) blocks the interaction of RBD of spike protein and human ACE2 and can significantly inhibit the entry and replication of SARS-CoV-2 (Wuhan strain) from clinical samples. Broad anti-SARS-CoV-2 activity of rfhSP-D was established with a significant inhibition of the entry and replication of SARS-CoV-2 (Delta and Omicron strains) from clinical samples (n=15). Therapeutic and prophylactic anti-SARS-CoV-2 potential of rfhSP-D was established *in vivo* in the hamster (n=9) and transgenic mouse models (n=15) with a significant reduction in mortality, viral load and lung damage.

10. MISCELLANEOUS

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

The national snakebite project is an interventional study being conducted in Shahapur and Aheri blocks of Maharashtra, Khordha and Kasipur blocks of Odisha. Two years of data from 1415 retrospective cases were collected. A total of 188 Medical Officers and Community Health Officers were trained by national experts on prevention, diagnosis and management of snakebite envenomation. IEC material for healthcare workers and community awareness has been developed in English, Marathi, Hindi and Odiya languages. Flowchart for management of snakebite envenomation for the Medical Officers has been developed in consultation with national experts. Survey of 38 public health facilities (30 PHCs, 3 RH/CHC and 5 SDH/DH) for snakebite management preparedness completed. International Snakebite Awareness Day was celebrated by conducting training sessions for ASHAs. Interviews to assess the knowledge of healthcare workers were completed.

10.2 Nationwide Study to Estimate Incidence, Mortality, Morbidity and Economic Burden due to Snakebites in India

NIRRCH is implementing the snakebite study in Raigad, Pune and Nanded districts in Maharashtra state. Training of ASHA workers was completed during the reporting period. Out of the 6862 ASHAs at the study sites, training was attended by 5414 (78.9%). The proportion of ASHAs attending the training out of the total ASHAs in the district was highest in Raigad [1520 (80.6%)], followed by Pune [2625 (78.3%)] and Nanded [1269 (78.1%)]. From 01st April 2022 till 31st January 2023, 2096 snakebite cases and 56 deaths have been recorded at the study sites. Out of these, the maximum number of cases have been recorded at Raigad (n=1300) followed by Pune (n=476) and Nanded (n=320) districts. The case fatality rate was highest in Pune (4.8%) and lowest in Raigad district (0.9%).

10.3 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

Schizophrenia (SCZ) is a heritable complex neuropsychiatric disorder. Although, it is the most common mental disorder, its pathophysiology is still elusive. In the last year, we reported a copy number loss in 7q35-q36.1 region, encompassing the entire CNTNAP2 gene in one family of patients. Both the affected sisters in this family were carrying the same deletion. Further, iPSCs (induced Pluripotent Stem Cells) have been generated from the blood samples of two sisters with CNTNAP2 deletion. These iPSCs colonies were extensively characterized for the expression of pluripotency markers. It was then differentiated to cortical neuron in which RNAseq was carried out to see the differential expression of neuronally expressed genes. From the expression data, top 10 up and down regulated genes were selected for validation. Initial analysis showed that the expression of CNTNAP2 gene was found to be reduced to almost half compared to control.

FEMALE INFERTILITY AND ASSOCIATED REPRODUCTIVE DISORDERS

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

PCOS is a prevalent condition in women of reproductive age, characterized by hyperandrogenemia, skewed gonadotropins, insulin resistance (IR) and other reproductive and metabolic anomalies. Many of them are infertile and opt for IVF to conceive. However, IVF outcome is poor in them, which can be attributed to poor oocyte quality; and altered glucose metabolism dynamics in the follicle may contribute towards this. Granulosa cells (GCs) are the companion somatic cells of the oocyte, which act as the metabolic drivers of oocyte. Oocyte has limited capacity to utilize glucose and it is GCs, which metabolize glucose to supply oocyte with energy substrates like pyruvate. This crucial metabolic cooperation is regulated by gonadotropins and insulin. GCs along with follicular fluid (FF) provide a specialized niche to support the growth of oocyte hence they are appropriate candidates to be investigated to reflect follicular metabolism dynamics. Previously, we have reported lower glucose uptake (Annual report 2017-2018, p. 3), lower levels of GLUT4 transcript (Annual report 2018-2019, p. 7) and upregulated polyol pathway (Annual report 2020-2021, pp. 1-2) in GCs from women with PCOS. We found higher glucose level in follicular fluid (FF) of women with PCOS than that of controls. Hence, we further wanted to explore if

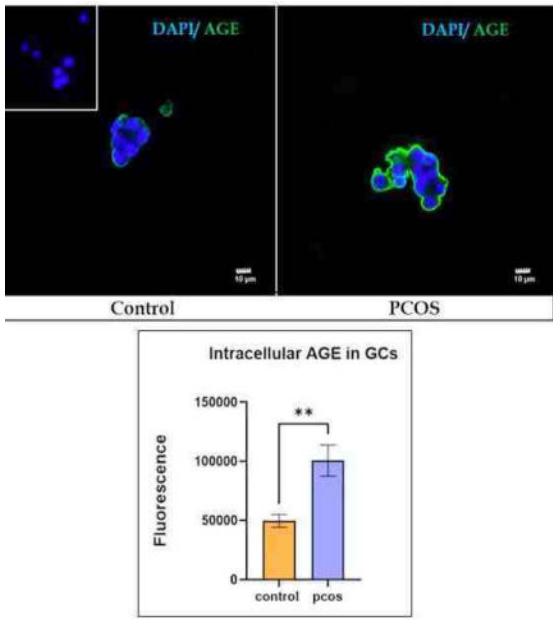


Figure 2: Levels of Advanced Glycation End products (AGE) in GCs of controls (n = 6) and women with PCOS (n = 7), represented as mean ± SEM, *P<0.05

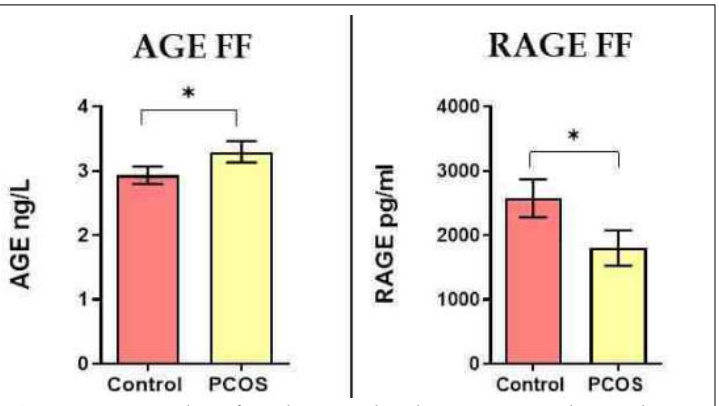


Figure 1: Levels of Advanced Glycation End products (AGE) and its decoy receptor (RAGE) measured in FF of controls (n=30) and women with PCOS (n=32) by ELISA, represented as mean ± SEM, *P<0.05

these women possess IR at follicular level as well. The unabsorbed glucose and upregulated polyol pathway can lead to glycation of macromolecules and give rise to advanced glycation end products (AGE). We studied AGE and RAGE (decoy receptor for AGE, protective in nature) in FF (by ELISA) and found higher AGE and lower RAGE levels in FF of PCOS women (Fig. 1).
Additionally, AGE showed negative correlation with pregnancy rate in control group and showed negative correlation with pregnancy outcome in both control and PCOS group indicating negative influence of higher AGE levels on IVF prognosis. In addition, AGE levels were studied by immunofluorescence and were found to be higher in GCs of women with PCOS (Fig. 2). Higher AGE levels can further lead to glycation of macromolecules, activation of pro-inflammatory and oxidative stress pathway and may further damage the cells. Overall, these results indicate IR and metabolic stress at follicular level in women with PCOS, which may in turn influence oocyte quality and IVF prognosis/outcome.

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

Granulosa cells (GC) participate in various processes including oocyte development and maturation, angiogenesis, maintaining redox homeostasis, ECM remodeling, etc., and thus provide the microenvironment essential for folliculogenesis. Thus, GC are the best candidate to investigate molecular mechanisms of impaired follicular growth in PCOS. To decifer the molecular mechanisms impacting the development and progression of PCOS, we performed transcriptome analysis of granulosa-lutein cells (GLCs) from women with PCOS by RNA-Seq. This led to the identification of 27,756 genes that are expressed in the GLCs. Out of these 876 genes were found to be significantly differentially expressed by at least 1.5 fold in GLCs of PCOS in comparison to controls. The GO term extraction of the differentially expressed genes (DEGs) revealed mitotic spindle organization, microtubule cytoskeleton organization, cell proliferation, and neutrophil activation, cell cycle processes, etc in the biological processes category. The pathway analysis of DEGs showed major dysregulated pathways including progesterone-mediated oocyte maturation, cytokine-cytokine receptor interaction, focal adhesion, ECM-receptor interaction, AGE-RAGE, IL-17, TNF, PI3K-Akt and Hippo signaling, etc in PCOS. Furthermore, metabolism-related pathways like glutathione, cholesterol, fructose and mannose, purine metabolism, insulin resistance were observed to be dysregulated in the GLCs of women with PCOS. All these pathways are crucial for the metabolic flux and redox homeostasis maintenance in follicles. Alterations of these metabolic processes may impair oocyte growth and maturation in women with PCOS. Additionally, genes involved in complement and

coagulation cascade, mTOR, P53, NF-Kappa B, Jak-STAT, HIF-1, Wnt signaling, and angiogenesis were observed to be altered. We selected 5 genes from the DEGs based on their biological significance in the pathophysiology of PCOS, viz., growth differentiation factor 15 (GDF15, prokineticin 1(PROK1), sphingosine-1-phosphate receptor 3 (S1PR3), S100 calcium binding protein A9 (S100A9), and angiomotin like 2 (AMOTL2). The PROK1 and S1PR3 genes are involved in the regulation of angiogenesis. Another gene, AMOTL2 was selected from the Hippo signaling process, which is important for cell proliferation and growth of follicles. Additionally, GDF15 and S100A9 genes were selected from transforming growth factor-beta receptor signaling pathway and inflammatory response respectively. We observed decreased transcript expression of PROK1 and GDF15 whereas increased expression of AMOTL2 and S100A9 in GLCs of women with PCOS (n=18) compared to controls (n=20) (Fig. 1). Although, S1PR3 gene expression was observed to be up-regulated in RNA-seq data, upon validation its transcript levels were found to be comparable between both groups.

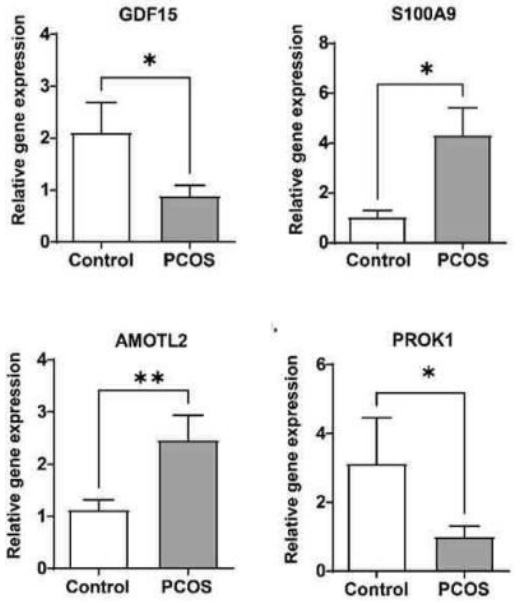


Figure 1: Expression of transcripts in GLCs of women with PCOS and controls using quantitative PCR

The protein levels of PROK1 were also down-regulated, supporting our gene expression findings (Fig. 2). Overall, our transcriptome analysis emphasizes alteration in several crucial pathways necessary for follicular development in the GLCs of women with PCOS. In PCOS, increased expression of S100A9, AMOTL2, decreased expression of PROK1 and GDF-15 along with dysregulated biological processes associated with angiogenesis, metabolism, and ECM remodeling suggests impaired folliculogenesis in PCOS.

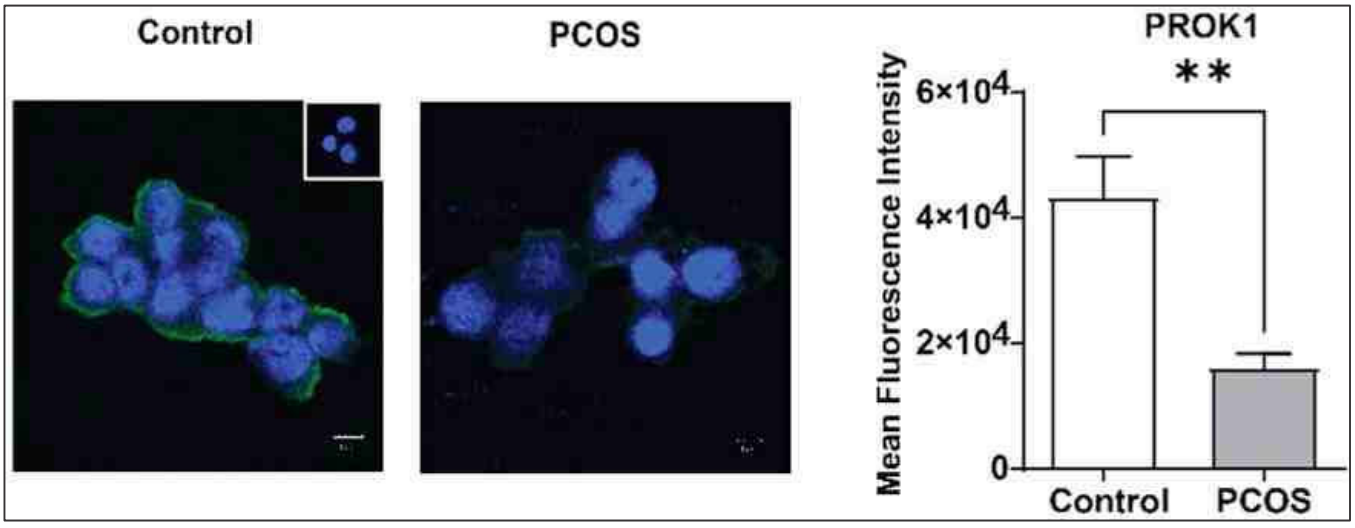


Figure 2: Representative image of PROK1 protein in GLCs obtained from women with PCOS and controls using immunofluorescence analysis.

1.3 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
Duration	:	2021-2026

Women with PCOS have a high risk of developing central obesity, glucose intolerance or type 2 diabetes, atherogenic dyslipidemia, hypertension, pro-thrombotic conditions, and cardiovascular disease, at a young age, which has been seen by reports of aggravated markers of sub-clinical atherosclerosis in them. A hypercoagulable prothrombotic state with impaired coagulation and fibrinolysis may contribute to the heightened risk of cardiovascular disease and cerebrovascular disease (CeVD) events, as reported in recent meta-analyses. Common pharmaceutical therapies given to women with the condition, such as oral contraceptives, metformin, etc., may also alter hemostatic components. Apart from their role in clot formation and breakdown, components of the coagulation and fibrinolytic pathways play an important role in the ovary by aiding ovulation, cumulus oocyte complex expansion, extracellular matrix remodelling and corpus luteum function. Altogether, altered functioning of systemic and ovarian hemostatic factors may underlie PCOS pathophysiology. Screening tests can help pinpoint anomalies in components of extrinsic, intrinsic and common pathways of coagulation.

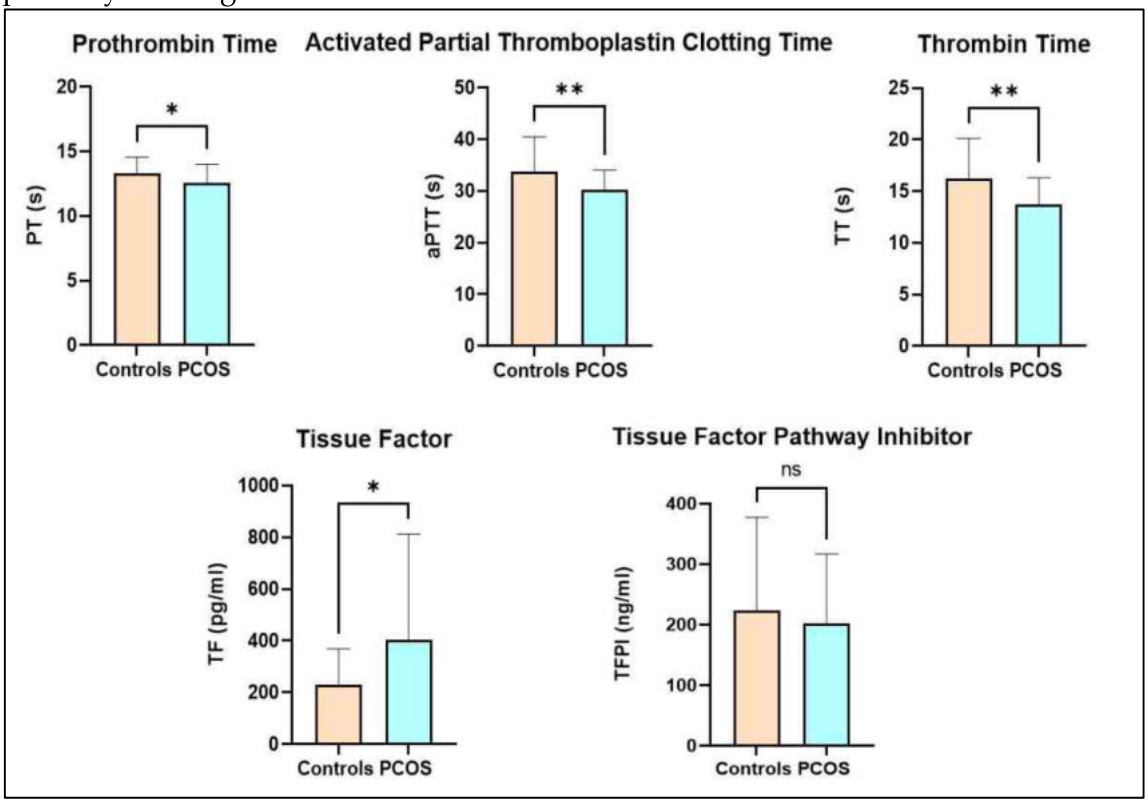


Figure 1: Comparison of coagulation parameters in plasma between controls and women with PCOS.

It was observed that prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time (TT) are significantly lowered in plasma of women with PCOS compared to controls, indicating that there may be an increased activation of procoagulant state. Furthermore, tissue factor, a key initiator of extrinsic coagulation cascade, is significantly elevated in women with PCOS, but the levels of its inhibitor were comparable between the two groups (Fig. 1).
Plasminogen activator inhibitor-1 (PAI-1), which inhibits fibrinolysis, was significantly raised in women with PCOS. Interestingly, both tissue plasminogen activator enzyme and plasminogen levels were also increased in women with PCOS. This suggests that enzyme activity for catalyzing conversion of plasminogen to plasmin may be diminished in women with PCOS, which may result in hypofibrinolysis (Fig. 2). The recruitment of more participants and data collection are ongoing.

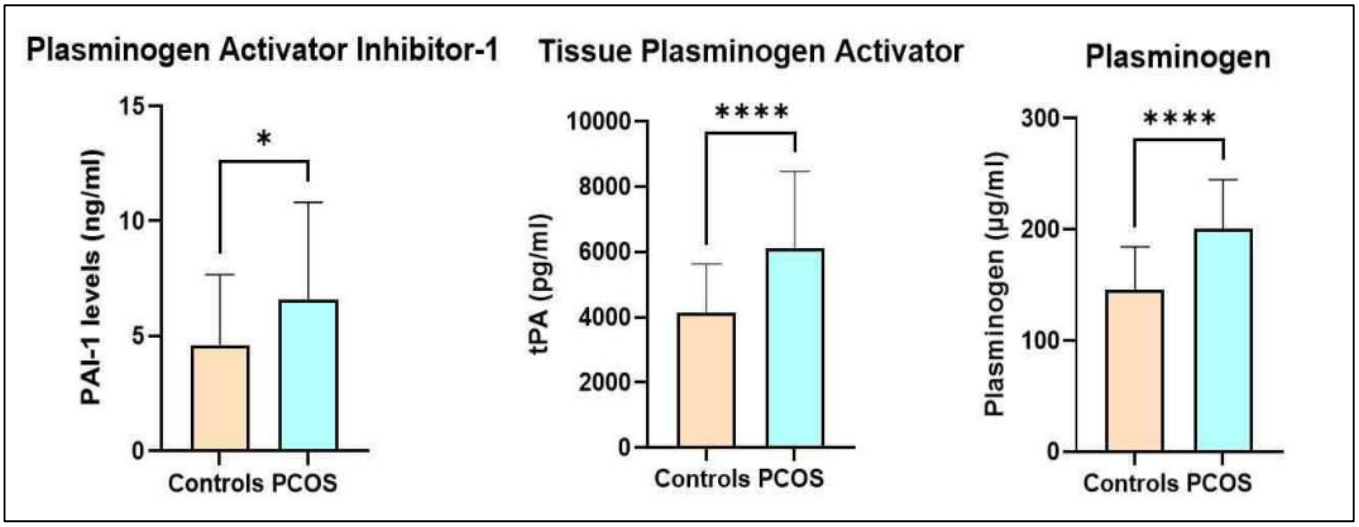


Figure 2: Comparison of levels of fibrinolytic factors in plasma between controls and women with PCOS. (PAI-1: plasminogen activator inhibitor-1, tPA: tissue plasminogen activator)

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

Principal Investigator	:	**Srabani Mukherjee**
Project Associates	:	Snehal Bhingardeve, Gayatri Shinde
Collaborators	:	Sadhana Desai, V Mangoli, Richa Jagtap
Duration	:	2020-2025

The systemic manifestation of PCOS is attributable to a cumulative impact of altered genetic and epigenetic profiles. Epigenetic modulators like DNA methylation, non-coding regulatory RNAs like microRNAs (miRNAs) govern the expression profiles of tissues. Several studies have identified differential expression of miRNAs in serum, follicular fluid and other tissues of women with PCOS. Aberrant DNA methylation of miRNA encoding genes could potentially alter their expression and thus may affect their target gene expression. Our earlier DNA methylome analysis of granulosa cells identified differential methylation of many miRNA encoding genes. Hence, we aim to investigate the role of DNA methylation in regulating miRNA expression, and in turn their target gene expression in

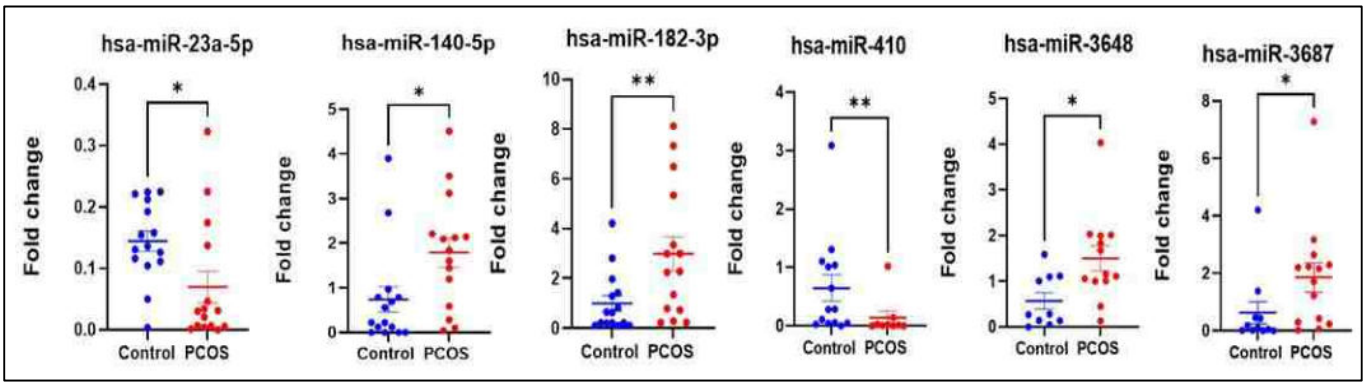


Figure 1: Quantitative real-time PCR analysis of miR-23a-5p, miR-140, miR-182, miR-410,miR-3648 and miR-3687 in GCs of women with PCOS and controls performed using the Mann-Whitney U test. p values < 0.05 are considered significant, *p < 0.05, **p < 0.01. Data represented as 'mean ± SEM'

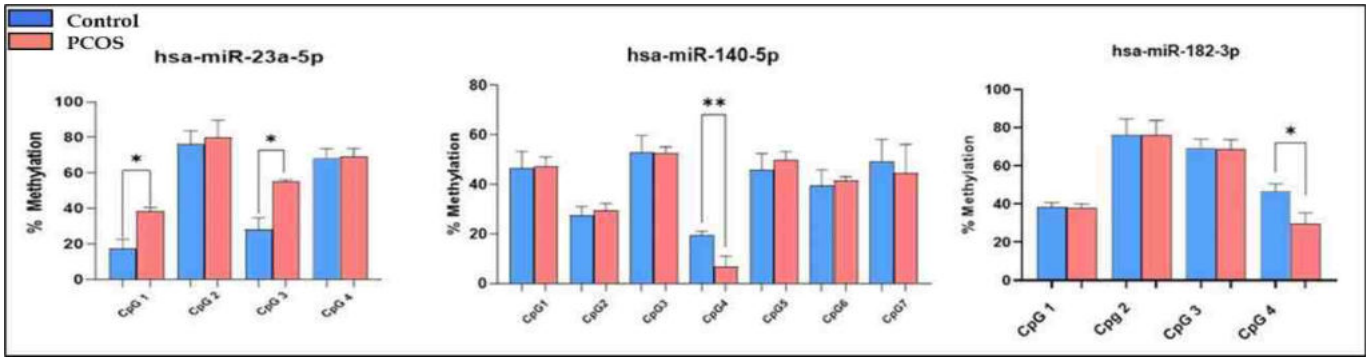


Figure 2: The percent methylation level of each CpG is compared between controls and PCOS. The p-value is calculated using Mann Whitney U test, p values < 0.05 are considered significant, *p<0.05, **p<0.01. Data represented as mean ± SE.

PCOS pathophysiology. Previously, we reported expression and methylation status of few miRNAs in granulosa cells of women with PCOS (Annual report 2021-2022, p. 7). This year we investigated few more miRNA. The levels of miR-23a-5p and miR-410 transcripts were decreased and of miR-140-5p, miR-182-3p, miR-3648 and miR-3687 were increased in the granulosa cells of women with PCOS compared to controls (Fig. 1). Methylation analysis by pyrosequencing showed hypermethylation of miR-23a-5p and hypomethylation of miR-140-5p, miR-182-3p in PCOS compared to controls thereby suggesting the role of DNA methylation in regulating the expression of these miRNAs in granulosa cells (Fig. 2). Further, it was aimed to evaluate the expression of the genes regulated by the differentially expressed and methylated miRNAs. Using bioinformatics tool such as miRTarBase, the target genes of the above-mentioned miRNAs relevant to PCOS pathophysiology were predicted. The target of miR-23a is IGF-2, which acts as a ligand for integrin, required for IGF2 signaling. Foxo3, which plays a vital role in follicular development, is targeted by miR-182-3p. The epigenetic mediators such as like DNMT1, HDAC4 are reported to be targeted by miR-140-5p. Overall, the target genes are found to play a role in regulation of different ovarian functions like follicular angiogenesis, insulin signaling, as well as regulation of epigenetic mediator enzymes. Thus, this present study will help us understand the influence of DNA methylation on the regulation of miRNA expression and their target gene expression in PCOS.

1.5 Unravelling Pathogenetic Mechanisms of Polycystic Ovary Syndrome by Whole Exome Sequencing *(Partly Funded by Department of Science and Technology)*

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

PCOS is a heterogenous disorder where both genetic and environmental factors contribute to the pathophysiology. To understand the genetic predisposition profile of Indian women, the present study on whole exome sequencing (WES) was initiated to discover gene variants associated with PCOS and its phenotypes in well characterised women with PCOS. Exome sequencing was carried out in 60 women with PCOS using the Agilent SureSelect CREV2. The sequenced data was annotated to analyse gene variants and develop a genotype-phenotype association. Annotated variants were analyzed by two approaches which are the Phenotype First Approach where PCOS associated terms are taken as inputs in the analysis pipeline. The second approach, the Phenotype Last Approach, enabled us to get a broader range of variants from the exome. Gene filtering strategy was developed where out of 2,86,321 total variants of gene; exonic, splicing, 5'UTR and 3'UTR variants were retained. From this list of 1,11,135 variants, a total of 47,386 including nonsynonymous, frameshift deletion, frameshift insertion, stopgain and stoploss variants were selected. These variants were catagorized based on their gnomAD MAF range, 17,288 variants were present in the range of 0 to <0.01 (ultra-rare variant) and 5,922 variants were in MAF range of 0.01 to 0.05 (rare variant). VarElect prioritization identified 275 rare and 13 polymorphic variants. We have shortlisted a few polymorphic and rare variants, which may have a role in pathogenetic mechanisms of PCOS. The distribution of variants of the significant genes among our cohort of 60 PCOS women is portrayed in the heatmap (Fig. 1).

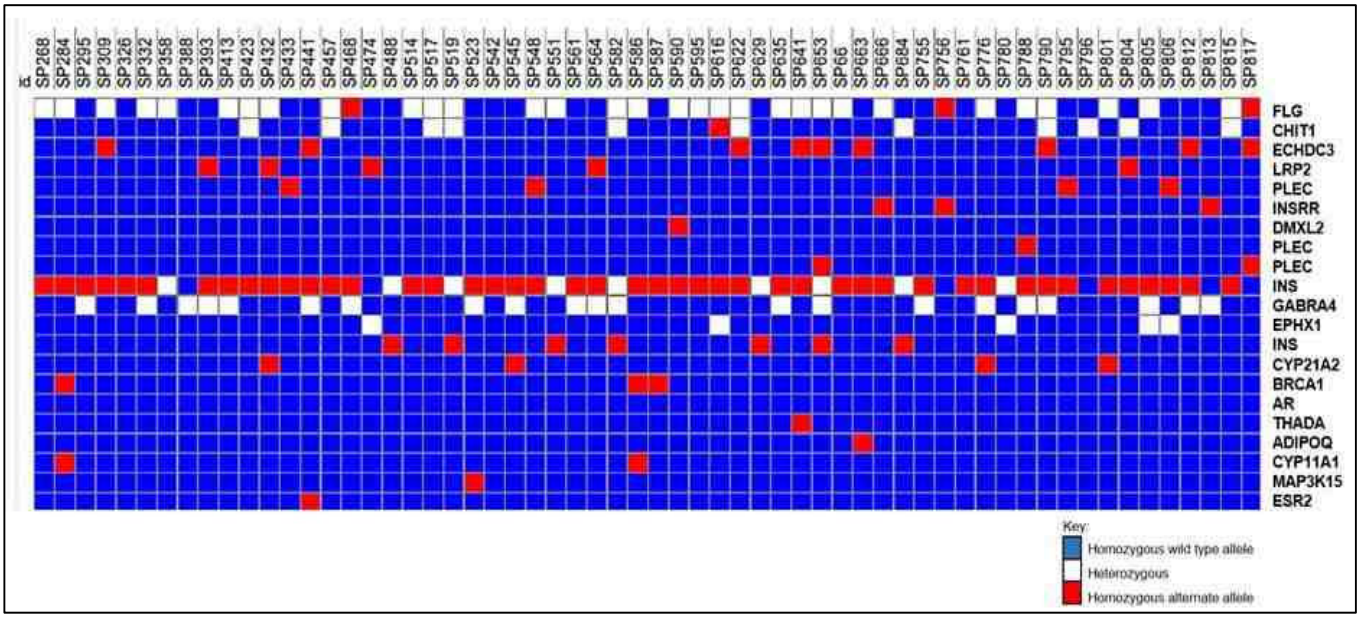


Figure 1: Heatmap of genetic burden of alternate alleles across our cohort of women with PCOS. The participant name indicated along the X-axis as SPID (e.g. SP268) the gene name indicated along the Y-axis

Some of these genes were FLG, ECHDC3, THADA and CYP21A2. FLG (Filaggrin) is an intermediate filament-associated protein that aggregates keratin intermediate filaments in mammalian epidermis. ECHDC3 (Enoyl-CoA Hydratase Domain Containing 3) is predicted to enable enoyl-CoA hydratase activity. It may play a role in fatty acid biosynthesis and insulin sensitivity. THADA is likely to be involved in the death receptor pathway and apoptosis. Variants in this gene are shown to be associated with type 2 diabetes and PCOS. CYP21A2 (Cytochrome P450 Family 21 Subfamily A Member 2) protein is a monooxygenase which is involved in the production of cholesterol, steroids and other lipids. Some of the identified variants in exome sequencing have been confirmed by Sanger sequencing (Fig. 2). These variants will be validated in a suitably sized replication cohort of PCOS and control samples. The exome sequencing will be carried out on more samples and further in-depth analysis.

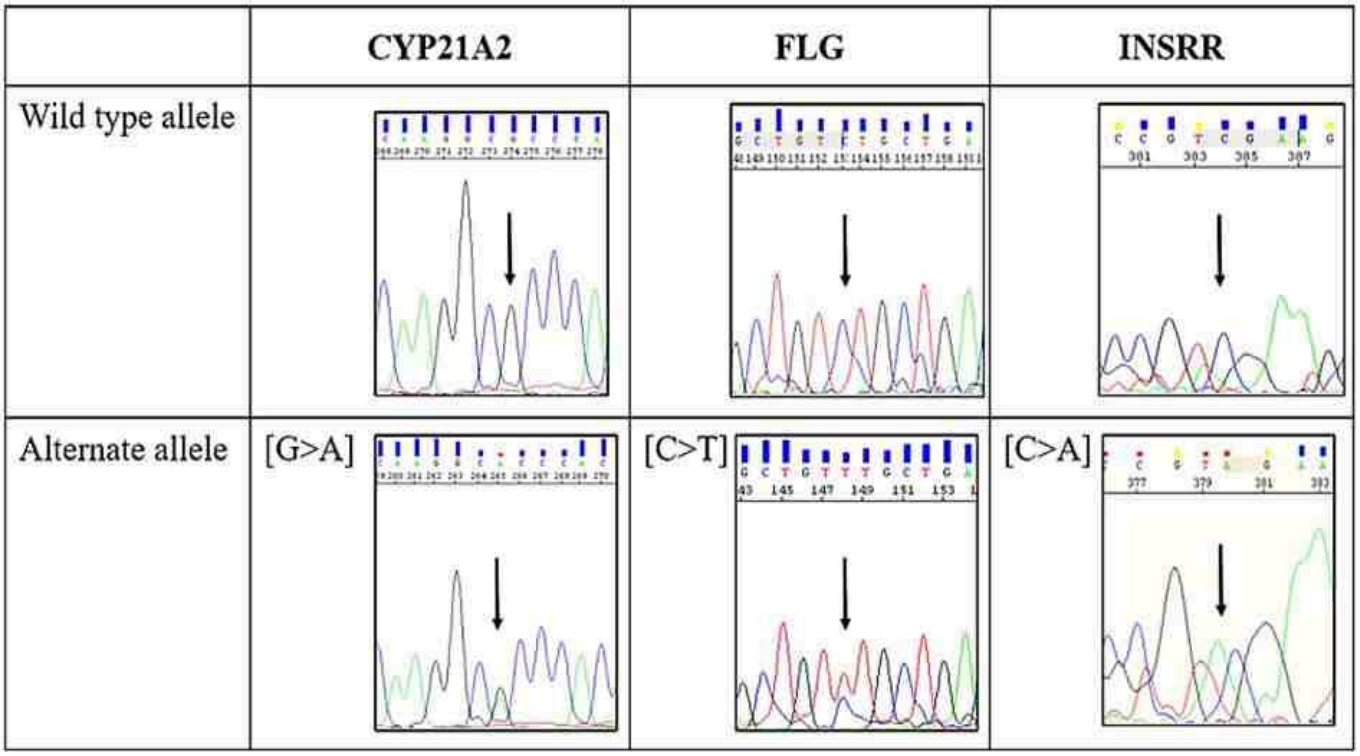


Figure 2: Electropherogram of the variants of genes validated by Sanger sequencing analysis

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

Principal Investigator	:	**Pallavi Shukla**
Project Associate	:	Srabani Mukherjee
Collaborators	:	Anushree Patil, Beena Joshi
Duration	:	2018-2023

Role of mitochondria in the etiopathophysiology of Polycystic ovary syndrome (PCOS), a common cause of infertility is not understood fully. In the current year we compared mtDNA copy number in peripheral whole blood in 85 women with PCOS who were insulin resistant (PCOS- IR) and insulin

sensitive (PCOS-IS) and 65 non-PCOS women who were insulin resistant (non-PCOS-IR) and insulin sensitive (non-PCOS-IS). The study revealed that mitochondrial DNA (mtDNA) copy number was significantly reduced in PCOS-IR group compared to PCOS-IS group (P=0.045) and non-PCOS-IR group (P=0.0059) group. Furthermore, mtDNA copy number in PCOS-IS was significantly reduced compared to non-PCOS IS (P=0.0032). This implies that mitochondrial dysfunction in women with PCOS plays a crucial role in its pathophysiology, more importantly in insulin resistant women. Comparison of mtDNA copy number between frank PCOS and normoandrogenic PCOS phenotypes identified that frank phenotype showed reduced mtDNA copy number (1.18 ± 0.34) compared to normoandrogenic PCOS phenotype (1.38 ± 0.28) though the change was not significant (p=0.06). Investigation of mitochondrial ROS in PCOS participants (n=44) and healthy control participants (n=17) using a fluorescent probe, MitoSOX, a red mitochondrial superoxide indicator by flow cytometry analysis, suggests that mitochondrial ROS production was significantly increased in women with PCOS (86.5± 19.9) compared to non-PCOS control women (28.6±15.2) (p=0.001) (Fig. 1B).

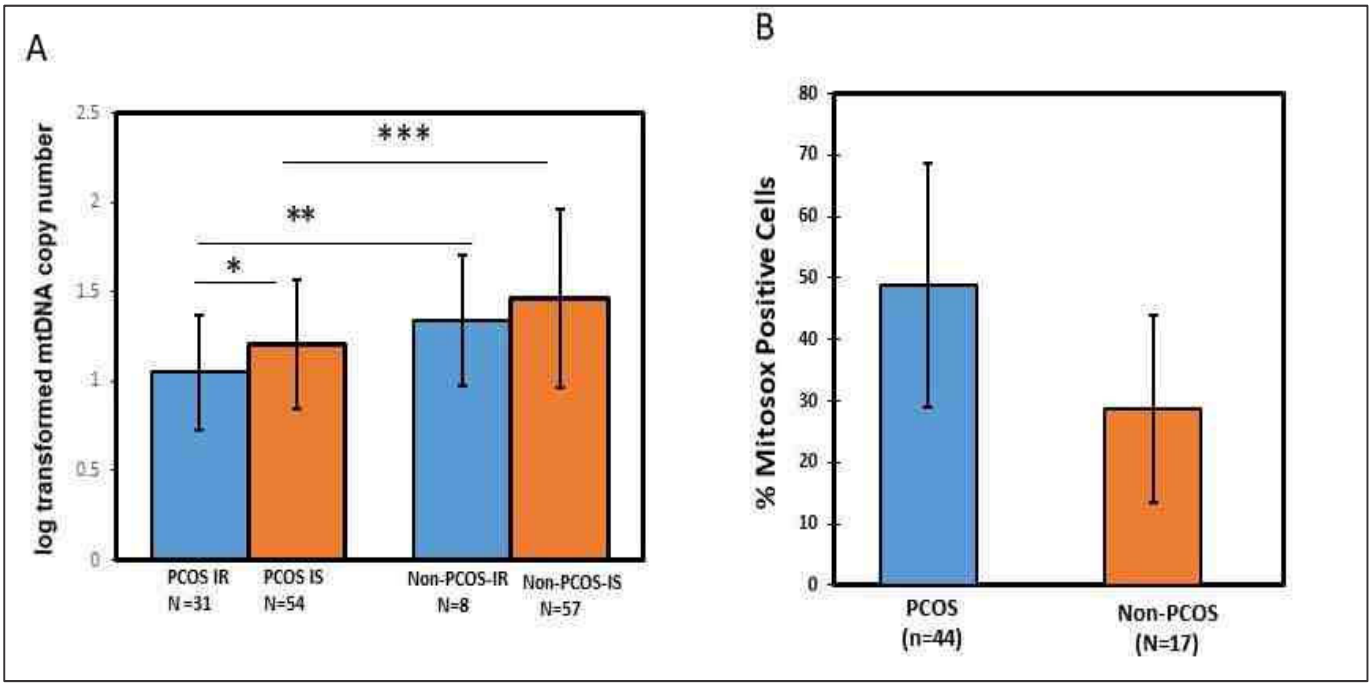


Figure 1: A. mtDNA copy number was significantly reduced in PCOS-IR group compared to PCOS-IS group (P=0.045) and non-PCOS-IR group (P=0.0059) group. B. Mitochondrial ROS production was found to be increased in women with PCOS compared to Non-PCOS group.

1.7 Study of Maternally Inherited Mitochondrial DNA Variants in Women with Polycystic Ovarian Syndrome *(Partly Funded by Indian Council of Medical Research)*

Principal Investigator	:	**Pallavi Shukla**
Co-Principal Investigators	:	Srabani Mukherjee, Anushree Patil, Beena Joshi
Collaborator	:	V Patil
Duration	:	2021-2024

Around 20%-40% of women with PCOS have an affected mother or sister with PCOS. As mitochondrial DNA (mtDNA) is exclusively maternally inherited, mtDNA variants may contribute to missing heredity of PCOS and may be implicated in the pathogenesis of PCOS. The study groups include PCOS mother daughter pairs as following; Group I: PCOS Daughters- PCOS Mothers (N=5), Group II: PCOS Daughters- Non-PCOS Mothers (N=33), Group III: Non-PCOS daughters- Non-PCOS mothers (N=9). The study revealed significant mtDNA copy number alterations in PCOS daughters with non-PCOS mothers compared to non-PCOS daughters with non-PCOS mothers (P=0.025). We did not find any significant difference in mtDNA copy number between comparisons among other groups. However, more participants have to be recruited and mtDNA copy number analysis has to be done in large number of samples to finally reach to conclusion. mtDNA variant sequencing analysis in more mother-daughter pairs is going on.

1.8 Building and Analyzing Gene Network for Polycystic Ovary Syndrome *(Partly Funded by Department of Science and Technology - Women Scientist Scheme (WOS-A)*

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

Polycystic Ovary Syndrome (PCOS) is a reproductive endocrine disorder with multifactorial etiology. Ovarian folliculogenesis is one of the major processes affected in this condition. Maintenance of the follicular intra microenvironment is essential for the proper growth and development of the oocyte. Follicular fluid which surrounds the oocyte is rich in many proteins, enzymes, metabolites etc. Dysregulation of some of these proteins in the follicular fluid has been reported in PCOS. *In-silico* prioritization of secretory proteins using reported proteins in PCOS as the training dataset identified an enrichment of complement and coagulation factors. Expression of one of the high scoring factor, Complement Factor, C1QA was found to be dysregulated in PCOS.

1.9 Investigating DNA Methylation and Histone Modifications in Cystic Ovarian Murine Model

Principal Investigator	:	**Srabani Mukherjee**
Project Associates	:	Gayatri Shinde, Sushma Khavale, P More
Duration	:	2021-2024

Environmental factors and hormones can exert their effects via epigenetic machinery such as changes in DNA methylation, histone acetylation, and regulation by noncoding RNAs. Exposure to estrogen is known to modulate the Hypothalamic-Pituitary-Ovarian (HPO) and HP-adrenal (HPA) axes. PCOS is a systemic disorder leading to reproductive and metabolic abnormalities. This study aims to investigate estrogen induced global epigenetic alterations in different tissues like the ovary, uterus, hypothalamus–pituitary, adrenal, liver, and visceral adipose tissue in an established neonatally estrogen-induced mouse model of cystic ovary. Last year we reported that the global 5 methylcytosine (5mC) level is not altered in hypothalamus-pituitary tissue (HP) of this cystic mouse model (Annual report 2021-2022, p. 6). This year we found that the global 5hmC level was comparable in HP tissue of

mouse model of cystic ovary and control ovary by both ELISA and Flow cytometry (Fig. 1A&B). Further expression of DNA demethylation machinery enzymes (TET1, TET2, and TET3) in E2T animals was not altered (Fig. 1C). This suggests that at hypothalamic-pituitary level the DNA methylation machinery is not affected in neonatally estrogenized animals. In liver, global 5mC level was found to be significantly decreased in E2T animals compared to control animals whereas 5hmC levels were found to be comparable in both the groups (Fig. 2). Thus, it indicates that methylation machinery may be altered in liver of estrogenized treated animal.

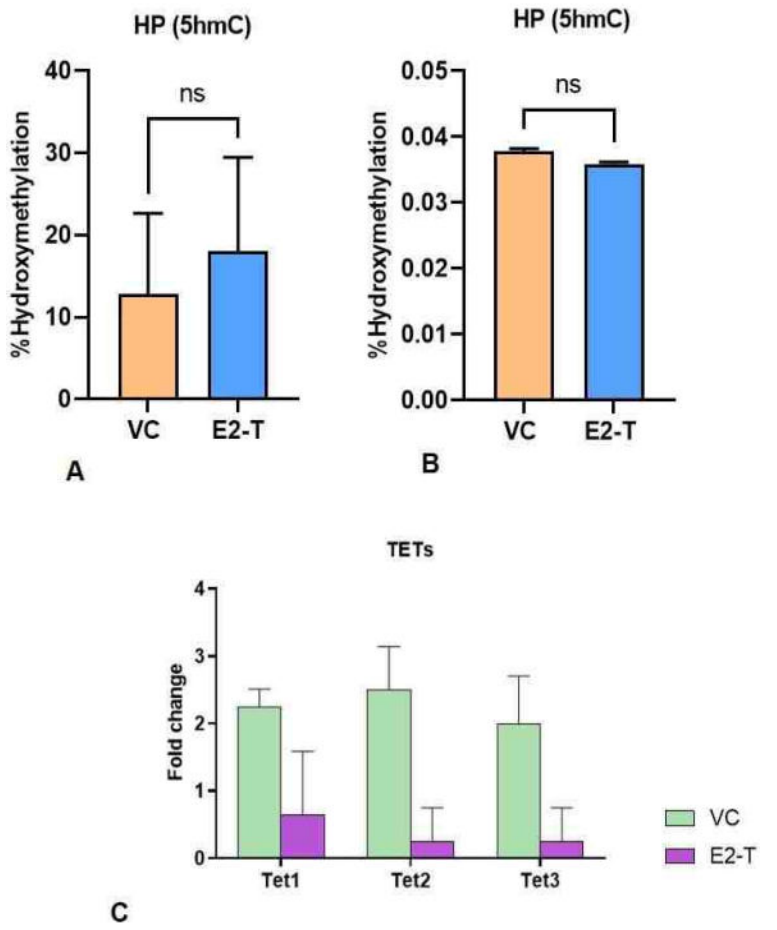


Figure 1: Global DNA 5-hydroxymethylcytosine (%5hmC) levels assessed in Hypothalamus-Pituitary of vehicle-treated controls (VC, n=4) and E2-treated adult mice (E2T, n=4) at 12 weeks post-treatment. A) represents the assessment of %5hmC by Flowcytometry and B) by ELISA.C) Transcript levels of Ten Eleven Translocases (TET1, TET2 and TET3) as measured in the Hypothalamus-Pituitary of vehicle-treated controls (VC, n=4) and E2-treated adult mice (E2T, n=4) at 12 weeks post-treatment. Fold change was evaluated using Gapdh as an internal housekeeping gene.

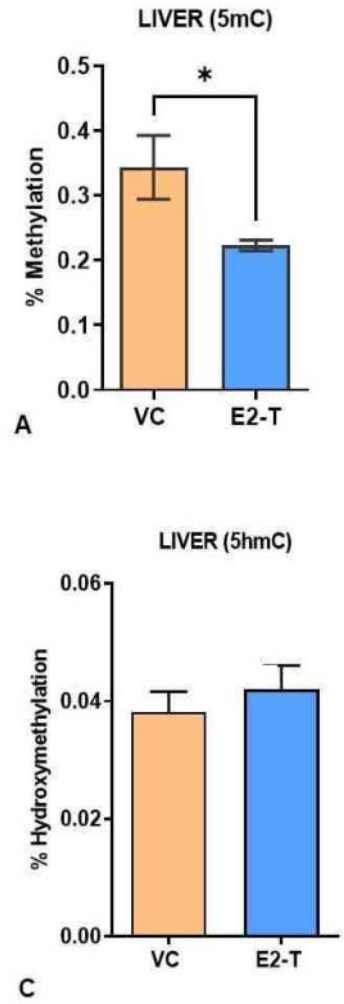


Figure 2: Global DNA 5 methylcytosine (%5mC) levels and 5-hydroxymethylcytosine (% 5hmC) assessed in liver of vehicle-treated controls (VC, n=4) and E2-treated adult mice (E2T, n=4) at 12 weeks post-treatment. A and B represent the assessment of 5mC and 5hmC respectively by ELISA.

1.10 Clinical Phenotypes and Genetic Regulation of Endometriosis in Indian Women *(Funded by Wellcome Trust Department of Biotechnology India Alliance, 2019-2024)*

Principal Investigator	:	**R Gajbhiye**
Project Associates	:	Geetanjali Sachdeva, Shahina Begum, Shagufta Khan, Akshata Shetty, Ashwini Patel, Arti Kushwaha, Kiran Kharsodiya, Komal Khade, Tabassum Khan, Naffifa Rehman, Sheetal Dubey, Sindhya Raju, Teesta Banerjee
Collaborators	:	Mentor: G Montgomery, Institute for Molecular Biosciences, The University of Queensland (UQ), Australia Smita Mahale, Scientist Emiritus Gita Mishra, School of Public Health, UQ, Australia
Duration	:	2019-2024

Endometriosis is a chronic, estrogen-dependent, inflammatory condition associated with pelvic pain, subfertility, dysmenorrhea, and dyspareunia, affecting 6-10% of women of reproductive age. It affects an estimated 247 million women worldwide and ~42 million women in India. We have undertaken a large-scale case-control study to investigate the clinical phenotypes and genetic risks associated with endometriosis in the Indian population. Replication analyses for genetic risk factors proposed for our Endometriosis Clinical and Genetic Research in India (ECGRI) study will consider new results from the latest IEGC meta-analysis. 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. The study has established a National network of endometriosis experts, a National Clinical Database, and a Biorepository of Endometriosis, engaged with clinics across the country, and conducted extensive staff training to ensure consistent and high standards of data collection essential for the study. To manage the data collection across multiple sites, we developed an electronic database for clinical data management. During the reporting year, a total of 436 endometriosis cases and 424 hospital controls were recruited making a total of 1133 cases and 1064 controls till March 31, 2023. Clinical and surgical data of the first 540 Endometriosis cases and 540 hospital controls were exported for data analysis. Endometriosis cases were classified as minimal, mild, moderate and severe disease [rASRM stages: I (n=168, 31.9%), II (n=55, 10.4%), III (n=101, 19.1%) and IV (n=203, 38.5%)]. All three 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 the subtypes of endometriosis was seen in Indian women. The distribution of isolated lesion types reported in our study cohort was as follows: 39.1 % OMA, 12.4 % SUP and 1.9 % DIE (Fig. 1).

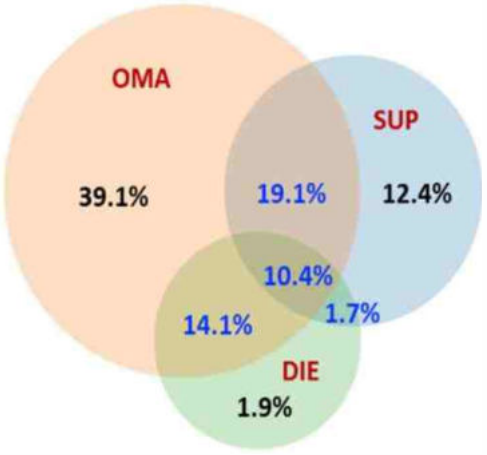


Figure 1: Frequency of endometriosis cases as per endometriosis lesion type.

The risk of endometriosis was highest in the underweight group. There was no association with age at menarche and endometriosis in our study cohort. Mild, moderate, and severe pelvic pain during menstrual period was significantly higher in women with endometriosis compared to controls. A significant difference was observed in endometriosis lesion types and pelvic pain during menstrual period. Following co-morbidities were significantly higher in endometriosis cases as compared to controls: congenital anomalies of the uterus, cervix, vagina, thyroid disorders, PCOS, hypertension, fibroid, cancer.

1.11 Determining the Role of HOXA10 in the Pathogenesis of Endometriosis

Principal Investigator	:	**DN Modi**
Collaborators	:	S Majumdar, N Ganguli, NIAB, Hyderabad MK Jolly, IISc, Banglore
Project Associate	:	Anuradha Mishra
Duration	:	2021-2025

Endometriosis is defined as the presence of endometrial-like tissues in any extrauterine region. It affects women of reproductive age and the cause of endometriosis is still unknown. HOXA10 is a transcription factor that belongs to the homeobox gene family and is required for implantation and decidualization in adult endometrium. Studies have shown that there is an altered expression of HOXA10 in both eutopic and ectopic endometrium of women with endometriosis. The aim of the project is to dissect the role of HOXA10 in endometrium and pathogenesis of endometriosis. To address the role of HOXA10, transgenic mice that expressed a shRNA against HOXA10 were developed using testicular transgenesis method. We observed that in these animals, there was almost 70% reduction in the expression of HOXA10 and hence, we termed these animals as HOXA10 hypomorphs.

In the reporting year, we analyzed these mice in greater details. We also observed that downregulation of HOXA10 led to the development of endometrial hyperplasia and most animals developed well-differentiated endometrial adenocarcinoma with age (Fig. 1). There was an increased proliferation of the uterine glands and stromal cells in the hypomorphs in both young and aged groups. In the aged animals (but not young) there was gain of OVGP1 expression and increased levels of ERα and ERβ which are similar to those observed in women with endometrial cancer. In parallel, there was increased expression of Wnt4 and β-Catenin, SOX9 and YAP1 in the endometria of young and aged animals. These observations indicate that chronic reduction in HOXA10 expression disrupts multiple pathways in the uterus that aids in the development of endometrial hyperplasia which progresses to endometrial cancer with age (Fig. 2). This part of the study is published in the Journal of Molecular Endocrinology (https://doi.org/10.1530/JME-22-0051). Studies are ongoing to investigate how the loss of HOXA10 affects the endometrium at ectopic locations simulating endometriosis.

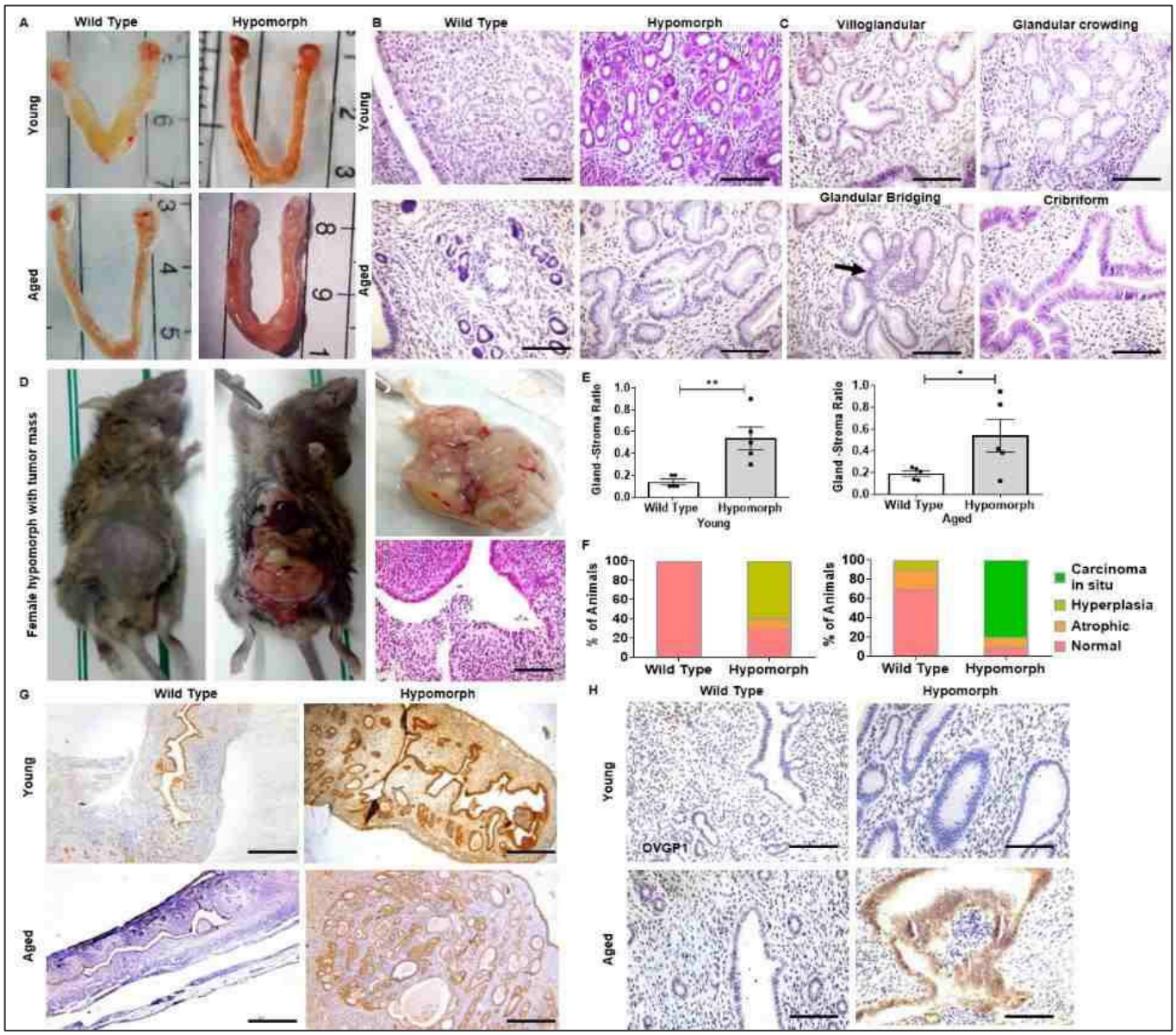


Figure 1: Endometrial hyperplasia progresses to endometrial cancer in mice hypomorphic for HOXA10. (A) Representative images of uteri from young (3 months) and aged (15 months) WT and HOXA10 hypomorphs (B). Hematoxylin and eosin-stained endometrium of WT and hypomorphic female mice. Bar, 50 μm. (C) Histology of the endometrium for aged (15 months) hypomorphic female mice representing the abnormally (villoglandular) shaped glands, glandular crowding, glandular bridging, and cribriform glandular cells. Bar, 50 μm. (D) Image of an aged (15 months) hypomorphic female mouse with a bulky abdominal region. Uterine horn with a large bulky mass from the same animal. Histology of the mass representing infiltration of leucocytes. Bar, 50 μm. (E) Gland–stroma ratio in the endometrium of young and aged hypomorphs as compared to WT. Each dot represents one animal. Mean and ±S.E.M. is shown for n = 5 WT and n = 5 hypomorphs. Statistically significant differences between the groups are shown by *P ≤ 0.05, **P ≤ 0.001. (F) Graphs representing the proportion of mice with normal, atrophic endometrium, uterine hyperplasia, and carcinoma *in-situ* endometria in WT and hypomorphs (n = 10 biological replicates per group). Immunohistochemistry for cytokeratin (G) and OVGP1 (H) in endometrial tissues of young and aged hypomorphs (brown staining). Bar, 50 μm for OVGP1 and 100 μm for cytokeratin.

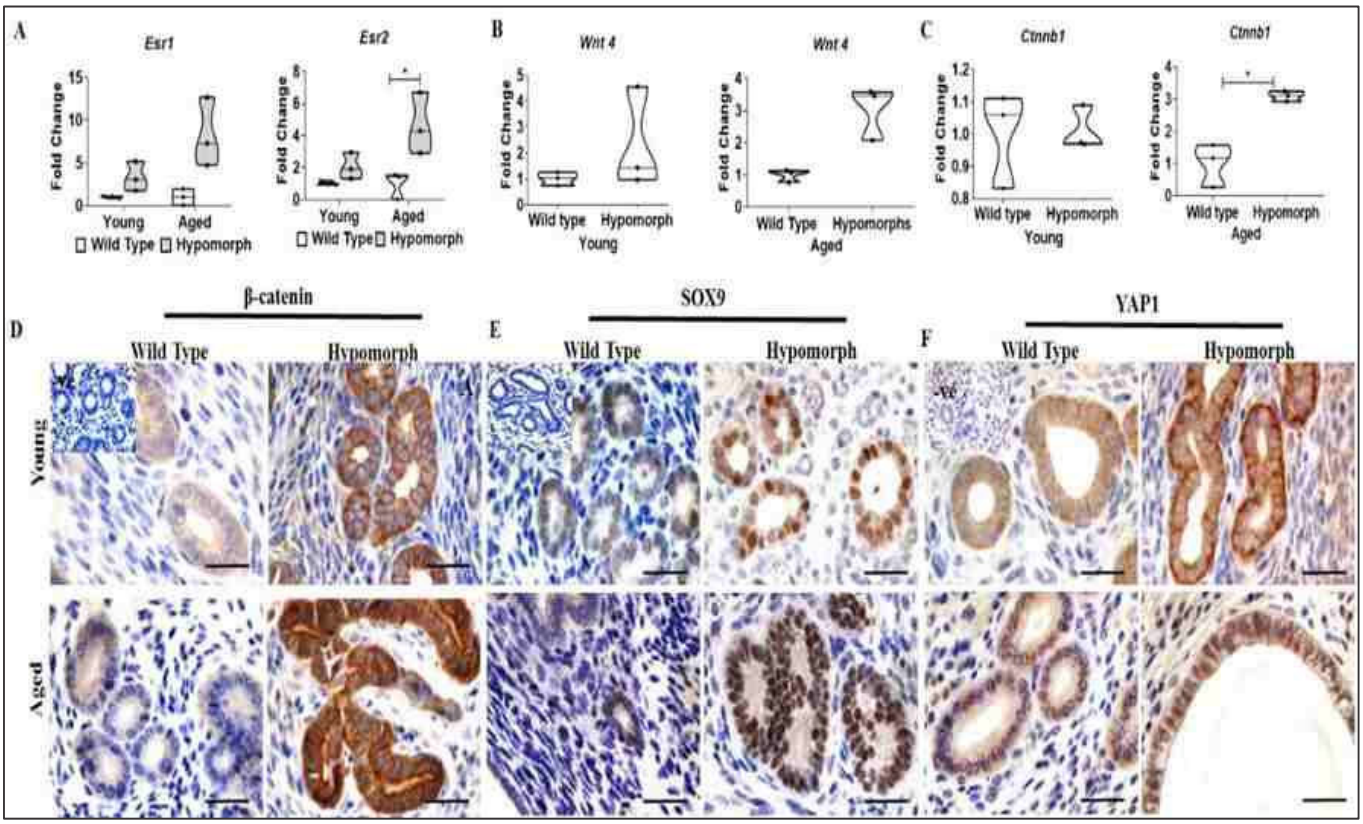


Figure 2: qPCR for Esr1 and Esr2 (A), Wnt4 (B) and Ctnnb1 (C) in endometrial tissues of young (3 months) and aged (15 months) hypomorphs and their age-matched controls. In all graphs, values on Y-axis are fold change in mRNA levels where the mean value of wild type controls is taken as 1. Each dot represents data from one animal. Mean and ±S.E.M. for each group are shown (n = 3/ group). Statistically significant differences between the groups are shown by *P ≤ 0.05. Immunohistochemistry for β-catenin (D), SOX9 (E), and YAP1 (F) in endometrial tissues of young (3 months) and aged (15 months) WT and hypomorphic mice (brown staining). Uterine sections are counterstained with hematoxylin (blue staining). Negative (-ve) in the inset is incubated without a primary antibody. Bar, 20 μm. This work is published in the Journal of Molecular Endocrinology (https://doi.org/10.1530/JME-22-0051).

1.12 Omics of Serum Exosomes in Endometriosis: An Attempt to Identify a possible Biomarker *(Partly Funded by Indian Council of Medical Research)*

Principal Investigator	:	**D K Das**
Co-Principal Investigator	:	Geetanjali Sachdeva
Project Associate	:	Kanchan Sharma
Collaborators	:	V Salunke, Shinjini Pande, Nalini Speciality Hospital, Mumbai
Duration	:	2022-2025

Endometriosis is a chronic estrogen dependent disorder that affects women of reproductive age. Women with endometriosis exhibit symptoms like dysmenorrhea, dyspareunia, cyclic pelvic pain and secondary infertility. Definite diagnosis of endometriosis is only by laparoscopic examination, which is the gold standard. There are no simple non-invasive/less invasive test that can detect the disorder

with an ease. This poses a challenge to both the treating doctor as well as the patient. Moreover, recurrence occurs at a rate of 20 to 40% within 2-5 year following conservative surgery. Therefore, there is a need to develop a simple non-invasive technique that can identify/diagnose the recurrence of the disorder. The objective of the project is to identify differentially expressed exosomal miRNA or protein in endometriosis. During this reporting year, 8 cases and 6 control samples have been collected. Exosomes have been isolated from the serum samples. Exosomes were characterized using nanoparticle tracking analysis (NTA) for size distribution and concentration of exosomes. Exosomes from 4 patients and 4 control cases had average size 114.68±68 nm and the average concentration was found to be 3.86 x 1010 particle/ml (Fig. 1a). Further, transmission electron microscopy was carried out for analysis of size and shape of exosomes. Cup-shaped structures with 50-100 nm size were identified which were within the size range of exosomes (Fig. 1b). Further, candidate miRNAs analysis was carried out using real-time PCR. Some miRNAs such as miR-210, miR-223, miR-181, miR-145 were used for differential analysis. Initial results showed amplification of all these miRNAs in serum exosomes. Overall, exosome characterization has been done during reporting year. Analysis of miRNAs are still ongoing in larger number of samples.

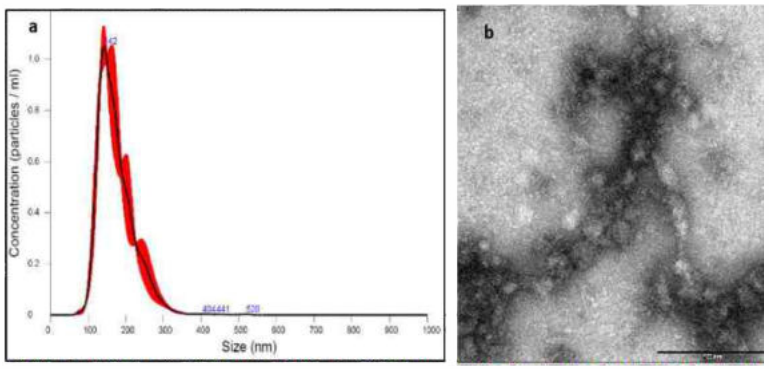


Figure 1: Characterization of serum Exosomes. (a) NTA of patient's exosome showing the average size of 142nm. (b) Transmission electron microscopy of exosomes

1.13 Investigating the Contribution of DNA Damage, Repair and Demethylation in Pathogenesis of Endometriosis *(Partly Funded by Department of Biotechnology)*

Principal Investigator	:	**Geetanjali Sachdeva**
Co-Principal Investigator	:	R Gajbhiye
Project Associates	:	Itti Munshi, U Chaudhari
Collaborators	:	A Mangeshikar, Jaslok Hospital, Breach Candy Hospital, ACI Cumballa Hill Hospital and Mangeshikar's Clinic Pratima Thamke, MGM Hospital, Navi Mumbai
Duration	:	2019-2024

Endometriosis, an estrogen dependent condition, is characterised by presence of endometrium like cells outside the uterus. Higher oxidative stress, inflammation and higher proliferation are well-established traits of eutopic and ectopic endometrium from women with endometriosis. These attributes together with higher levels of *in-situ* estrogen can contribute to higher DNA damage in the eutopic (EUE) and ectopic endometrium (ECE) in endometriosis. Our group previously demonstrated that the EUE encounters higher DNA damage than its control counterparts (EUC) (Bane et al. 2022). In response to this higher DNA damage, the DNA Damage Response (DDR) machinery is activated. DNA

repair genes like GADD45A, GADD45G, MCPHI, PPPIRI5A, XRCC3, ERCC1, MAPK12, MDC1, MLH3, RAD9A and TP73 showed significant upregulation in the mid-proliferative phase EUE compared to EUC. Whereas PRKDC was found significantly upregulated in the mid-secretory phase. In addition a higher trend in the expression of GADD45A, ATR, ATRIP, CDKNIA, DDIT3, FANCA, MAPK12, NTHLI, TP73, XRCC3, MLH3 and PMS1 was observed in the mid- secretory phase EUE compared to EUC. The present study was initiated to explore DDR in paired EUE and ECE. Endometrial stromal cells (CD10+) were isolated from paired proliferative phase EUE and ECE (endometrioma) (Fig. 1).

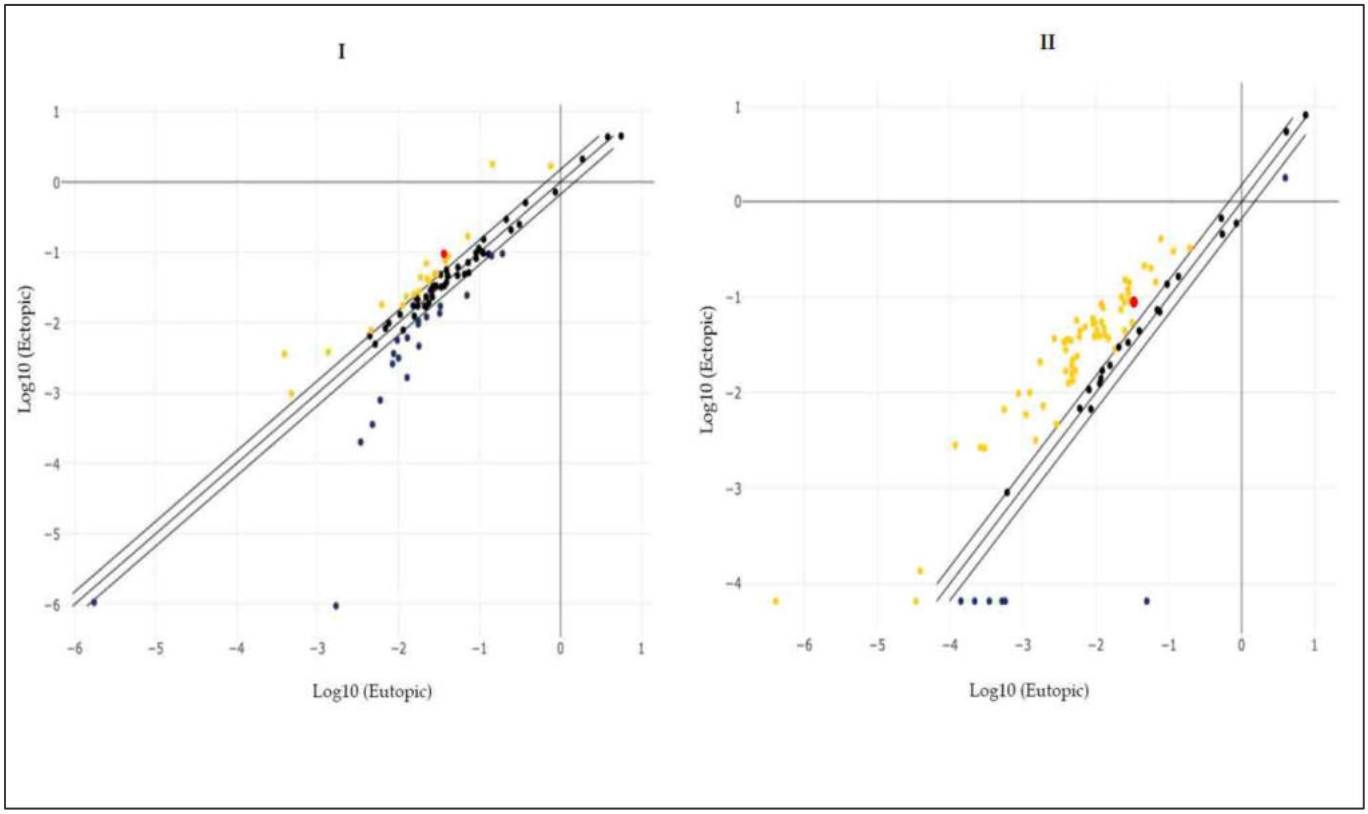


Figure 1: Scatter plots showing differential expression of DNA damage and repair (DDR) genes in stromal cells from paired proliferative phase eutopic and ectopic endometrium from women with endometriosis. Yellow dots represent genes with higher expression in ectopic vs eutopic endometrium. (MBD4 highlighted in red).

RNA samples from these sorted stromal cells were utilized to access the status of DDR genes. DDR genes DDIT3, ERCC1, MBD4, PPM1D, RAD51B, SIRT1, XPC and XRCC3 were upregulated in the stromal cells isolated from ectopic lesion compared to their respective paired eutopic endometrium, in proliferative phase, whereas BLM, BRIP1, CDC25A and EXO1 had reduced expression. In the previous year, we reported a significantly higher expression of MBD4 (Methyl CpG Binding domain 4) protein in the EUE compared to EUC (Annual report 2021-2022, pp. 18-19). Overall, these investigations collectively indicate higher DNA damage response in the lesions as compared to their paired eutopic endometrium. MBD4, popularly known as one of the guardians of CpG island, has been reported to correct any mismatch present in CpG islands. Studies will be undertaken to identify endometrial cells.

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

Principal Investigator	:	**U Chaudhari**
Co-Principal Investigator	:	Geetanjali Sachdeva
Project Associates	:	A Khandvilkar, SM Metkari, RR Katkam
Collaborators	:	Vandana Bansal, Deepti Tandon
Duration	:	2022 - 2025

Heavy Menstrual Bleeding (HMB) or Menorrhagia is defined as menstrual blood loss > 80 ml or periods lasting more than 7 days. Menorrhagia affects quality of life and is one of the most frequently encountered symptoms in women of reproductive age. Menorrhagia is caused due to an altered or inadequate endometrial repair during menstruation. The initial events of endometrial repair are independent of steroid hormones. Damage Associated Molecular Patterns (DAMPs) are molecules that are released extracellularly from damaged cells. Extracellular DAMPs are shown to activate immune cells and help in tissue repair. The aim of the present study is to understand the role of DAMPs (HMGB1) and their receptor (RAGE) in endometrial repair. We previously reported the development of a rat model of endometrial repair and breakdown (Annual report 2019-2020, p. 18). In the last year, we showed an increase in the expression and secretion of HMGB1 along with increased expression of RAGE after 8 hours of progesterone withdrawal in a rat model of endometrial breakdown and repair (Annual report 2021-2022, pp. 12-13).

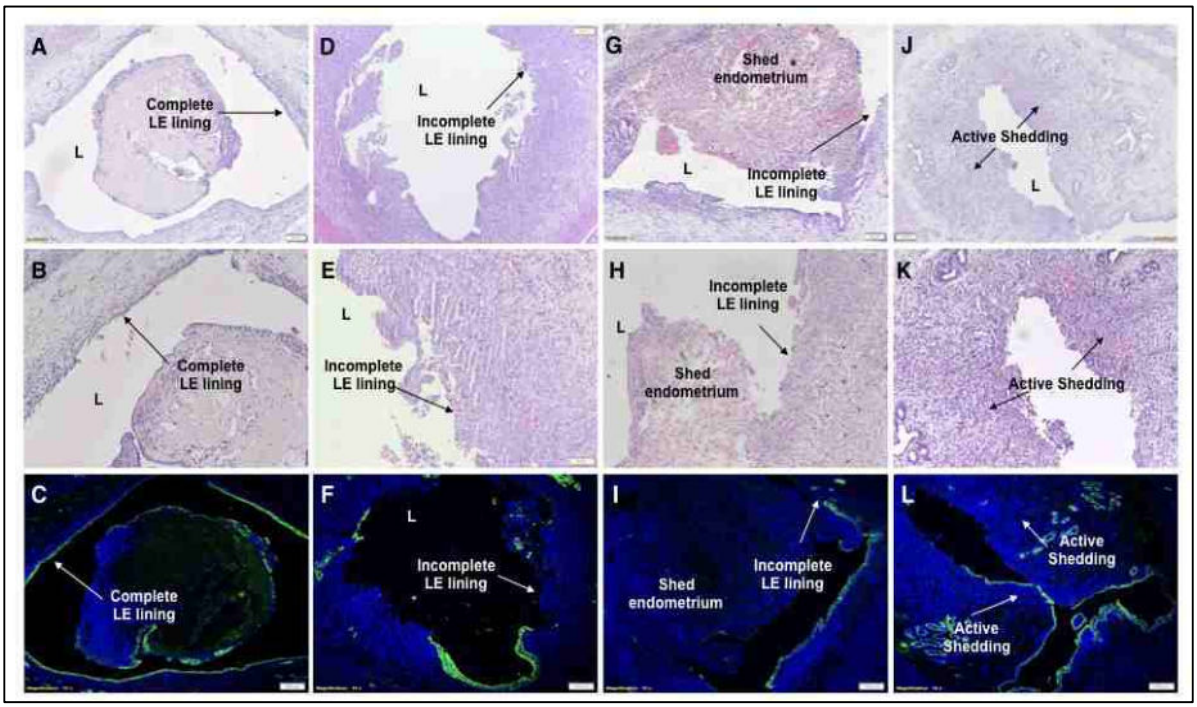


Figure 1: Representative image of Hematoxylin and Eosin stained uterine sections from control (A, B), HMGB1 inhibitor (glycyrrhizin) treated (D, E), RAGE inhibitor FPS-ZM1treated (G, H) and in combination of both (J, K. Pan-cytokeratin immunostaining of the rat uterine horn section of a vehicle control (C), glycyrrhizin treated (F), FPS-ZM1 treated (I) and in combination of both (L). Rats were sacrificed 48 hrs after P4 implant removal. Magnification 4X (A, D, G & J) and 10X (B, E, H, K, C, F, I & L). L - lumen, LE - luminal epithelium

To further understand the role of HMGB1 and RAGE in endometrial repair, the rats were treated with glycyrrhizin a HMGB1 inhibitor and FPS-ZM1, a RAGE inhibitor alone or in combination at 24 hours post progesterone withdrawal. All rats were sacrificed at 48 hours post-progesterone withdrawal and uterine horns were processed for histology and pan-cytokeratin (epithelial cell marker) immunofluorescence analysis. Uterine histology of HMGB1 inhibitor (Fig. 1C,D), RAGE inhibitor treated rats (Fig. 1E,F) and of rats treated with both (Fig. 1G,H) showed active shedding, breakdown of decidualized endometrium, and incomplete closure of luminal epithelium compared to control rats (Fig. 1A,B). Pan-cytokeratin immunostaining showed complete luminal re-epithelializationin in control rats (Fig. 1C) and incomplete luminal re-epithelization in the rats treated with inhibitors of HMGB1 (Fig. 1F), or of RAGE (Fig. 1I) alone and combination (Fig. 1L) treated rats.

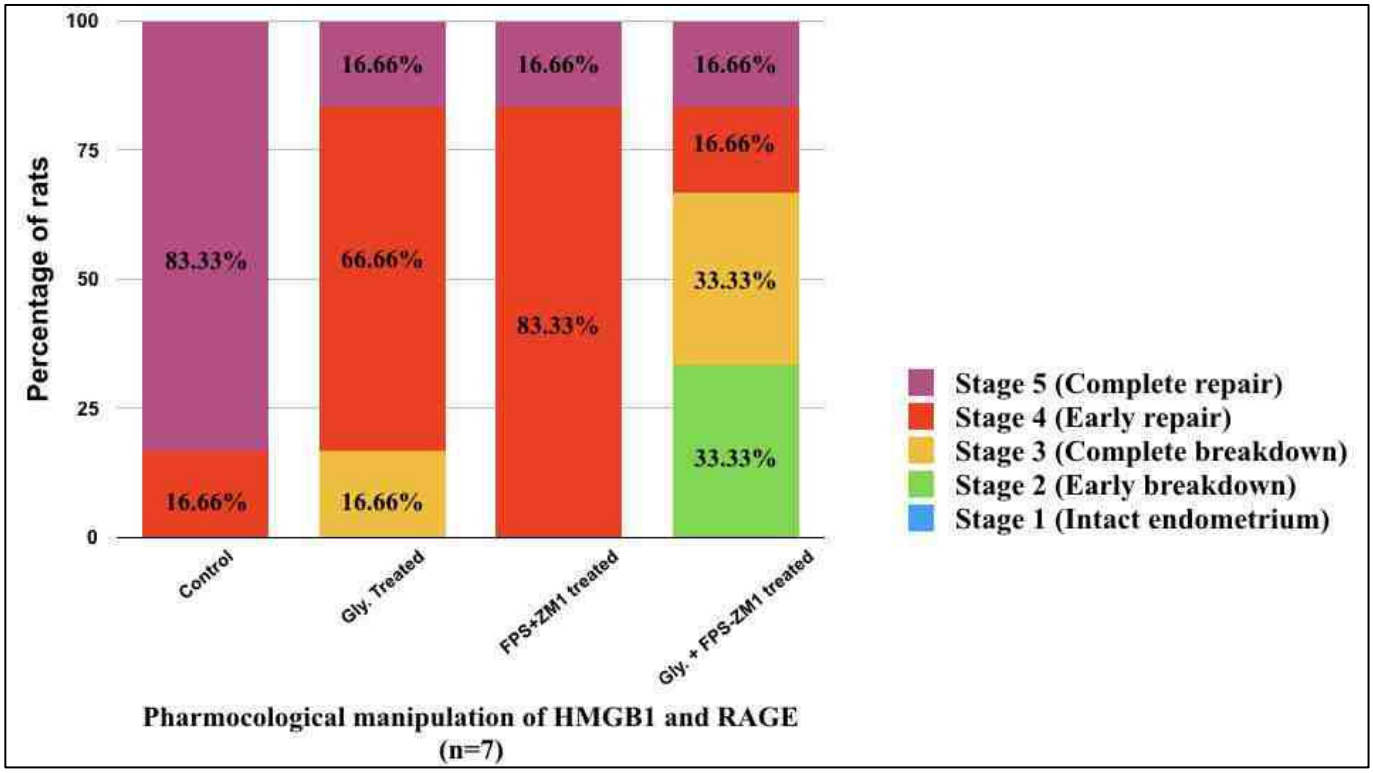


Figure 2: Graph showing percentage of rats displaying histological staging 1-5 treated either with glycyrrhizin (HMGB1 antagonist), FPS-ZM1 (RAGE antagonist) or both, along or with a vehicle.

Further, the events of endometrial repair were staged histologically into 1-5 stages. Where 1 means no breakdown (intact endometrium post progesterone withdrawal) and 5 means complete breakdown as well as re-epithelization. In the control group, 83% (n=7) rats showed stage 5 (compete breakdown, repair and re-epithelization). However, HMGB1 inhibitor treated rats showed 67% in stage 4 (early repair) and RAGE inhibitor treated rats showed 83% in stage 4. Rats treated with HMGB1 and RAGE inhibitors in combination showed 33% stage 2 (early breakdown) 33% stage 3 (complete breakdown) and 16% (early repair) (Fig. 2). These findings suggest blocking of HMGB1 and RAGE, either alone or in combination inhibits endometrial breakdown, shedding and re-epithelialization in rat model. Studies are ongoing to understand downstream mechanism of endometrial repair in HMGB1 or RAGE inhibitor-treated rats.

1.15 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, primarily an antidiabetic agent, has multiple effects on metabolism and is used in the treatment of various metabolic disorders, in addition to diabetes. Metformin is also shown to be effective for the treatment of Polycystic Ovarian Syndrome (PCOS), a reproductive metabolic disorder. Metformin is known to modulate androgen synthesis in ovaries in women with PCOS. Metformin treatment is also shown to restore menstrual cyclicity and improve pregnancy rates in PCOS women. However, limited data are available on the direct effects of metformin on endometrium. This study was initiated to evaluate the direct effects of metformin on endometrium using *in-vivo* and *in-vitro* models. We previously demonstrated low dose of metformin (50 µM) induces the proliferation of endometrial epithelial cells (Annual report 2017-2018, p. 18).

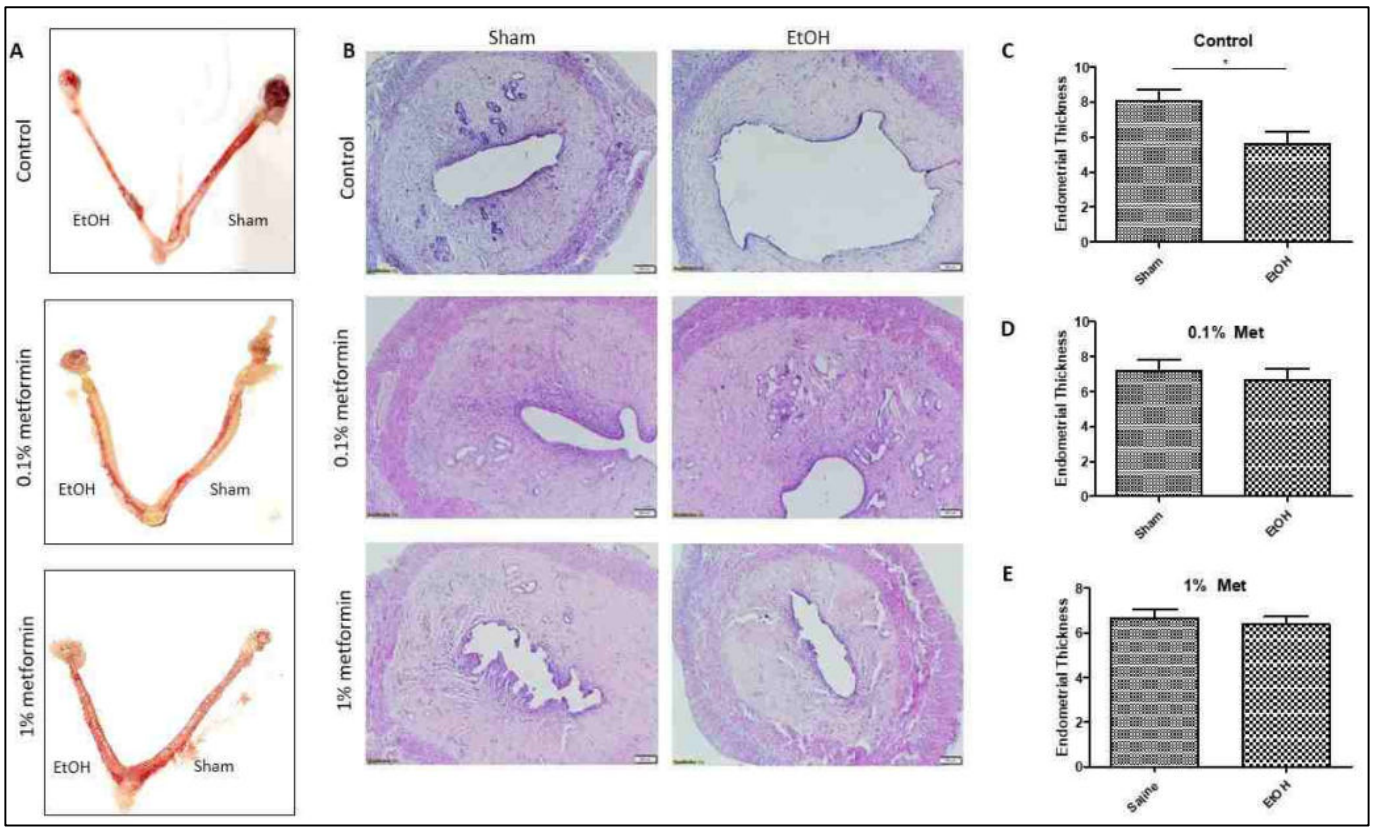


Figure 1: Histological analysis of uterine horn treated with metformin. Control and metformin (0.1% and 1%) treated uterine horns. In each animal, one horn was treated with ethanol (EtOH) and another was treated with saline (A). Hematoxylin-Eosin stained endometrial sections (4X) from control, 0.1% and 1% metformin treated animals. 0.1% and 1% metformin treated endometrium showed restoration of endometrial thickness (B). Histographs (C, D, E) comparison of endometrial thickness between sham and EtOH treated horns of control, 0.1% and 1% metformin treated animals. Saline treated control horn does show decreased endometrial thickness (*p<0.05) indicating absence of endometrial restoration.

In this reporting year, a rat model of thin endometrium was used to study *in-vivo* effects of metformin on endometrial thickness. Rat uterine horns were treated with ethanol (95%) to develop a thin endometrium rat model. Thin endometrium rats were orally treated with (0.1% or 1%) (w/v) metformin. Fifteen days of metformin treatment showed restoration of endometrial thickness in thin endometrium rats compared to control horn (Fig. 1). However, there were no differences between the number of implanted embryos and corpora lutea between control and metformin (0.1% and 1%) treated mated rats (Fig. 2). To conclude, metformin treatment could restore endometrial thickness but did not improve implantation and pregnancy outcomes. Studies are underway to assess the expression of PCNA and VEGF in the endometrium of rat model for thin endometrium that did or did not receive metformin treatment.

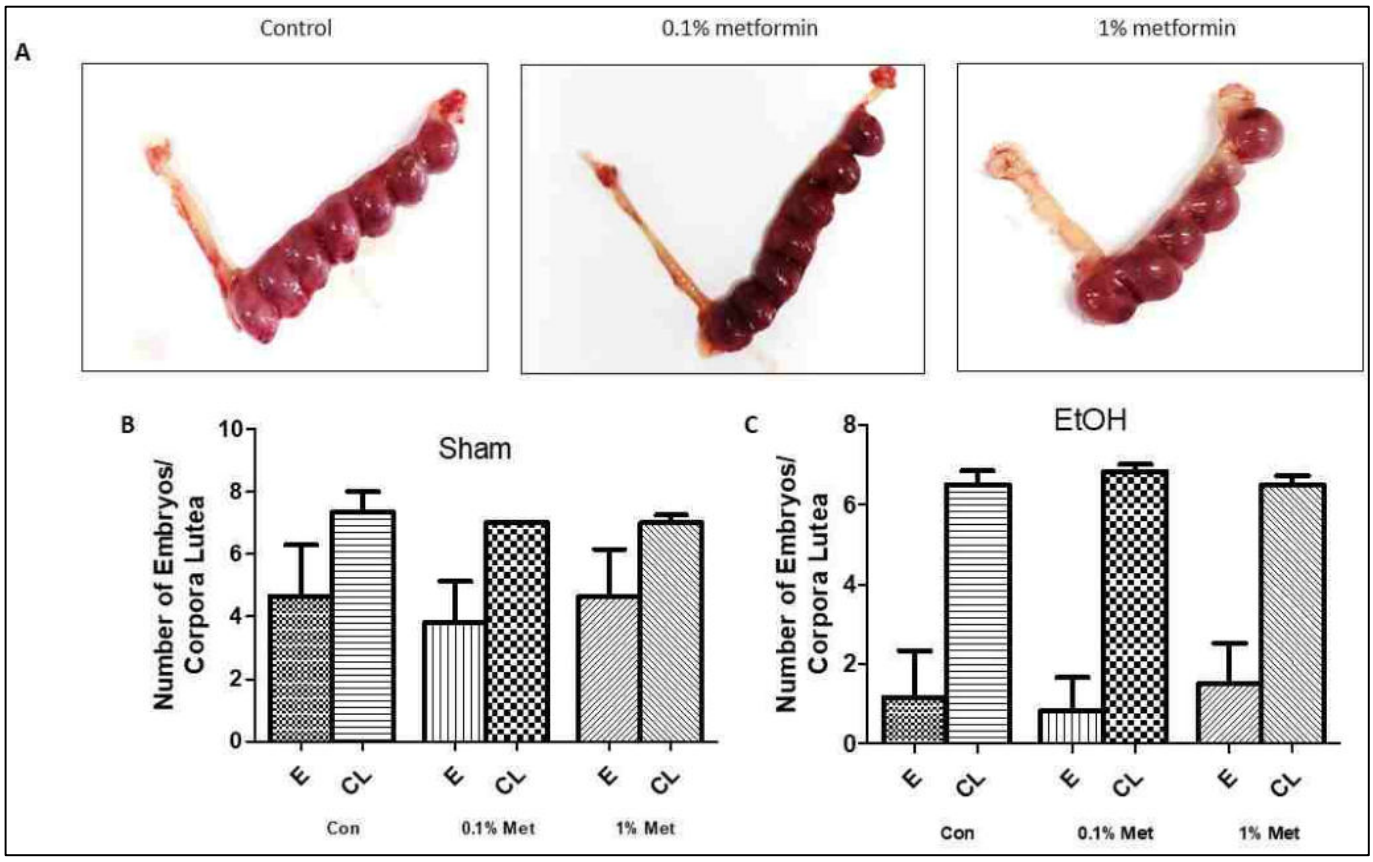


Figure 2: Pregnancy outcomes in metformin treated and control horns. Implanted embryos in the sham and ethanol treated uterine horn in control, 0.1% and 1% metformin treatment (A). Graphs represent the number of corpora lutea (CL) and implanted embryos (E) in sham and EtOH treated horns in control 0.1% and 1% metformin treated animals.

1.16 Effects of Metformin on Reproductive Aging in Rats

Principal Investigator	:	**U Chaudhari**
Co-Principal Investigator	:	Geetanjali Sachdeva
Project Associates	:	G Paswan, S M Metkari
Duration	:	2022-2026

The ovary contains a finite number of oocytes, which progressively decreases from birth to menopause. Ovarian reverse start declining from age 30's and exhaustion of oocytes occurs at age 40 to 45 s in humans. Ovary is one of earliest aging organ and ovarian aging accelerates the aging of multiple other organs in body. There is a great interest in identifying the molecules which can delay the ovarian aging to preserve the fertility and drive healthy aging. Metformin is antidiabetic drug known to affect many anti-aging pathways in dose-dependent manner. Low dose of metformin (0.1%) is found to increase longevity in mice. However, limited data is available on the effects of metformin on ovarian aging. The aim of the present study is to investigate whether metformin can delay spontaneous ovarian and reproductive aging in the rats. The rats were studied longitudinally throughout their reproductive lifespan beginning at age 3 to 12 months (M). Metformin at different concentration (0.1% and 0.5%) were administered orally in the drinking water at the age of 6M and continued up to 12M. Blood samples were collected at 3M, 6M, 9M, and 12M to assess the levels of anti-mullerian hormone (AMH), a marker of ovarian aging. Within the control group plasma levels of AMH showed significant increase at 12 M compared to 3 ($p<0.05$), 6 ($p<0.01$) and 9 ($p<0.05$) months. Within the metformin treated rats (0.1%) and (0.5%) showed no significant difference in the plasma AMH levels throughout the reproductive lifespan. The comparison of AMH levels between control, 0.1% and 0.5% at 3M, 6M, 9M, and 12M does not showed any significant difference in AMH levels, however, there was increase trend in the AMH levels in control compared to 0.1 and 0.5% metformin treated rats. Further studies are going on to evaluate the estrogen, progesterone, LH and FSH and histological analysis of ovary and endometrium in metformin treated and control rats.

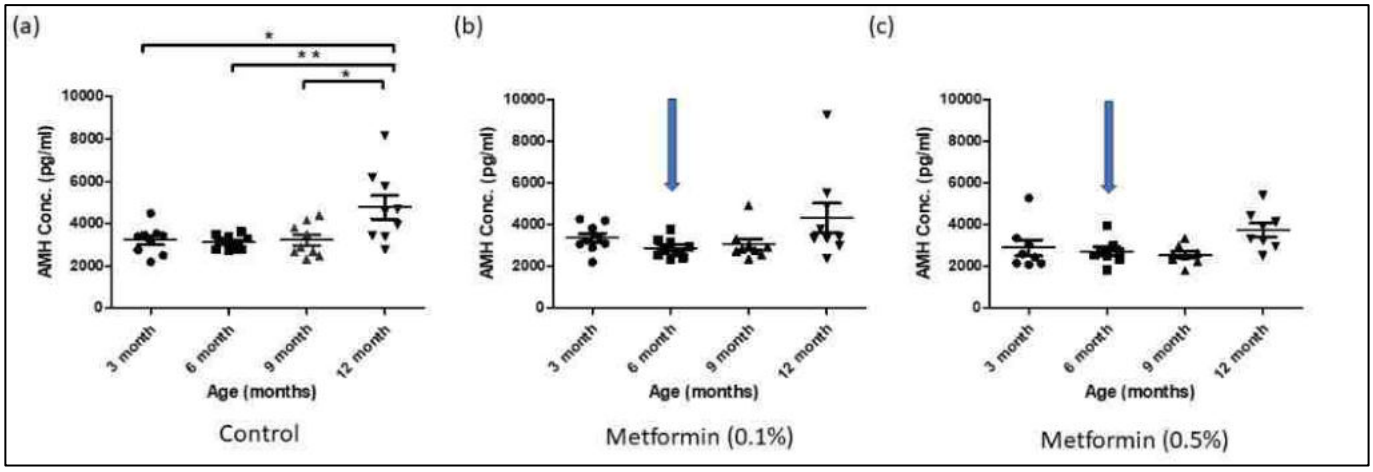


Figure 1: Scatter plots show the plasma levels of AMH at 3M, 6M, 9M, and 12M in (a) control, (b) metformin (0.1%) and (c) metformin (0.5%) treated groups. Arrows in the plots represent the start of metformin treatment. Significance within in the longitudinal data of control animals. *p<0.05 and **p<0.01

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

Principal Investigator : **Geetanjali Sachdeva**
Project Associates : U Chaudhari, V Patel, SM Metkari, RR Katkam, Rithika Rajendran, Sheetal Singhania

Collaborators : SK Adiga, P Narayan, Anjali M, Kasturba Medical College, Manipal

Duration : 2019-2024

Embryo implantation is a synchronized event in which an embryo forges physical contact with a receptive endometrium and the maternal vasculature for nourishment. Initial physical interactions are succeeded by embryonic invasion. These events involve participation of the uterine immune cells, of which uterine natural killer (uNK) cells form the major population. uNK cells are less cytotoxic and more cytokine-secreting and play a crucial role in remodelling of the maternal vasculature, regulation of trophoblastic invasion and stromal cell decidualization. Derangements in the uNK cell frequency have been reported in women who have experienced recurrent implantation failure (RIF). Our laboratory had previously demonstrated that excess of the alarmin HMGB1 in the uterine fluid led to implantation failure in rats (Annual report 2013-2014, pp. 23-24). Differential levels of HMGB1 were detected in the pre-receptive and receptive phase uterine fluid samples in humans and rats (Annual report 2010-2011, pp 25-26). In the current study, we proposed to investigate the effect of excess of HMGB1 on the uterine NK cell profile during implantation by the intrauterine administration of 0.8µg/15µL of recombinant HMGB1 (rHMGB1) on day 3 post coitum (p.c.). It was observed that excess of HMGB1 in the uterine lumen reduced the frequency of DBA-lectin+ uNK cells in the uterus of rats on day 5 p.c. (Fig. 1A; $p=0.0197$) as wells as day 8 p.c. (Fig. 1B; $p<0.0001$).

This observation was further validated by *in-vitro* studies, in which peripheral blood PBMCs isolated from healthy control women were treated with 2, 5, 10 and 50 ng/mL of HMGB1, and the flow cytometric analyses of the NK cell population revealed that treatment of PBMCs with 2 ng/mL of HMGB1 resulted in a decrease in the cytokine-secreting CD16dimCD56bright NK cells (Fig. 2B; $p=0.0257$) and a trend towards increase in the frequency of the cytotoxic CD16brightCD56dim NK cell population (Fig. 2C). These results suggested that higher levels of extracellular HMGB1 skews the NK cells to a more cytotoxic profile. Further experiments will be carried out to determine whether the altered levels uNK cells in HMGB1-treated uterine horns are associated with the impaired decidual response at the time of implantation.

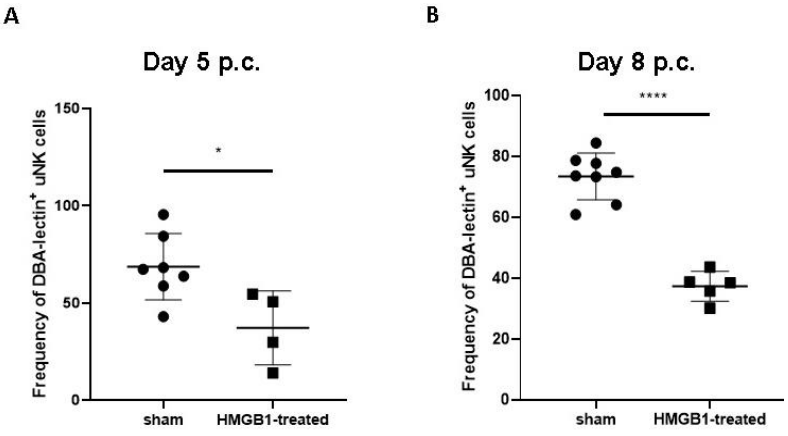


Figure 1: Effect of excess of HMGB1 on the frequency of DBA-lectin+ uNK cells on day 5 p.c. (panel A) and day 8 p.c (panel B) as determined by flow cytometry of endometrial cells isolated from sham and rHMGB1-treated animals. * in panels A ($p=0.0197$) and B ($p<0.0001$) indicate statistical significance of the difference between the two groups.

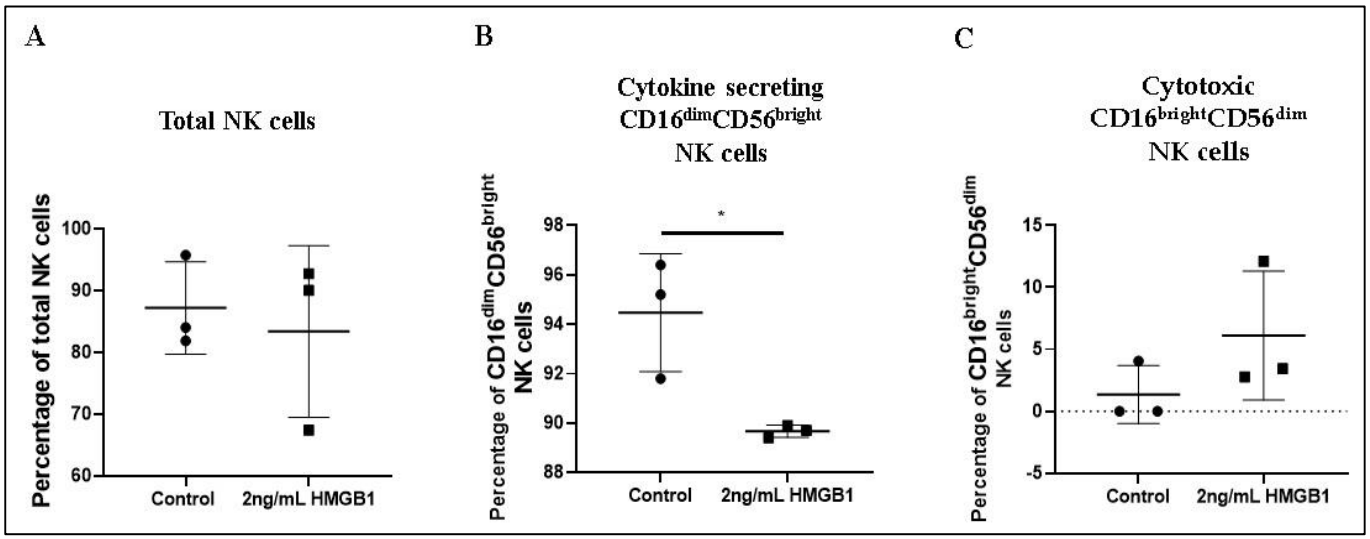


Figure 2: Effect of HMGB1 treatment to PBMCs on the frequency of total NK cells (panel A), cytokine-secreting CD16dimCD56bright (panel B) and cytotoxic CD16brightCD56dim (panel C) NK cell populations. * in panel B (p=0.0257) indicates statistical significance of the difference between the two groups.

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, Babita Negi
Collaborator	:	S Chauhan, ILS Bhubhaneswar
Duration	:	2020-2025

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-1β, suggesting that HOXA10 may be a key regulator of the inflammatory process. Herein, we aim to investigate the involvement of HOXA10 in the regulation of inflammatory processes during embryo implantation and decidualization.

We previously reported that a key proinflammatory cytokine IL1β is expressed specifically at the implantation site mainly in the decidua and not at the inter-implantation site. IL1β 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. In the reporting year, we investigated the expression of TXNIP. Decidualization is accompanied by unfolded protein response (UPR) and reticular stress (RS) response. TXNIP (thioredoxin-interacting protein) which gets activated in response to UPR and RS is known to in turn activate inflammasomes. We checked the expression of TXNIP during embryo implantation. We observed that TXNIP expression in the mouse endometrium starts from Day 4 and peaks day 6 during embryo implantation. Like inflammasomes, TXNIP is also expressed specifically at the implantation site and decidua and not at the inter-implantation site. We also observed co-localization of IL1 β and TXNIP in the decidua of Day 6 and Day 7 of embryo implantation.

To investigate the role of NLRP3 in embryo implantation, we treated the animals with MCC950, which is a chemical inhibitor for NLRP3. In the previous year, we had reported that by blocking NLRP3 there is the failure of embryo implantation and the embryos were misoriented and the implantation cup had not correctly formed. In the MCC950 treated group, in most animals, there were no clear implantation sites. To investigate the NLRP3 inhibition, in the reporting year, we checked the expression of Gasdermin D (GSDMD) that is processed by NLPR3 inflammasome. We observed that the expression of GSDMD was drastically reduced in the treated animals as compared to controls. Similarly, expression of Caspase 1 was also reduced in the treated animals versus control animals. 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 *(Partly Funded by Department of Science and Technology, Science and Engineering Research Board)*

Principal Investigator	:	**DN Modi**
Project Associates	:	Nancy Ashary, Pranya N
Collaborator	:	M K Jolly, IISc Banglore
Duration	:	2020-2023

Embryo implantation occurs in three stages, which include adhesion, attachment, and invasion. This process eventually allows the trophectoderm to demolish the luminal epithelial (LE) barrier and make direct contact with the underlying stroma. Genetic studies show that blocking trophectoderm passage through the LE barrier is a contributing factor to implantation failure. Even while trophectoderm-LE interactions have been researched for decades, it is still unknown how LE cells get cleared up. We have earlier shown that at the site of implantation in the mouse the luminal epithelial (LE) cells undergo epithelial-to-mesenchymal transition (EMT) that may cause the luminal epithelium to dislocate, leading to embryo invasion (Annual report 2021-2022, p. 22). In the reporting year, we tested if loss of HOXA10 causes EMT in the endometrial epithelial cells. Towards this, we knocked down HOXA10 expression in the endometrial epithelial cell line and observed that downregulation of HOXA10 leads to altered E-CAD and N-CAD expression in endometrial epithelial cells. There was increased expression of transcription factor TWIST2, SNAIL and SLUG in HOXA10 knockdown cells compared to control.

In the reporting year, we carried out experiments to understand how HOXA10 affects EMT *in-vivo*. To investigate that we used the HOXA10 hypomorph model. We demonstrate that, in the hypomorphs, when compared to controls, the mesenchymal marker N cadherin expression was increased and E cadherin expression was decreased in non-pregnant state. Further, during embryo implantation, there was precocious removal of LE in the hypomorphs. These findings showed that the absence of HOXA10 causes EMT during embryo implantation. The result reveals that embryo implantation is associated with EMT and loss of HOXA10 induces EMT in the endometrial epithelium *in-vivo* and *in-vitro*.

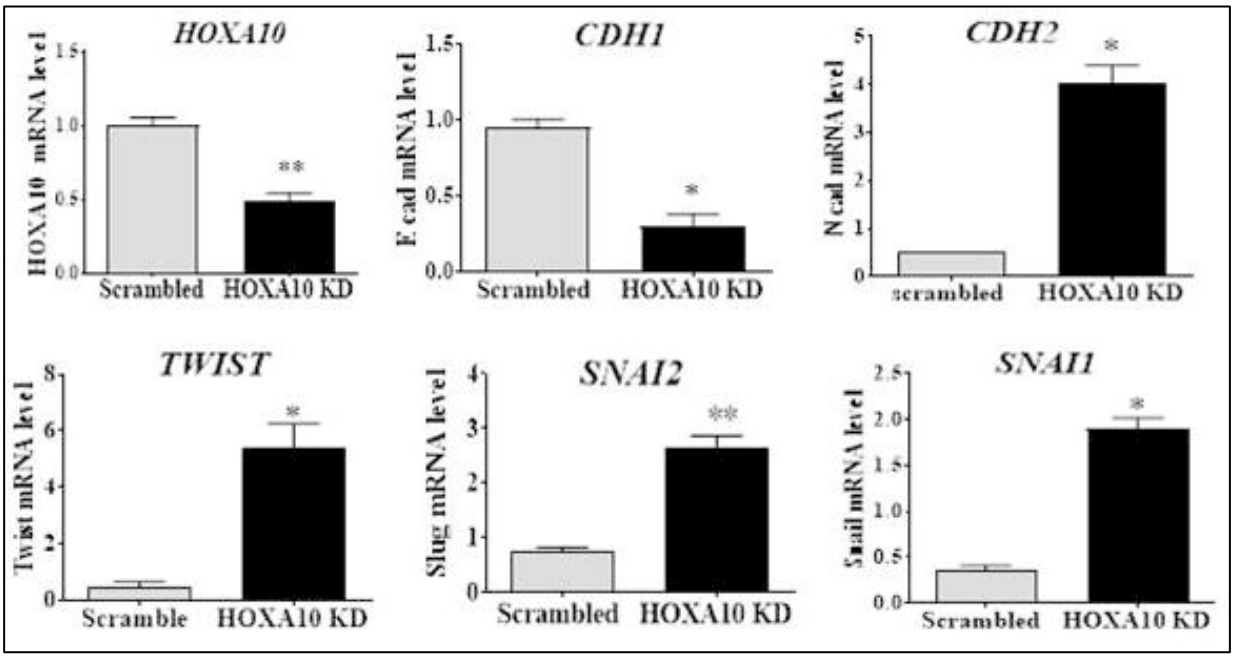


Figure 1: Effect of loss of HOXA10 in endometrial epithelial cells mRNA levels of HOXA10, CDH1, CDH2, SNAI1, SNAI2 and TWIST in control (scrambled) and HOXA10 knockdown (HOXA10 KD) RL-95 (endometrial epithelial cell line). Y-axis is fold change where values obtained from scrambled cells were taken as 1. *p<0.05, **p<0.001.

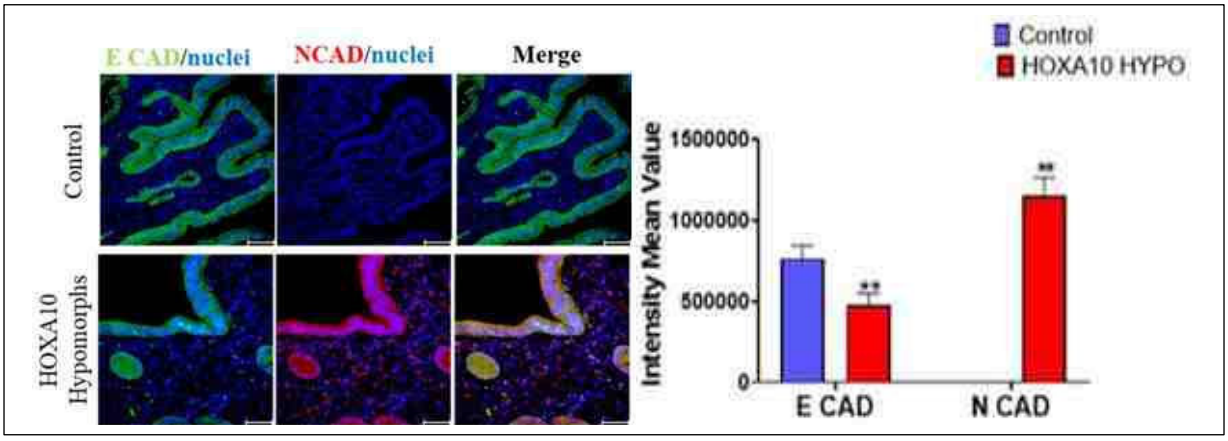


Figure 2: Expression of EMT markers in HOXA10 hypomorphs: Immunofluorescence images for E CAD (green), N CAD (red) and nucleus (blue) of control and HOXA10 hypomorphs non-pregnant uterus. Graph represents fluorescence intensity mean value of E cad and N cad in non-pregnant uteri of control and HOXA10 hypomorph with X axis represent E cad and N cad and Y axis represent intensity mean value. ** p<0.001.

1.20 Development of a Microfluidic-Based Tool for Assessing Placental Functions and Evaluating its Potential Application in Pregnancy-Related Disorders *(Funded by Imprint II C Department of Science and Technology - Science and Engineering Research Board)*

Principal Investigator : **DN Modi**
Project Associate : A Bhide

Collaborators : S Majumder, Debjani Paul, IIT-Bombay
Duration : 2020-2025

Placental dysfunctions are one of the causes of many pregnancy-related complications. Research on placental (patho-) physiology is impeded due to a lack of good model systems that simulate the human placenta structurally and functionally *in-vitro*. This project aims at developing placenta-on-chip devices that would aid biologists and the pharma industry to study various aspects of placental biology including high throughput screening. We had designed several devices that mimicked various aspects of the feto-maternal interface including embryo implantation, floating villus, and stem villus. In the reporting year, we benchmarked and validated two such designs that enable the study of the process of placentation. The first design was to mimic the anchoring/stem villus to study extra villus trophoblast migration in response to decidual stimulus which is an essential aspect of placental biology. In this design, there are two chambers connected by a microchannel and the design allows the formation of morphogen gradients. COMSOL simulation was done to optimize the device`s parameters to allow gradient formation in a stipulated time. Based on the results, the design was optimized and used as a proof of concept to study cell migration in response to a chemical gradient. The migration of extravillous trophoblast cell line HTR-8/SVneo in response to HGF, EGF, FGF2, and BMP2 and compared to the wound healing assay (Fig. 1A).

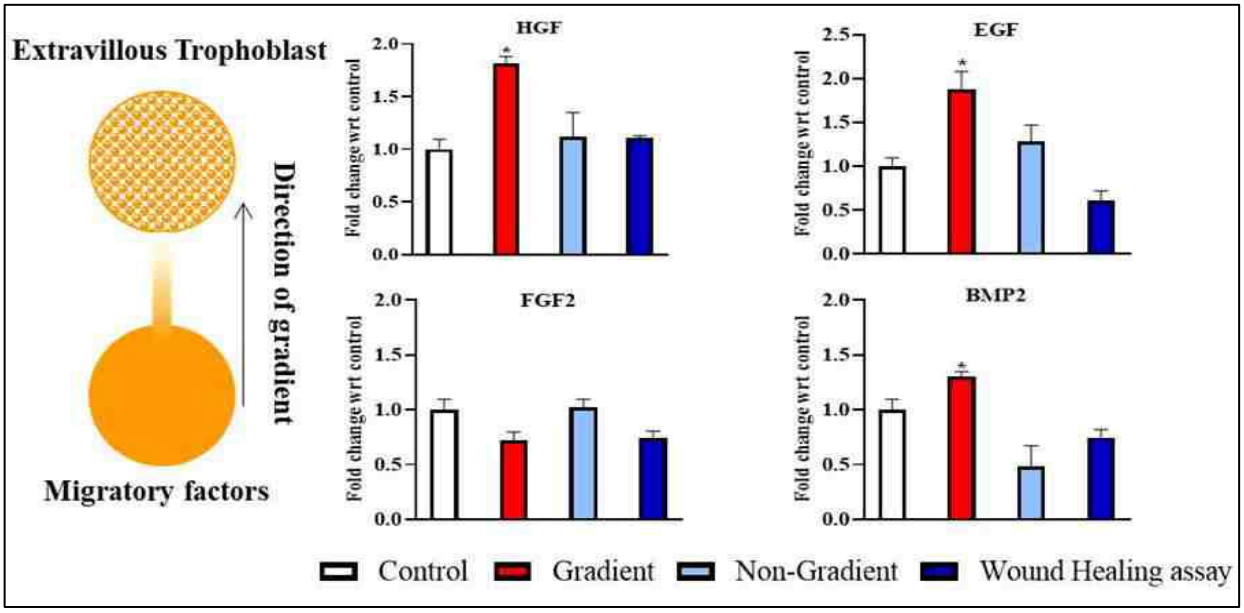


Figure 1: EVT migration in response to morphogen gradient on a placenta on chip device. Data is presented as mean ± SD. Y axis is the fold change with respect to control, *is $p<0.05$.

As evident, all the growth factors significantly induced migration within 24h in response to a gradient while no major increase was observed in non-gradient conditions as compared to untreated controls. We have developed and validated another device mimicking the barrier function of the placenta. These devices will help in understanding transplacental drug transport under static and flow conditions. We first optimized the device parameter using COMSOL simulation to define the kinetics of glucose transport across the membrane. In these devices, BeWo (model cell line for syncytiotrophoblasts) and

HUVEC cells (endothelial cells) were co-cultured mimicking the placental barrier. These cells formed a monolayer on either side of the membrane (Fig. 2A). We investigated the structural integrity of the barrier by studying the transport of glucose, FITC, and high molecular weight molecule FITC-Dextran. As evident (Fig. 2B), glucose showed active transport, FITC transport was not impeded by the cells while FITC-Dextran did not cross the barrier in the presence of the cells. These benchmarked devices are currently being used to investigate drug transport across the barrier, and study effects of infections and high glucose conditions.

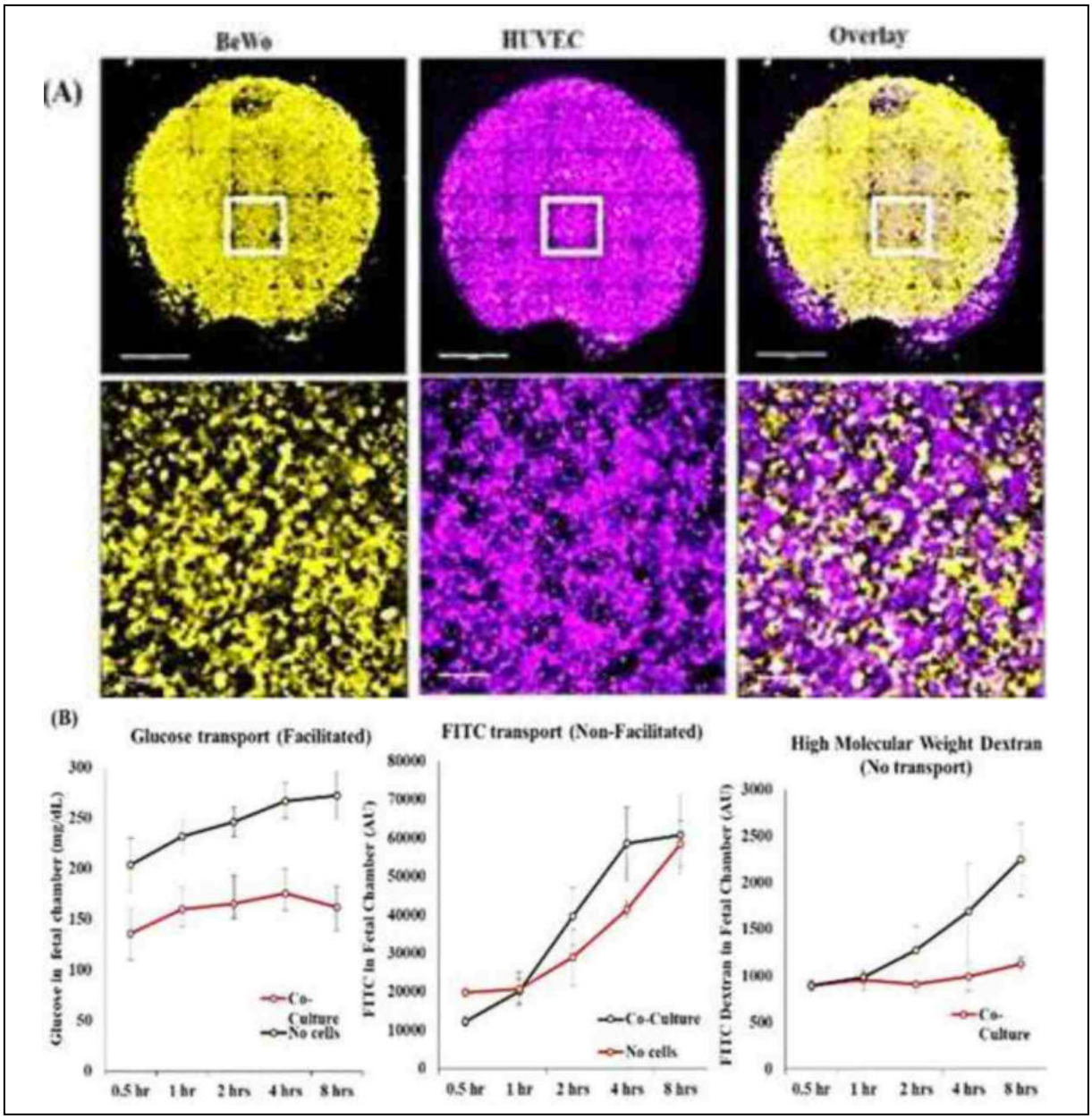


Figure 2: Placenta-on-chip to assess placental barrier function (A) Co-culture of Trophoblast cells (BeWo, Yellow) and fetal endothelial cells (HUVEC, Pink) (B) Placenta-on-chip device validation by Glucose, FITC and FITC-Dextran for barrier integrity and function. Data is presented as mean ± SD. Each dot represents one time point.

MALE INFERTILITY AND ASSOCIATED REPRODUCTIVE DISORDERS

2. MALE INFERTILITY AND ASSOCIATED REPRODUCTIVE DISORDERS

2.1 Molecular Mechanisms Involved in Prolactin and Dopamine Signaling in Male Reproduction *(Funded by Science and Engineering Research Board, Department of Science and Technology)*

Principal Investigator	:	**Nafisa H Balasinor**
Co-Principal Investigators	:	Priyanka Parte, Dipty Singh
Project Associates	:	Sanketa Raut, Kushaan Khambata
Duration	:	2020-2023

Hyperprolactinemia is prevalent in upto 16% of the infertile males. Prolactin and dopamine receptors (PRLR and D2R) are present on various testicular cells, including spermatogonia, spermatocytes and spermatids. The aim of this study is to delineate the mechanisms by which prolactin and dopamine affect male reproduction. In the previous year, we reported downstream signaling and gene alteration associated with PRLR in seminiferous tubule culture (STC). PRLR activated JAK2/STAT5 pathway, but not MAPK/ERK and PI3K/AKT pathways in the testicular cells. Microarray identified a total of 692 differentially expressed genes (DEGs) regulated by prolactin. Furthermore, top-regulated genes along with genes involved in cell cycle processes were validated by qPCR (Annual report 2022-23, pp. 33-34). In the reporting year, in vitro gene alteration pertaining to D2R were studied. Microarray was performed to identify genes regulated by dopamine after treating STC for 24 hours with D2R agonist bromocriptine (5µg/ml). A total of 1077 DEGs were identified; of which, 511 were upregulated and 566 were down-regulated (fold-change>1Log2; $p<0.05$). Classification of DEGs into various testicular cell types showed that majority of the DEGs were expressed in the post-meiotic cells including early and late spermatids, and sperm. Gene ontology (GO) of DEGs performed using g:Profiler showed developmental processes, cell development, and cell motility to be enriched under biological processes. Additionally, processes related to extra cellular matrix (ECM) were enriched and supported by differential expressions of various collagens and laminins, thereby indicating a role of dopamine in ECM integrity. The top 3 up-regulated DEGs are Syt12, Sik1, LOC102555340; and the top 3 down-regulated DEGs are Tmem52b, LOC102556854, Slc39a5 (Table 1).

Table 1: List of top up-regulated and down-regulated genes obtained by microarray of STC after bromocriptine treatment

Gene Symbol	Gene Name	Fold Change (Log Base2)
Syt12	Synaptotagmin 12	3.188
Sik1	Salt-inducible kinase 1	3.184
LOC102555340	Uncharacterized LOC102555340	3.04
Tmem52b	Transmembrane protein 52B	-4.025
LOC102556854	Uncharacterized LOC102556854	-3.564
Slc39a5	Solute carrier family 39 member 5	-3.269

The top regulated genes were validated by qPCR and all the genes followed a similar trend in gene alteration as in microarray. Dopamine is known to play a role in sperm motility. This is further supported by enrichment of cell motility and microtubule motor activity by GO analysis that is

fundamental to sperm flagellum motility. Therefore, expression of eleven genes involved in sperm motility were validated by qPCR. The results obtained showed that seven genes (Akap4, Ccnyl1, Iqcf1, Klc3, Prss55, Tbc1d21, Tl18) were up-regulated, whereas four genes (Dnah1, Dnah5, Clxn, Fsip2) were down-regulated upon treatment with bromocriptine (Fig. 1). Furthermore, in vivo male rat model for hypo- and hyper-prolactinemia (Prl) were established by injecting D2R agonist (Bromocriptine) (0.5 mg/kg b.wt/day) and antagonist (Fluphenazine) (3 mg/kg b.wt/day), respectively, for 60 days. A significant increase in pre- and post-implantation loss (PIL and POL) was observed in both groups. Time taken to mate in hyper-Prl group was also increased, along with reduction in litter size, sperm count, and sperm motility (Fig. 2). Analysis of differential germ cell population by flow cytometry revealed that hyper-Prl group showed significant reduction in elongated and elongating spermatids and a concomitant increase in round spermatids indicating an arrest in differentiation of round to elongated spermatids. Hormonal profile and transcriptome analysis is ongoing. Results of this study demonstrate that dopamine and prolactin are crucial for different aspects of male fertility.

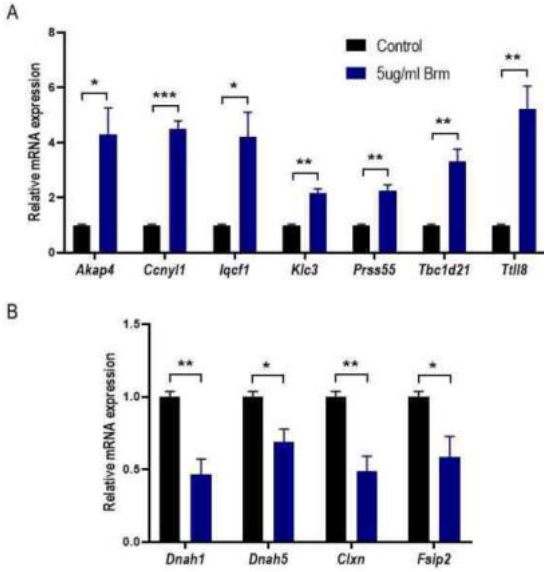


Figure 1: Relative expression of genes involved in sperm motility. Expression of genes (A) up-regulated and (B) down-regulated in seminiferous tubule (ST) culture 24 hours after treatment with bromocriptine (5µ/ml). ST without the drug were used as control. Values are represented as mean ± SEM; N = 6; * $p < 0.05$; ** $p < 0.01$; *** < 0.001.

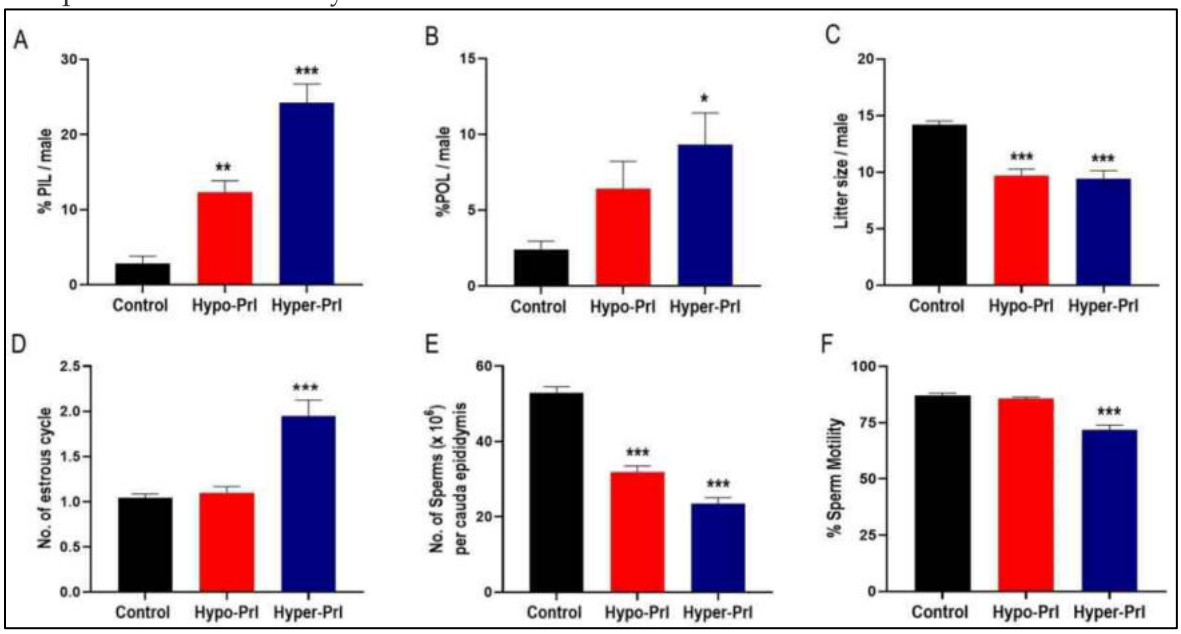


Figure 2: Fertility studies in hypo- and hyper-Prl group after treatment with Brm and Flu for 60 days. Pre- and post-implantation loss (A&B), litter size (C), time taken to mate (D), caudal sperm count and motility (E&F). Saline was used as vehicle control. Values are represented as mean ± SEM; N = 6; * $p<0.05$; ** $p<0.01$; *** <0.001

2.2 Unravelling Sperm Epigenetic Landscape Regulated by Estrogen Receptors in Adult Male Rats *(Partly Funded by Science and Engineering Research Board, Department of Science and Technology - Start-up Research Grant)*

Principal Investigator	:	**Kushaan Khambata**
Project Associate	:	Priyanka Bera
Duration	:	2021-2024

Estrogen through its receptors (ERα and ERβ) 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 ERα (PPT) and ERβ (DPN) agonist for 60 days led to decreased fertility in adult male rats resulting in pre- and post-implantation embryo loss. 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 ERα and ERβ on sperm DNA methylome in rat models. Whole genome bisulfite sequencing (WGBS) revealed 4653 differentially methylated genes (DMG) in PPT and 314 DMGs in DPN (Annual report 2021-2022, pp. 39-40). In the reporting period, gene ontology and pathway analysis for the DMG were done using g:Profiler software. Based on molecular functions, most of the DMGs for PPT were involved in catalytic and binding activities (Fig. 1A), whereas for DPN they were involved in transporter activities (Fig. 1B). For the biological processes, the DMGs were involved in several developmental processes, such as anatomical structure and organ development (Fig. 1C&D). Pathway analysis revealed that most of the pathways were involved in developmental biology for PPT and post-translational protein modification for DPN (Fig. 1E&F).

To obtain a better overall picture of the biological processes and pathways affected, the output for these ontologies was subjected to enrichment map analysis using Cytoscape software. Similar ontologies were grouped into clusters using AutoAnnotate, clusterMaker2, and WordCloud application of Cytoscape. Sub-networks of clusters involved in developmental processes were then manually curated from the annotated enrichment maps. For PPT, the largest cluster consisting of 55 ontologies were involved in transcriptional regulation of developmental and growth processes. Several clusters were involved in cell migration and cell proliferation; both of these processes are critical for embryogenesis. Further subgroups of clusters involved in organogenesis, neurogenesis, and heart development were also identified (Fig. 2A). Similarly, for DPN, the largest cluster in the enrichment map was involved in developmental process with 34 ontologies. These developmental processes were majorly involved in protein phosphorylation, transmembrane transport, cellular localization and transport, cell migration and morphogenesis. All these processes play a key role during embryo development. These results indicate that ERs can epigenetically regulate these processes during embryogenesis via the sperm DNA methylome. DMGs involved in the ontologies of embryo and placenta development were selected for validation by pyrosequencing. For PPT, imprinted genes such as Peg3, Igf2r, Sfmbt2, Cdkn1c, Tgfb1, WT1, and Gab1 were selected. Also, genes associated with histones in the spermatozoa in rodents such as Bmp4, Myc, Hoxc10 were selected. For DPN, genes such as Erf, Sox5, Ovol2, and Hoxa cluster were selected. All the selected genes showed similar trend of differences as compared to the control in pyrosequencing.

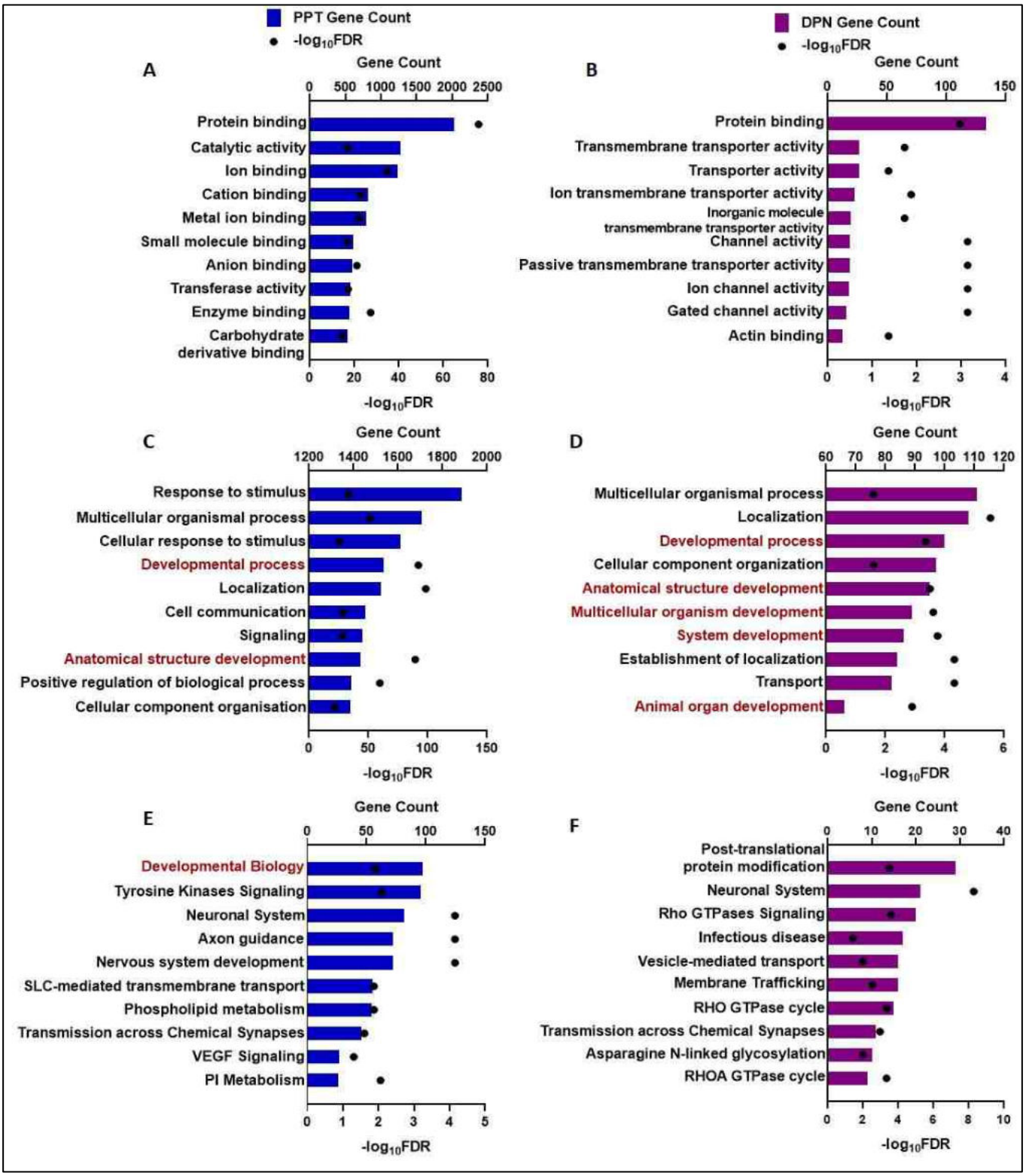


Figure 1: Gene ontology analysis: top 10 ontologies enriched for molecular functions (A and B), biological processes (C and D), and pathways (E and F) for differentially methylated genes in spermatozoa after PPT (blue bars) and DPN (purple bars) treatments. The bars represent the gene count and black dots represent the -log10FDR.

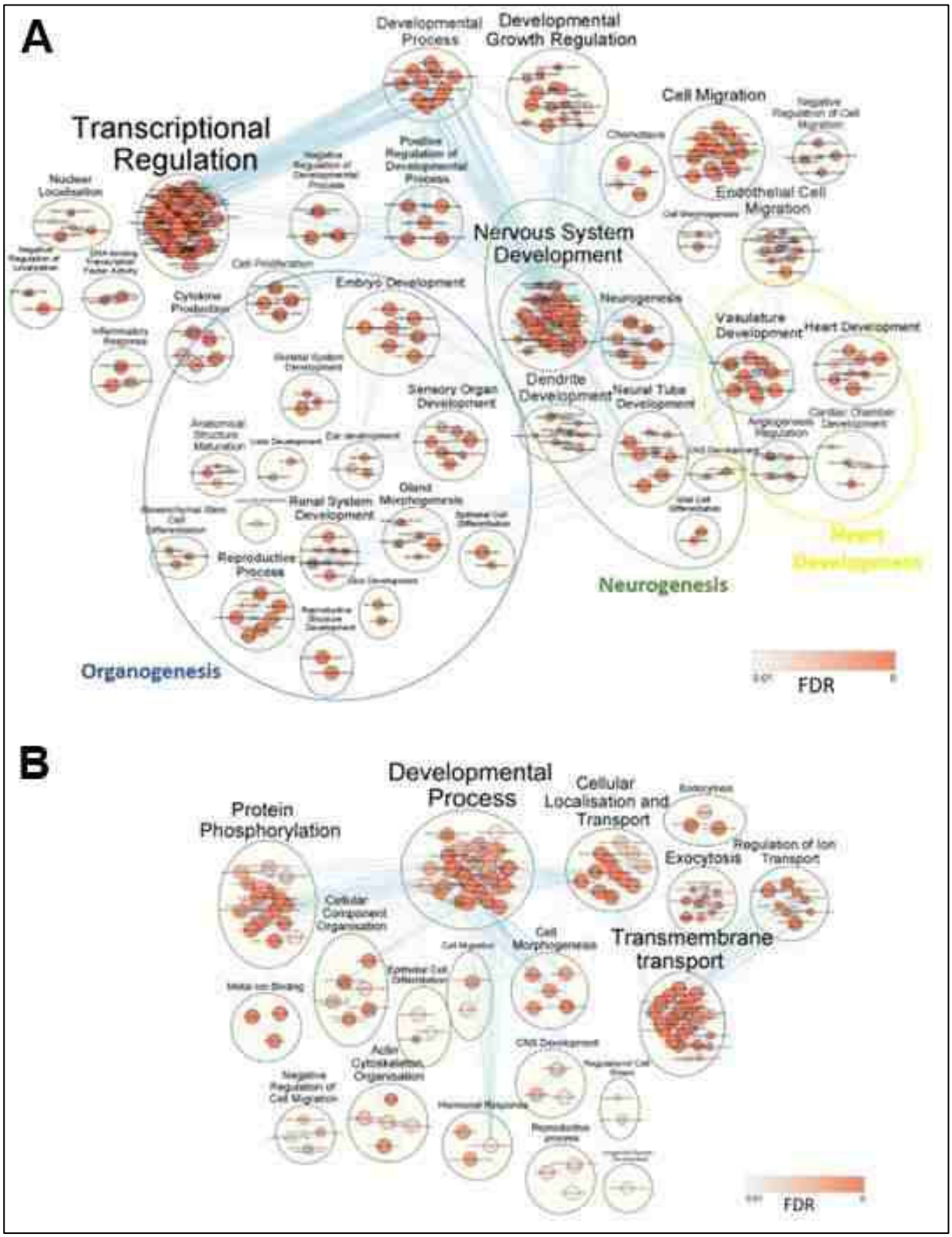


Figure 2: Developmental sub-network of the differentially methylated genes after PPT (A) and DPN (B) treatment. Each red node represents an ontology enriched and the blue lines (edges) represent the common genes. Similar nodes are further grouped in clusters and annotated.

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

Principal Investigator	:	**V Dighe**
Project Associates	:	Shruti Desai, Amruta Gadade, S Jadhav, Shilpa Kerker, P Salunke
Duration	:	2017-2023

Triclosan (TCS), or 5-chloro-2-(2, 4-dichlorophenoxy) phenol, an antimicrobial agent, is widely used in consumer products such as toothpaste, deodorants, hand-washes, furniture, and surgical scrubs. It is also reported to cause immune suppression and is responsible for deficient embryonic development. The present study was planned to decipher the effect of TCS on testis and prostate pathobiology in adult Wistar rats. A total of 24 Wistar male rats (8-10 weeks old) were administered with Triclosan (0.1, 4, and 150 mg/kg body weight) subcutaneous injection daily for 75 days, i.e. two spermatogenic cycles. A significant decrease in sperm count, motility, and daily sperm production was observed, suggesting an effect on testicular steroidogenesis. No significant changes were observed in the relative weight of the testis. TCS being an endocrine disruptor, decreased testosterone levels and increased estrogen levels in treated animals. PSA levels from serum were increased as compared to the control group. Changes in prostate histopathology indicate inter-papillary convolutions and multifocal functional hyperplasia of glandular epithelium with increased cellular layers of epithelium forming fingerlike projections in the lumen, which are the signs of prostate hyperplasia. TCS produces reactive oxygen species (ROS), leading to oxidative stress. MDA levels were elevated at 4 and 150 mg/kg b. wt. /day of TCS exposed animals as compared to the vehicle control. ROS production in the liver leads to a decrease in SOD levels in 0.1, 4, and 150 mg/kg of TCS exposure as compared to the vehicle control. A slight decrease was observed in the catalase level in treatment groups i.e. 0.1, 4, and 150 mg/kg of TCS treatment relative to the vehicle control.

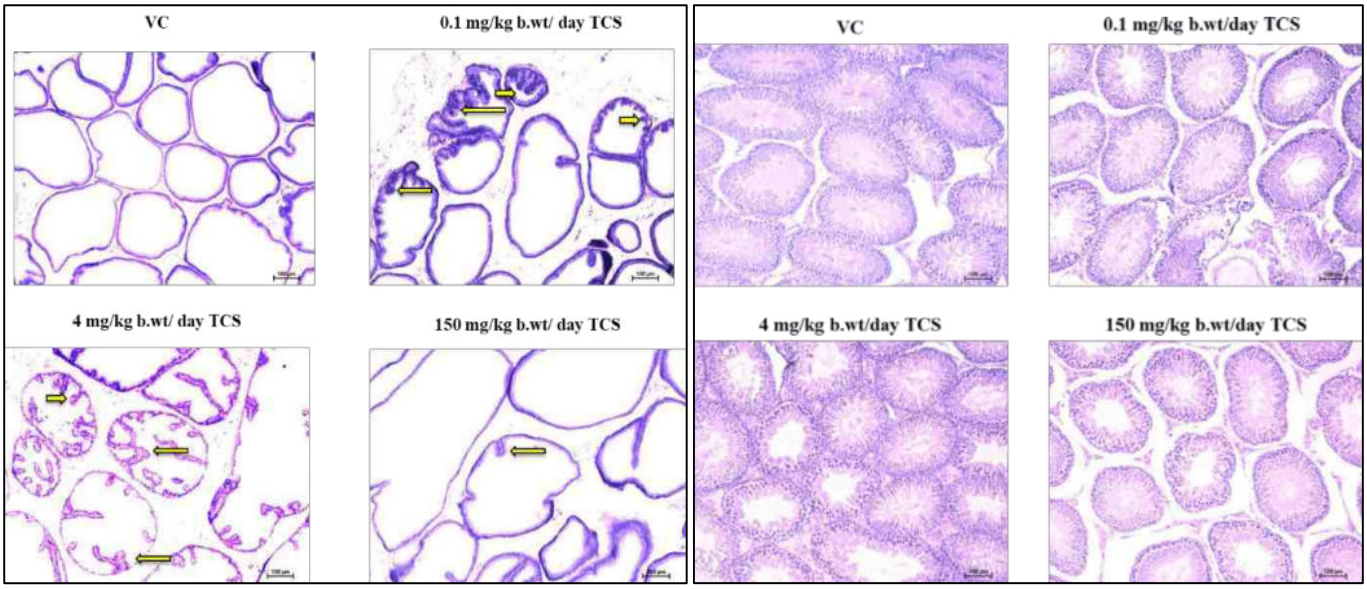


Figure 1: Histopathological alterations in the prostate of male rats exposed to TCS for two spermatogenic cycles (VC: vehicle control).

2.4 Implications of Gonadotropin and their Receptor Gene Variants in Male Infertility

Principal Investigator	:	**P Kuppusamy**
Project Associates	:	R Gajbhiye, S Pande, DVS Sudhakar, Shagufta A Khan
Collaborators	:	D Kale, G Desai, Nowrosjee Wadia Maternity Hospital, Mumbai P Kothari, V Kulkarni, Consultant Andrologists J Shah, Kamala Polyclinic and Nursing Home, Grant Road, Mumbai
Duration	:	2022-2026

Infertility affects 15% of reproductive-aged couples worldwide. Male factor infertility accounts for 50% of infertile couples and 7% of the general male population. The etiology of male infertility is diagnosed in 50-60% of men while more than 40% remain undetermined and referred to as idiopathic infertility. Generally, male reproductive hormones initiate and maintain spermatogenesis, and they can be used as surrogate markers of sperm quality. Several studies show that hormones are correlated with different cases of infertility. Idiopathic infertile men usually undergo hormonal treatment, followed by Assisted Reproductive Technology (ART) for fertilization. It is known that only 30-50% of men benefit from hormonal treatment. Non-response to hormonal treatment may be because of the changes in genetic material (mutations) in the hormonal (gonadotropins and their receptors) genes. Therefore, we have undertaken this study to investigate the gene variants in gonadotropin and their receptor genes in Indian men. The approvals from SAC and IECs were obtained during the reporting period. A MOU was signed with Collaborating Centers. The total number of study participants recruited till May 24, 2023 are Idiopathic cases (n= 10), healthy controls (n=3). We have tests such as Sperm Vitality assay (a), sperm function test like Sperm DNA Fragmentation (b), and reproductive hormone assays including FSH (c), LH, Testosterone, Estradiol, SHBG, and Inhibin B by ELISA methods (Fig. 1). Further, the recruitment of the study participants is ongoing.

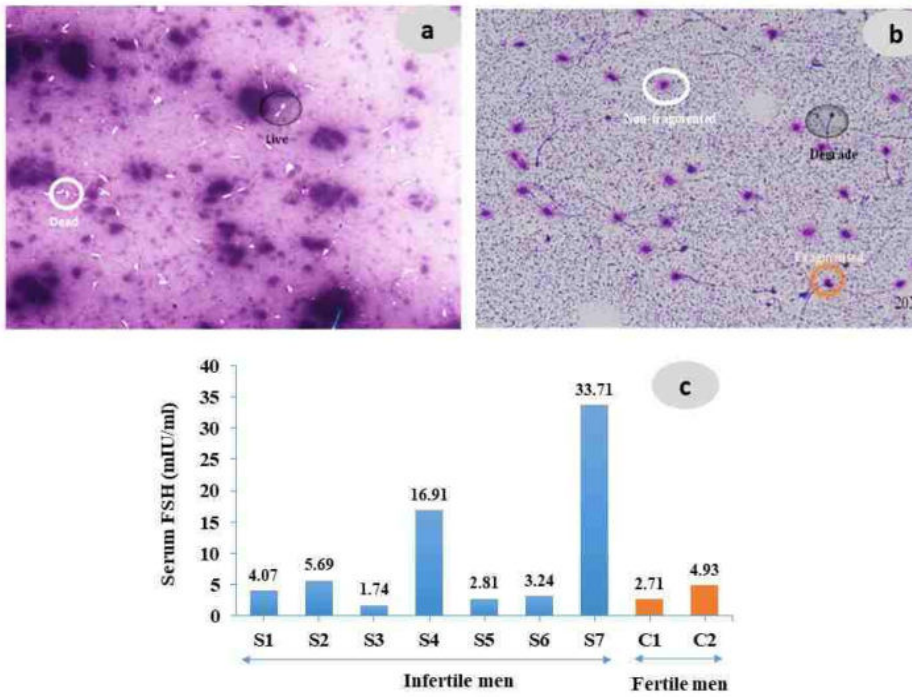


Figure 1: a) Sperm vitality test by Eosin-Nigrosin Stain, b) Sperm DNA fragmentation index by Sperm Chromatin Dispersion Assay, c) Follicle Stimulating Hormone levels

2.5 Deciphering the Role of PSP94 and CRISP Family Proteins in Ion Channel Modulation *(Partly Funded by Science and Engineering Research Board, Department of Science and Technology)*

Principal Investigator	:	**Bhakti R Pathak**
Project Associates	:	Vaidehi Miya, Antara Banerjee, Ananya Breed
Duration	:	2019-2024

CRISPs (Cysteine RIch Secretory Proteins) are evolutionary conserved proteins that show presence of 16 conserved cysteines. Out of these 16 cysteines, 10 reside in the C-terminal domain known as cysteine-rich domain (CRD). In several snake venom CRISPs, a C-terminal subdomain of the CRD is shown to possess ion-channel regulatory activity and this subdomain is also known as ion-channel regulatory (ICR) domain. Though there are many reports on ion-channels regulated by CRISPs present in the venom, information about channels regulated by mammalian CRISPs is limited. Mammalian CRISPs show expression bias towards reproductive tract tissue. Humans express three members of the CRISP family while rodents express an additional CRISP in the epididymis i.e. CRISP4. TRPM8 (Transient receptor potential cation channel melastatin 8) present on the sperm was reported to be regulated by mouse Crisp4. However, this channel is non-functional on ejaculated sperm. Apart from TRPM8, rodent CRISP1 purified from epididymis is shown to inhibit >50% of CatSer1 (Cation ion channel on Sperm 1) currents by patch-clamping and by yeast-two hybrid system mouse Crisp2 has been reported to interact with Catsper1 channel. In order to gain insights about ion-channels regulated by mammalian CRISPs as well as to identify novel binding partners, we employed proteomics-based approach. Understanding the interactome of CRISP protein may shed light on different cellular processes and pathways that it may affect. CRISP4 interactome was deciphered by immunoprecipitation followed by mass spectrometry.

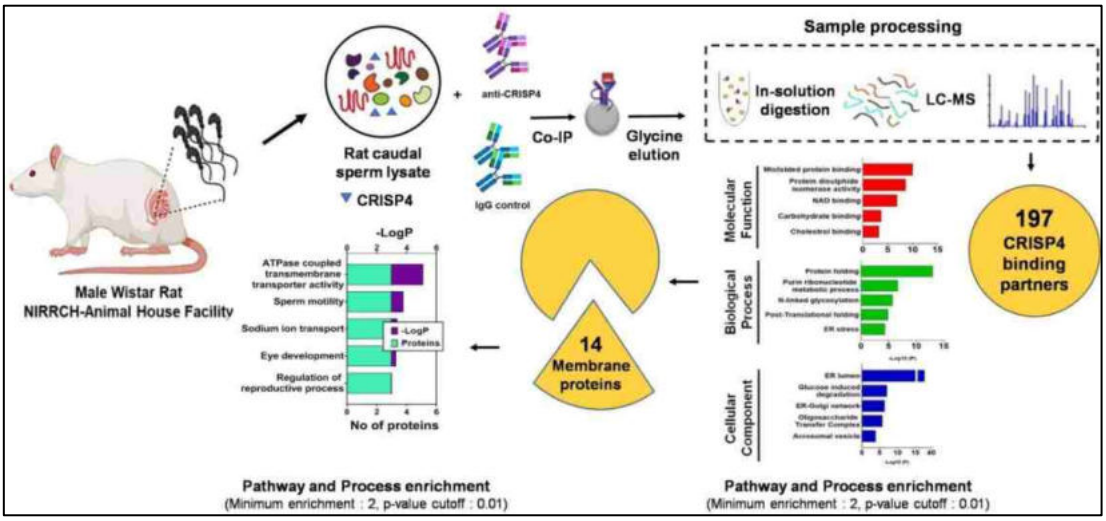


Figure 1: Protein-protein interaction and pathway enrichment analysis for rCRISP4 interactome The top panel shows the research methodology adapted for interactome analysis which yielded 197 unique CRISP4 binding proteins. Top 5 significantly enriched GO (−log10 (p-value)) annotated terms illustrated proteins to be involved in cholesterol binding, trafficking and protein folding. 14 membrane localizing proteins were shortlisted where ATPase coupled transmembrane transporter activity and sperm motility associated proteins were most enriched. Of these, PMCA4b was shortlisted for further validation.

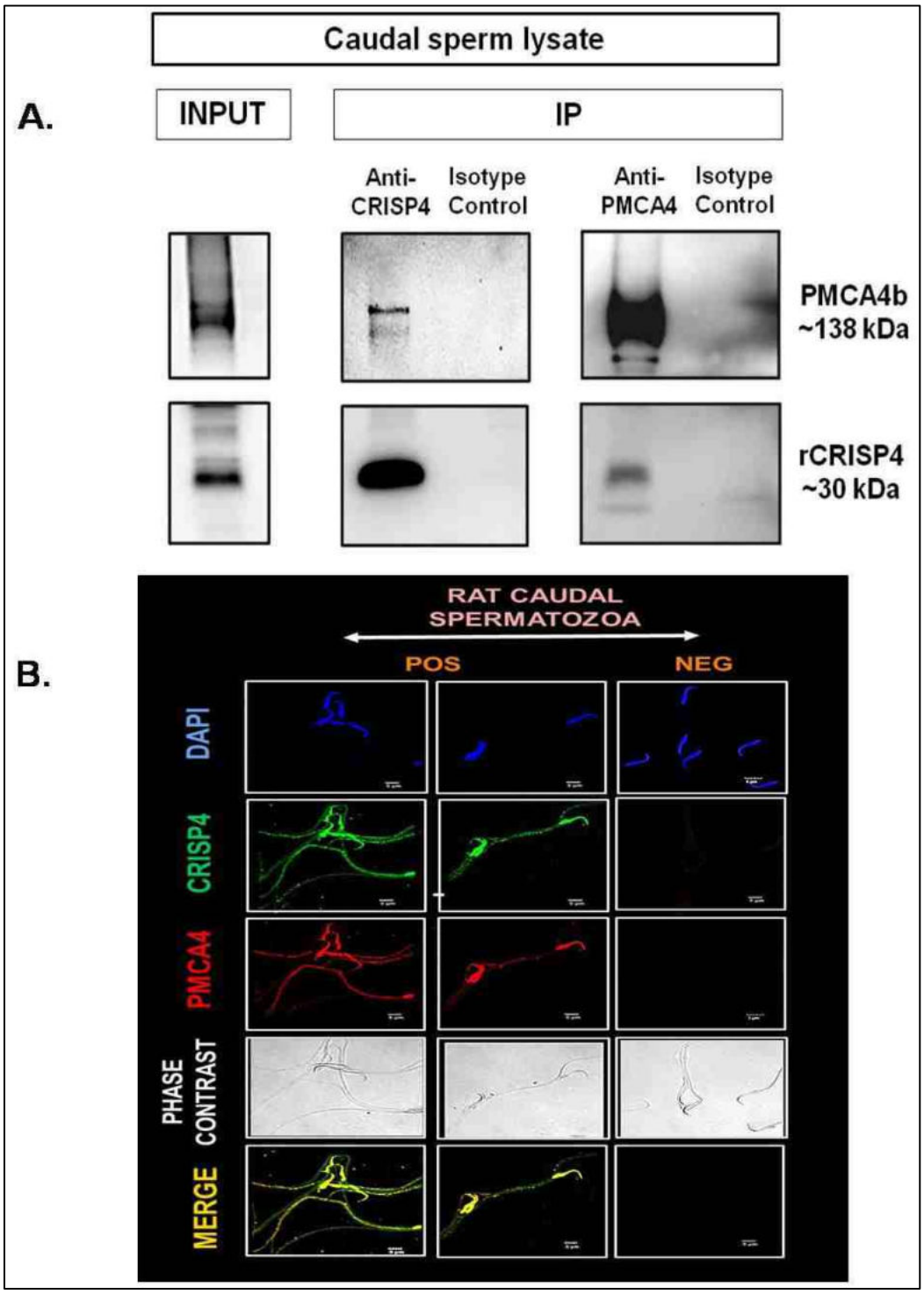


Figure 2: CRISP4 and PMCA4b interact with each other by co-immunoprecipitation a) Lysates from rat caudal spermatozoa were subjected to IP with antibodies against CRISP4 as well as PMCA4. The left panel indicates protein expression in 10% of the total lysate while the right panel shows enrichment of the bait proteins where PMCA4 and CRISP4, respectively got co-IPed. b) Immunofluorescence of rat caudal spermatozoa for CRISP4 (green) as well as PMCA4 (red) indicates that both the proteins co-localize on sperm head and faintly on the tail. Cell nucleus is stained with DAPI (blue) and sites of co-localization are depicted as yellow foci. Scale bar = 5 µm

In the reporting year, the MS data was manually curated and proteins unique to test with at least ≥2 unique peptides were filtered yielding 197 putative CRISP4 binding partners. Targets were subjected to Gene Ontology (GO) analysis using Metascape server. Fig. 1 highlights the top 5 cellular components, biological processes, and molecular functions that were significantly enriched in our analysis. Being a secreted protein, its interactome showed enrichment of proteins involved in ER-Golgi network and protein folding pathways. Molecular processes also showed enrichment for cholesterol binding which hints towards association of CRISP4 in cholesterol enriched microdomains. The list was further scrutinized for membrane spanning proteins which revealed significant enrichment of proteins involved in ATPase coupled with transmembrane transporter activity and sperm motility from which PMCA4 isoform b, was pursued for further validation. PMCA4 (Plasma Membrane Ca2+ ATPase) is the principle channel on sperm that ensures calcium homeostasis. Male mice lacking PMCA4 are reported to be sterile highlighting importance of this channel in sperm function. Interaction of CRISP4 with PMCA4b was validated via co-immunoprecipitation on caudal sperm lysates and co-localisation experiments (Fig. 2). Functional implications of this interaction are being investigated further.

2.6 Delineating the Role of Human β-Microseminoprotein in Male Reproduction *(Partly Funded by Indian Council of Medical Research)*

Principal Investigator	:	**Dhanashree Jagtap**
Co-Principal Investigators	:	Bhakti Pathak, Priyanka Parte, D Modi
Project Associate	:	B Kulkarni
Collaborators	:	R Gajbhiye, V Kulkarni, Anushree Patil, Deepti Tandon A Phadke, SRL Dr Avinash Phadke Labs
Duration	:	2023-2025

Beta-microseminoprotein (β-MSP also known as PSP94) is a non-glycosylated, cysteine rich protein secreted by the epithelial cells of the prostate. β-MSP is found in abundance in human seminal plasma and is also present on the spermatozoa. It is introduced into the semen during ejaculation and found to be absent in post-capacitated spermatozoa. Its abundance in semen along with the fact that it is present on the pre-capacitated spermatozoa suggests that it may have a function, which has not yet been elucidated. Previous studies from our lab using semen from fertile men has led to the identification of Prostatic Acid Phosphatase, CRISP-3 and CRISP-2 as binding partners of β-MSP in seminal plasma and on spermatozoa respectively. This study was initiated with the following objectives: (1) to investigate the difference in β-MSP levels in seminal plasma and spermatozoa of fertile versus infertile men; (2) to identify and characterize novel binding partners of β-MSP from spermatozoa of fertile versus infertile men; (3) to study the effect of β-MSP on sperm function and motility, capacitation and acrosome reaction in fertile and infertile men; and (4) to study the mechanism by which β-MSP is lost during capacitation. Men attending infertility clinic of ICMR-NIRRCH, Mumbai and SRL Avinash Phadke labs are included in the study. Recruitment for participants is done after assessment of the semen parameters following the WHO criteria (2021).

In the reporting year, 6 subjects were recruited. Localization of β-MSP on spermatozoa was studied by indirect immunofluorescence in normozoospermic samples. Spermatozoa were washed followed by capacitation and acrosome reaction as shown in Fig. 1(a-j). β-MSP was found to be present on the

surface of washed spermatozoa on head and tail region (Fig. 1a&b). Following capacitation and acrosome reaction, β-MSP was found to be absent on the head and retained on the tail region. Intense staining of β-MSP was observed on head region of abnormal spermatozoa even after capacitation and acrosome reaction indicating its possible role in sperm function.

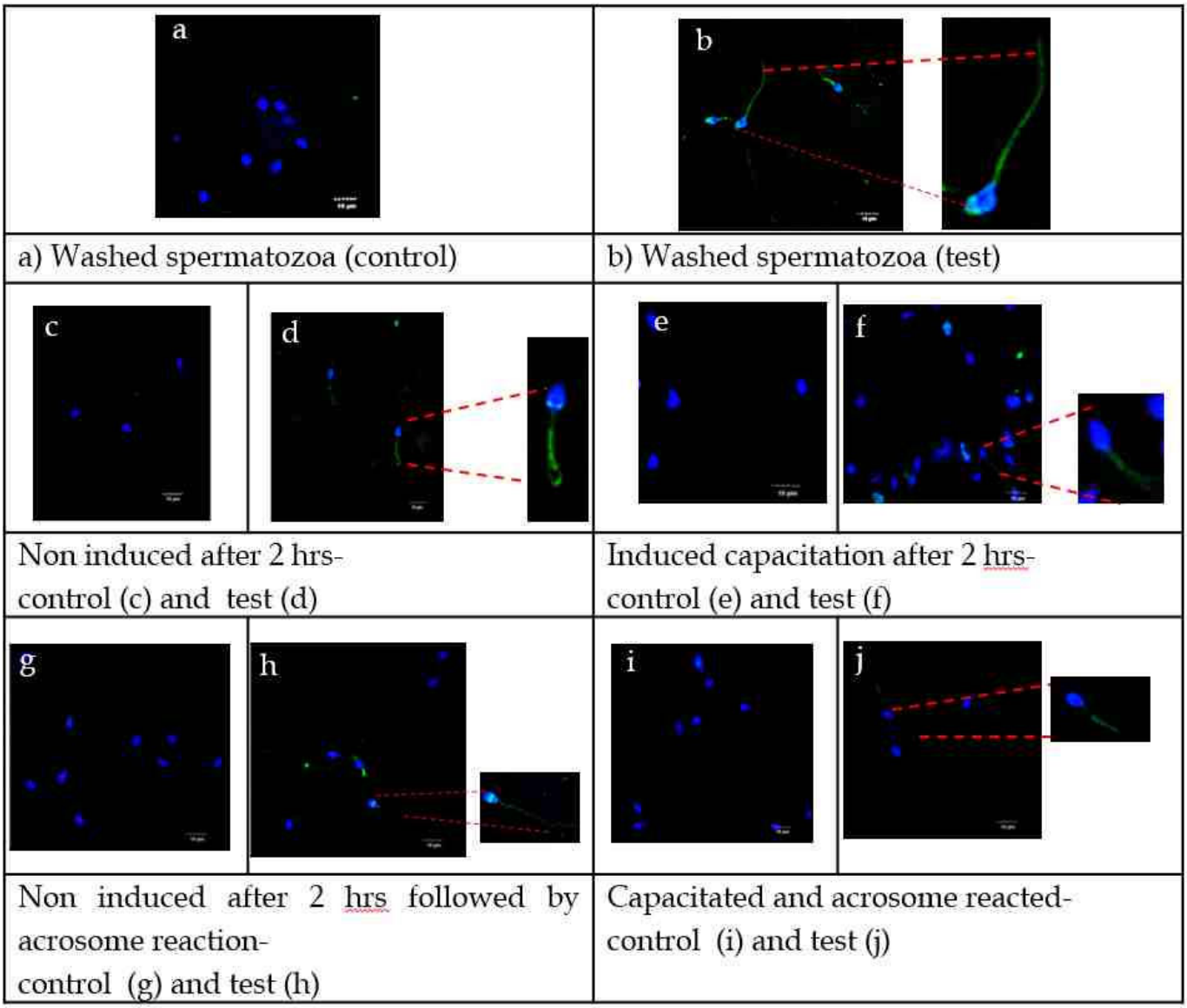


Figure 1: Spermatozoa without or with anti β-MSP and probed with FITC labelled secondary antibody were visualized by indirect immunofluorescence using confocal microscope (images scale bar=10μM). β-MSP is stained in green and nuclei are stained with DAPI (blue). Washed spermatozoa (a, b); capacitation reaction in the absence (c, d) and presence of BSA (e, f), non induced after 2 hrs followed by acrosome reaction (g, h), capacitated and acrosome reacted (i, j).

2.7 Role of Complex N Glycans and Functional Significance of Basigin (Glycoprotein possessing N Glycans) in the Testicular Germ Cells for Spermatogenesis *(Funded by Department of Science and Technology -INSPIRE)*

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

N-linked glycosylation is one of the major post-translational modifications that occur in a number of biological functions. N-glycans play a pivotal role in protein folding acting as a quality control mechanism to ensure that only properly folded proteins are trafficked to the Golgi. Basigin, also called CD147 or EMMPRIN, is a transmembrane glycoprotein, decorated with complex N glycans. One of the active monocarboxylate transporters (MCT) named MCT1 or SLC16A1 facilitates two-way proton coupled transport of L-lactate across plasma membrane. Anchorage and activity of MCT1 require interaction with basigin/CD147. Disruption of glycosylation may impair basigin-MCT1 interaction and failure of signaling of metabolites associated with basigin function.

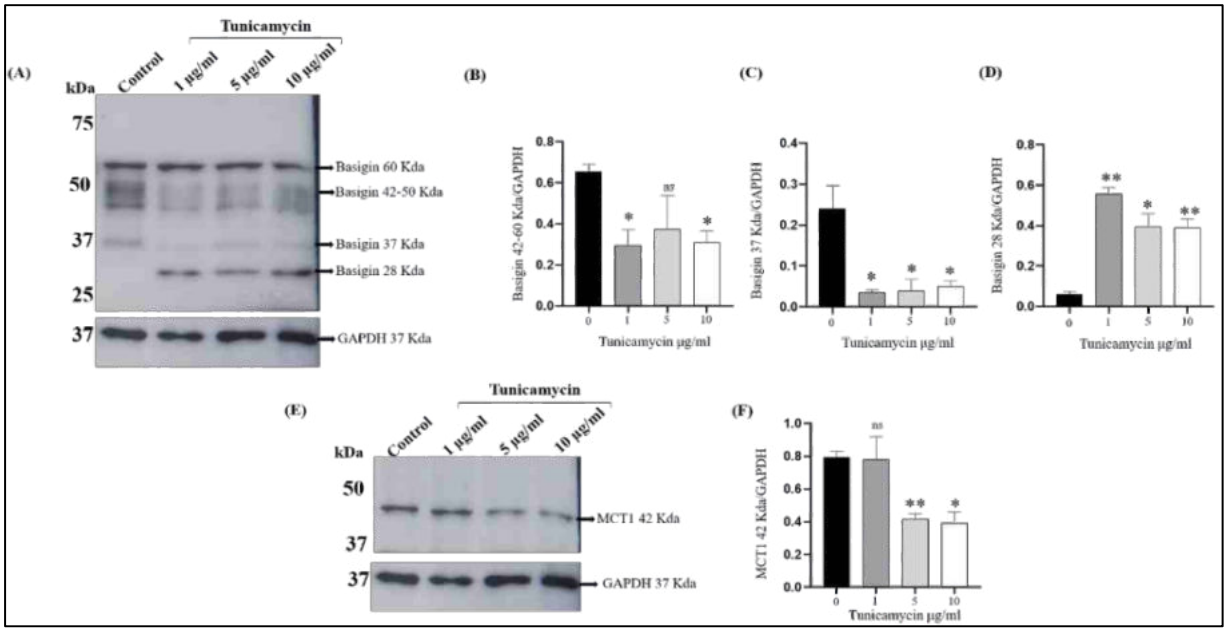


Figure 1. (A) Effect of different doses of tunicamycin on basigin N-glycosylation by Western blot analysis in PC3 cells. Cells were either treated with different doses of tunicamycin (1, 5 and 10µg/ml) or untreated for 5 hrs and expression of different glycosylated forms of basigin (28-60 kDa) was determined by Western blot using anti-basigin antibody. Bar diagram shows quantification of basigin for Complex forms at 42-60kda (Figure B); high mannose forms at 37 kDa (Figure C) and core glycosylated form at 27 kDa (Figure D) in tunicamycin treated cells as compared to control and measured by ImageJ software. (E) Representative Western blot showing MCT1 levels in PC3 cells untreated or treated with tunicamycin (1, 5 and 10µg/ml) for 5 hrs. Bar diagram (Figure F) shows quantification of MCT1 for tunicamycin treated cells as compared to control. In both cases, GAPDH was used as loading control. Data correspond to mean ± SEM from two independent experiments. * p value = 0.05, ** p value < 0.01, and ns = nonsignificant.

The study aims to investigate basigin- MCT1 interaction and L-lactate levels under glycosylation deficient condition. PC3 cells were used in the study as it shows stronger basigin and MCT1 expression. The cells were plated for 24hrs followed by addition of tunicamycin (glycosylation inhibitor) at concentrations of 1µg/ml, 5µg/ml and 10µg/ml for 5 hrs. The changes associated with tunicamycin treatment were analyzed at protein level. Tunicamycin treatment reduced expression of hyperglycosylated (42-50 kDa) and hypoglycosylated (37 kDa) forms of basigin in dose independent manner (Fig. 1A, B&C). An increase in expression of non-glycosylated basigin (28 kDa) was observed on tunicamycin treatment confirming glycosylation inhibition (Fig. 1A&D). MCT1 was also found to be reduced at tunicamycin concentrations of 5µg/mL and 10µg/ml (Fig. 1E&F).
As tunicamycin concentration of 1µg/ml did not affect the MCT1 levels, higher concentrations were taken into consideration for L-lactate assay. The extracellular L-lactate concentration was reduced in tunicamycin concentrations of 5µg/mL and 10µg/mL (Fig. 2A). The intracellular concentration of L-lactate also found to be reduced in dose dependent manner (Fig. 2B). Further experiments are ongoing to investigate the impact of N-glycosylation on transport of MCT1 on membrane and metabolic changes associated with glycolysis.

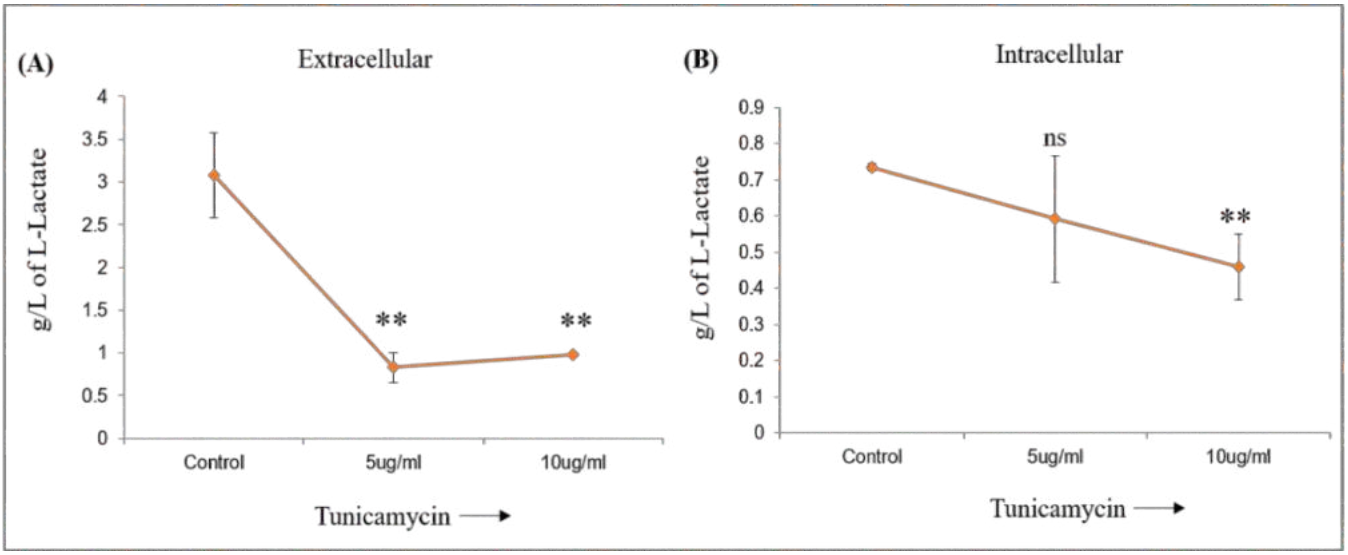


Figure 2. L-lactate production was determined for PC3 cells treated with different doses of tunicamycin (5µg/ml and 10µg/ml) for (A) supernatant (extracellular) and (B) cell lysate (intracellular) using the L-lactate Megazyme kit. Control cells were treated with the vehicle medium. The data represents the mean ± SEM of two independent experiments. ** p value < 0.01 and ns = nonsignificant.

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

Principal Investigator	:	**Dipty Singh**
Project Associates	:	Deepshikha Arya, R Gajbhiye, Deepti Tandon, P Kothari, Reshma Gaonkar, Kushaan Khambata
Collaborator	:	P Pawar, Lokmanya Tilak Municipal General Hospital, Mumbai
Duration	:	2020-2025

Varicocele has been associated with reduced male fertility. Currently it is managed by antioxidant treatment or varicocelectomy and/or assisted reproductive techniques (IUI/IVF/ ICSI). Treatment modalities for varicocele improve semen parameters, yet more than 50% cases remain infertile. In view of the crucial role of sperm epigenome in its functionality, this study aims to evaluate sperm genome wide DNA methylation signatures by whole-genome bisulfite sequencing (WGBS) in men with clinical varicocele. In this case-control study, control group include apparently healthy fertile men and case group includes infertile men with clinical varicocele (before and after 3 months of antioxidant or varicocele repair). A total of 25 healthy fertile men and 25 infertile men with clinical varicocele (Grade I-III) have been recruited in the study. Varicocele patients were followed up (n=15) and semen samples were collected after 3-8 months of treatment. Semen samples were analyzed for basic semen parameters as per WHO (2010) standards. Varicocele group had significantly lower semen parameters compared to controls, which was non-significantly improved after varicocele treatment (Table 1).

Table 1: Semen parameters of fertile controls and varicocele group

Parameters	Control group (n= 25)	Varicocele group (VBT; n= 25)	P value*	Varicocele follow-up patients after 3-8 months of treatment (n=15)		P value**
				Varicocele Before Treatment (VBT)	Varicocele-After Treatment (VAT)	
Age (years)	32.28±4.32	31.88±3.84	0.6329	33.73±4.59	33.73±4.59	>0.9999
Semen volume (ml)	1.88±1.03	3.26±2.23	0.0015	3.927±2.63	3.813±2.43	0.9920
Sperm concentration (Millions/ml)	108.6±99.86	13.44±10.58	<0.0001	11.15±10.89	20.89±30.02	0.4058
Sperm total motility (%)	71.16±20.89	37.60±26.97	<0.0001	28.07±22.52	32.07±28.11	0.9267
Sperm progressive motility (%)	33.28±11.68	11.52±12.76	<0.0001	6.40±5.76	8.33±8.09	0.7502
Sperm normal morphology (%)	8.184±2.37	3.344±0.64	<0.0001	1.83±2.37	2.20±2.37	0.4844
Sperm viability (%)	49.28±16.77	35.16±15.08	0.0031	34.74±15.47	37.77±12.76	0.4844

WGBS of sperm genomic DNA was performed to identify differentially methylated CpG sites (DMCs) in sperm of varicocele men compared to controls. 5 samples from each group were pooled and submitted for WGBS to Sandor Life Sciences, Hyderabad. Alignment was done with hg38 (Homo sapiens) reference genome. Differentially methylated cytosines (DMCs) were identified with >25% methylation change and with a Q-value <0.05. Upon further analysis, a total of 6414 DMCs were obtained, of which, 3730 were hypermethylated and 2684 were hypomethylated. Based on genomic context, differential methylation was observed in introns, exons, promoters and intergenic regions; with highest enrichment in intergenic and intronic regions. DMCs were also noted to be highly enriched within transcription binding sites. Differentially methylated genes (DMGs) were identified based on the location of the DMC to be either within promoter, intron, exon or/and 3' end regions. WGBS analysis identified a total of 1484 differentially methylated genes, which were further used for pathway analysis using KEGG and DAVID tools (Fig. 1). DMCs within genes relevant to spermatogenesis, sperm function and sperm mitochondria regulation will be further selected to validate their methylation levels in the study population by pyrosequencing. Assessment of histone retention in spermatozoa is underway.

The present study would be helpful to understand the epigenetic changes in sperm of infertile men with clinical varicocele before and after antioxidant treatment or varicocele repair. The genome wide sperm epigenetic study may identify some novel pathways/genes altered in case of varicocele.

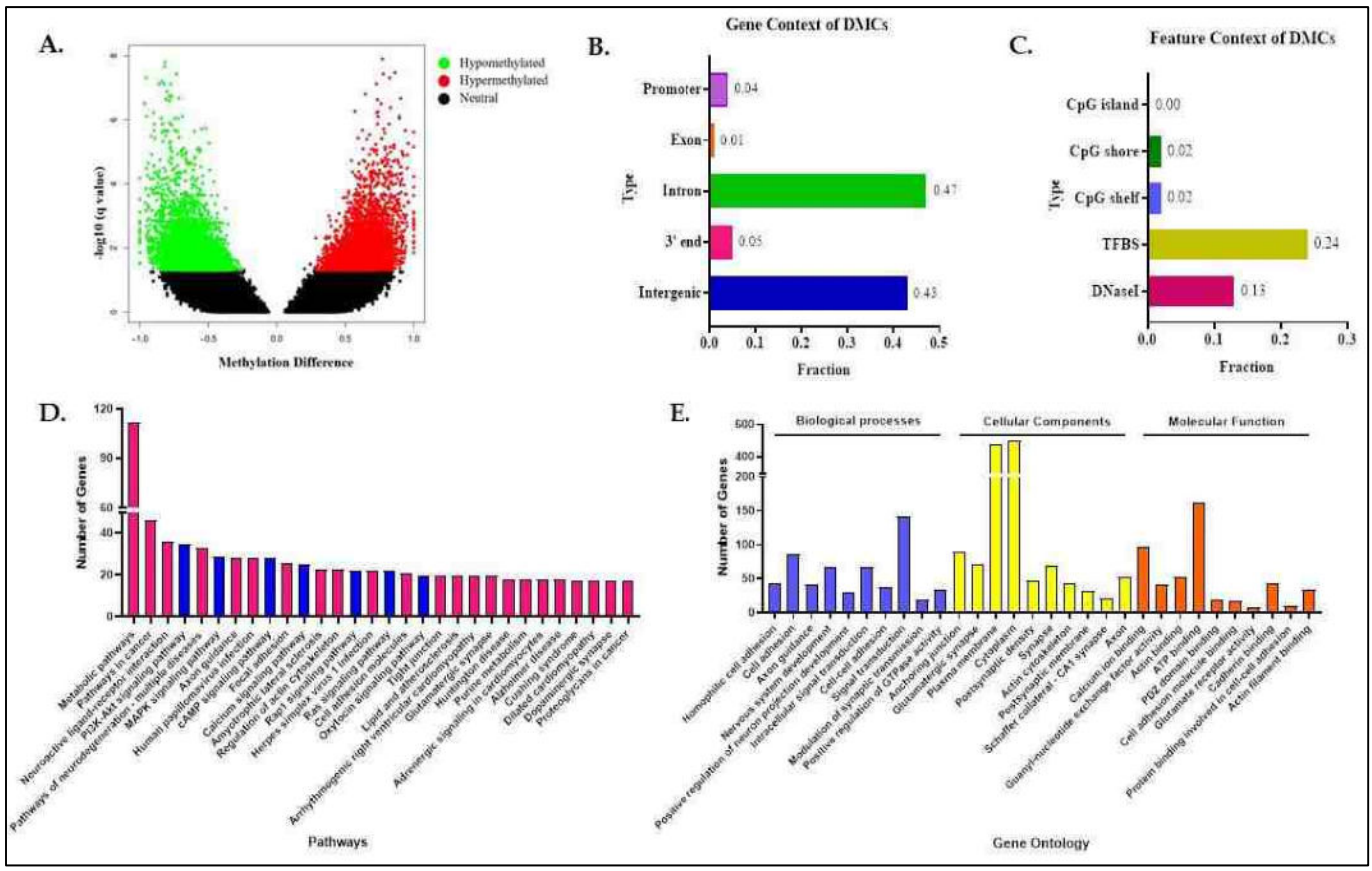


Figure 1: A. Volcano plot representing the distribution of DMCs as hypermethylated or hypomethylated, -log10 (q-value) vs. fraction of methylation difference B. Fraction of DMCs within promoter, exon, intron, 3' end and intergenic regions C. Fraction of DMCs within CpG island, CpG shore, CpG shelf, transcription factor binding sites and DnaseI hypersensitive regions D. Top 30 pathways identified by KEGG mapper tool with signaling pathways highlighted as blue bars E. Top 10 enriched pathways of biological processes, cellular components, molecular functions based on gene ontology analysis using DAVID bioinformatics tool (Bonferroni test, P≤5).

2.9 Functional Significance of Testis Specific Histone H2B Variant (TH2B) in Sperm and Early Embryonic Development *(Partly Funded by Department of Biotechnology)*

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

In the previous period, the epigenetic landscape of testis specific histone variants TH2B and TH2A was profiled in the murine caudal sperm. TH2B was enriched around TSS and gene ontologies associated with it were embryo development, RNA pol II transcription and spindle assembly. It was also enriched at developmentally important HOX cluster. A 26% evolutionary conservation was observed between human and murine TH2B-associated genes. These conserved genes were also found to be crucial for embryogenesis (Annual report 2021-2022, pp. 27-29). In the current reporting period, seven conserved TSH2B associated embryonically important genes were validated in fertile human sperm by ChIP-qPCR (Fig. 1). Mutants of most of these genes are known to show embryonic lethality. Presently, the TH2B dynamics in oocyte and murine preimplantation embryos is being profiled (Fig. 2).

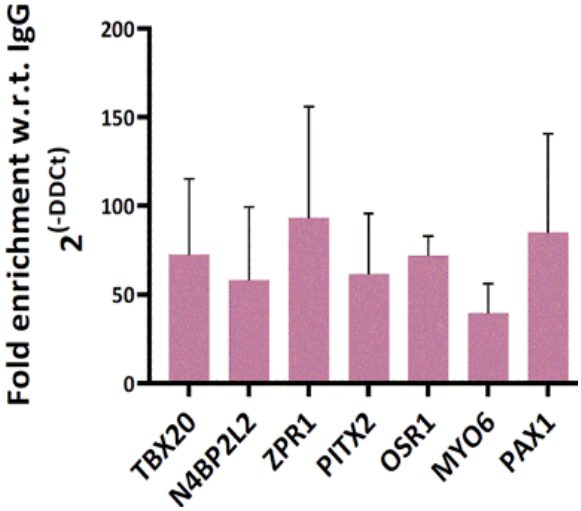


Figure 1: Validation of TH2B association in human sperm using ChIP qPCR; n=3; values represent Mean ± S.E.M.

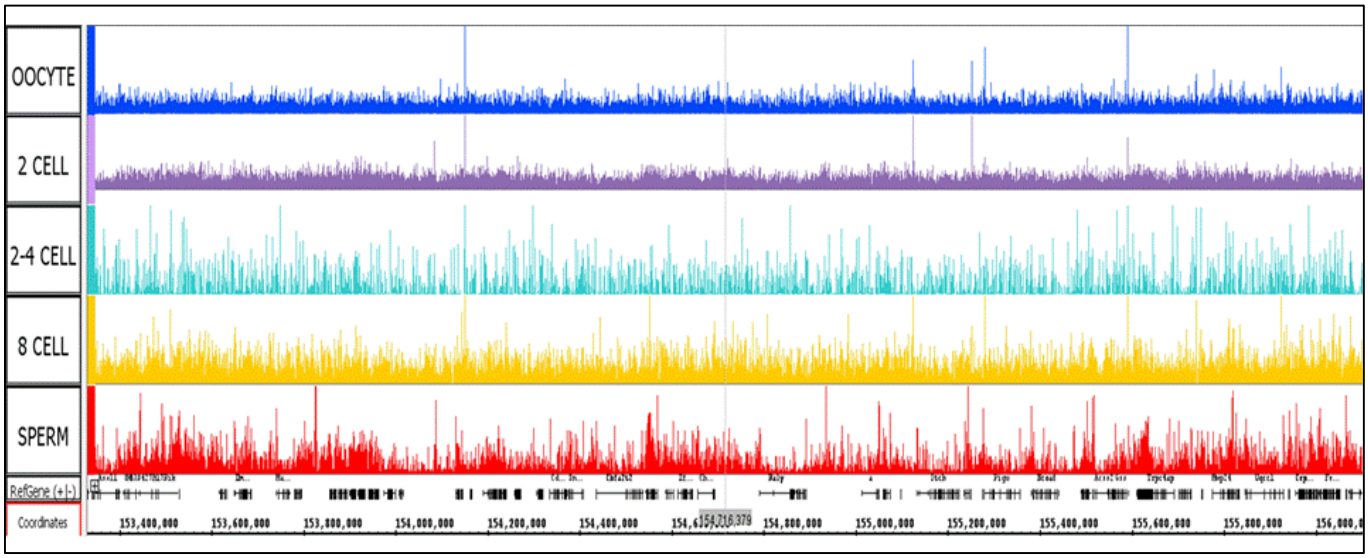


Figure 2: Dynamic landscape of TH2B in germ cells and early embryos. Genome browser view of chromosome 2 shows region (153,400,000-156,000,000).

Towards this, MII oocytes, 2 cell, 2-4 cell and 8 cell embryos were collected by mating C57BL6N/J female and DBA/2J male mice. These were then subjected to ultra-low input native chromatin immunoprecipitation sequencing (ULI-ChIP) to profile the dynamics of TH2B genome-wide. As is evident from Fig. 2, the landscape is highly dynamic across the germ cells (oocyte and sperm) as well as the early embryos. Data analysis in ongoing in order to understand the role of paternal TH2B in preimplantation embryogenesis towards which the parental specific TH2B dynamics is being explored. Concurrently, our studies with human sperm reported higher total histone retention but reduction in

TSH2B levels in sperm of oligozoospermic and oligoasthenozoospermic infertile individuals indicating altered incorporation of testis specific histone variants in sperm of these men (Annual report 2021-2022, pp. 27-29).
As TSH2B levels were unaltered in infertile asthenozoospermic men, in the current reporting period the status of phosphorylated TSH2B (pTSH2B) was investigated in sperm of these men. Intriguingly, pTSH2B was significantly reduced. Sequencing of the TSH2B gene H2BC1 in infertile and ethnically matched fertile individuals revealed 4 SNPs (rs4711096, rs4712959, rs4712960 and rs4712961) and 2 rare variants rs544942090 and rs368672899. The rare, non-synonymous variant; rs368672899 (p.Ser5Pro) results in amino acid change from Serine to Proline in N-terminal region of TSH2B. Interestingly, tandem mass spectrometry of TSH2B containing fraction of sperm lysate revealed that Ser5 site where rare mutation was observed bears phosphate group. Evidence of reduced pTSH2B in sperm of asthenozoospermic infertile men, the rare non-synonymous variant; p.Ser5Pro detected in an infertile individual, and the detection of phosphosite on Ser5 of TSH2B imparts significance to TSH2B phosphorylation in regulating sperm motility/ function.

2.10 Investigation of Potential Chemotactic Metabolites in the Follicular Fluid

Principal Investigator	:	**Priyanka Parte**
Project Associates	:	Durva Panchal, Aishani Bose, MT More
Collaborator	:	Grishma Desai, Nowrosjee Wadia Maternity Hospital, Mumbai
Duration	:	2019-2024

Previous research using a rat model revealed that ovulatory phase oviductal fluid (OV-OF) has a higher chemoattractant potential than pre-ovulatory phase oviductal fluid (preOV-OF), implying that some chemoattractants are released during ovulation and can attract sperm towards the oocyte. Metabolites were proposed as the most ideal candidate molecules to act as chemoattractants because being smaller in size they diffuse quickly, allowing them to establish a better and faster gradient in the oviductal milieu. In order to identify the chemoattractant/s, the metabolomic differences between OV- OF and preOV- OF were examined. Studies by our group have earlier reported NFA to be a sperm chemoattractant and deciphered the mechanism of sperm response to NFA (Annual report 2021-2022, pp. 24-25). At that point, only the hydrophilic fraction of the metabolite was explored.
In this reporting period, the metabolome of hydrophilic and hydrophobic fractions of oviductal fluid was investigated. Towards this, the oviductal fluid was separated into hydrophilic and hydrophobic fractions using the M-PLEX protocol, chloroform: methanol-based phase separation protocol. The separated hydrophilic and hydrophobic fractions were subjected to HILIC-MS/MS and RPLC-MS/MS respectively; in both the positive and negative ion mode. The data files were extracted into ion chromatograms, with peak matching, retention time correction and similar peaks were aligned. The base peak intensity chromatogram (Fig. 1A & 2A) illustrates the peak intensity profile of hydrophilic and hydrophobic fractions of OV- and PreOV- OF. Reserpine, an internal standard, was detected in all the samples, adding up to the quality control of the LC runs. Multivariate statistical analysis using the PCA approach (Fig. 1B & 2B) indicated close grouping of individual groups, and data quality. Furthermore, the two groups were clearly separated from one another (Fig. 1B), as well as in the two different ionization modes, demonstrating significant metabolomic variations and coverages not only

between the two different biological fluid types, but also between the two different ionization modes. In XCMS, univariate statistical analysis was performed using a p-value cut off of 0.001 and a fold change of 1.5. The metabolites that are either exclusive or enhanced in OV-OF will be researched further to get insight into their biological significance in terms of chemotaxis. The chemotaxis capacity of the short-listed metabolite/s will be confirmed further in the microfluidic device.

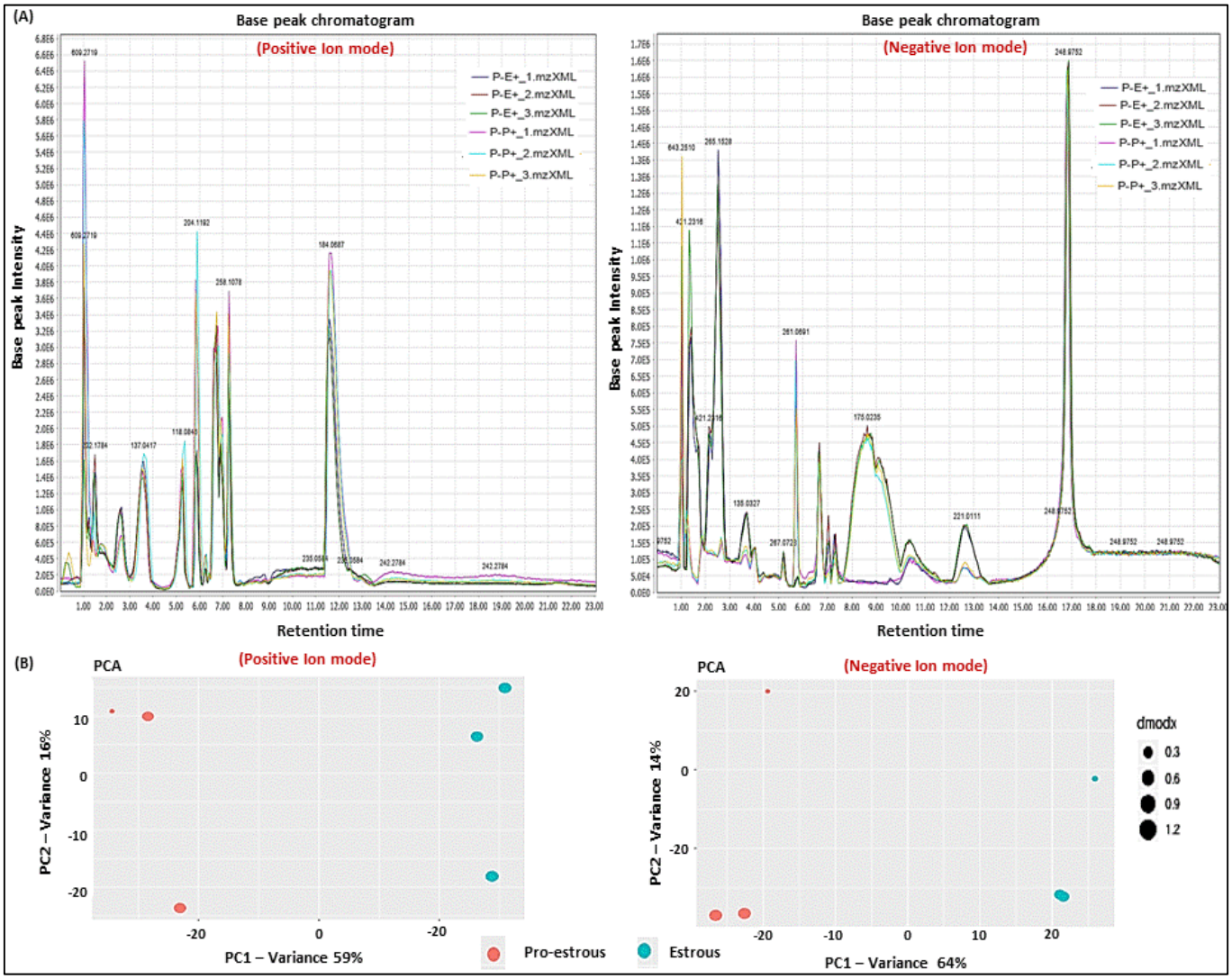


Figure 1: Metabolome profile of the hydrophilic fractions of OV- and preOV- OF. Base peak intensity chromatogram of all samples subjected to HILIC-MS/MS in positive ion mode and negative ionization mode. (A) Multivariate statistical analysis using Principal Component Analysis (PCA) score plot for OV- and PreOV-OF run-in triplicates (each technical replicate is represented as filled circle, red: OV and green: PreOV), with variances for PC 1 and PC 2 shown on X and Y axis respectively (B) The PCA plot indicates significant differences in the metabolomes between the two groups.

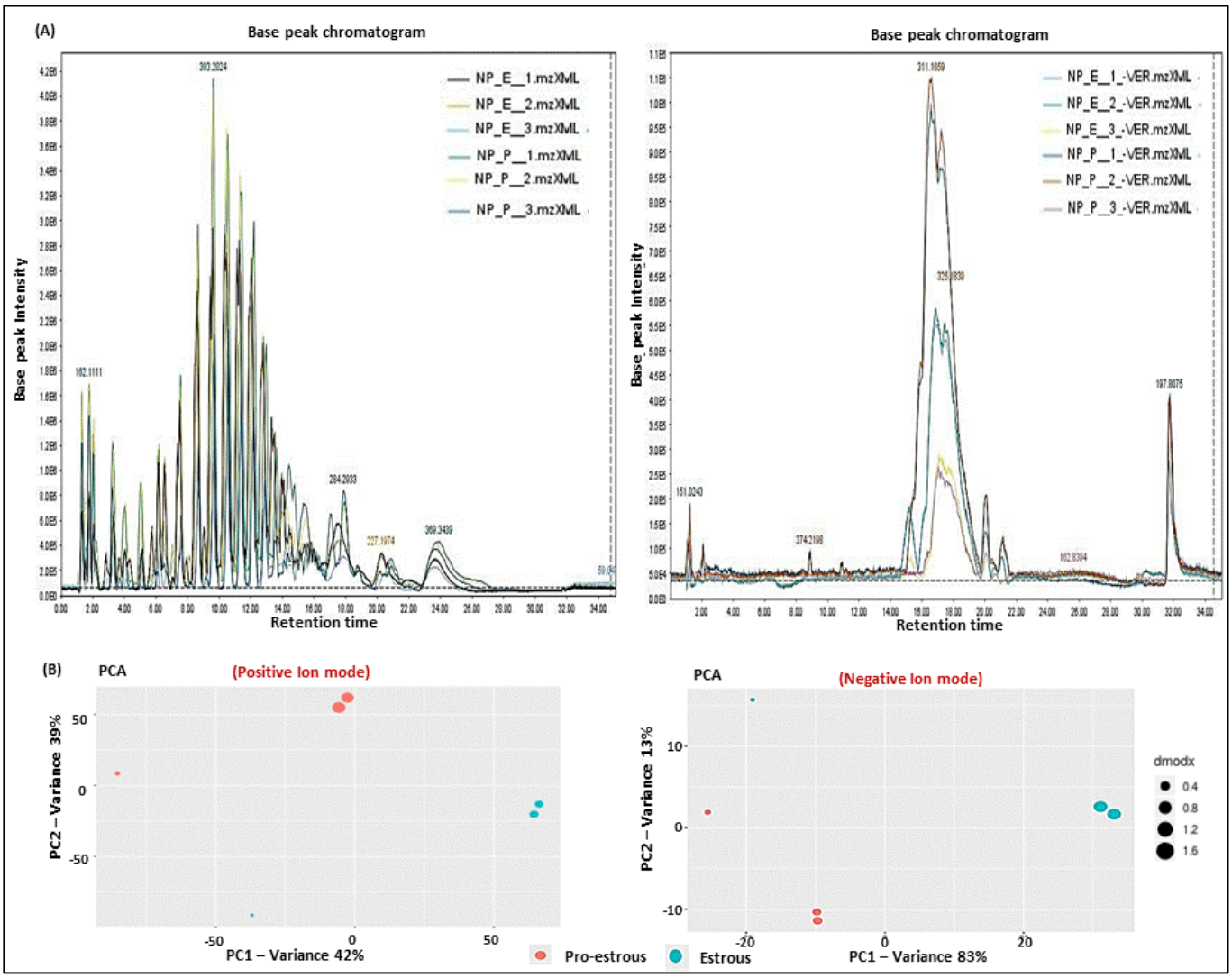


Figure 2: Metabolic profile of the hydrophobic fractions of OV- and preOV- OF using RPLC- MS/MS. Base peak intensity chromatogram of all samples subjected to RPLC-MS/MS in positive and negative ionization modes. (A) Multivariate statistical analysis using Principal Component Analysis (PCA) score plot for OV- and PreOV-OF run-in triplicates (each technical replicate is represented as filled circle, red: OV and green: PreOV), with variances for PC 1 and PC 2 shown on X and Y axis respectively (B) The PCA plot indicates that the metabolome is significantly different between the two groups.

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

Principal Investigator	:	Priyanka Parte
Project Associates	:	Shraddha Gandhi, Smita Yevate, D Gaikwad, M More
Collaborator	:	V Gundabala, IIT-Bombay
Duration	:	2021-2024

A growing body of evidence is available implying the role of sperm not just in fertilization but also in implantation, embryo development and placentation. 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 enhance the prevalent IVF success rate. In this study, we proposed to develop a microfluidic device to sort good quality sperm based on chemotaxis using N-formyl L-aspartate. The study is being conducted in collaboration with IIT-Bombay. In the previous report, we had simulated the device design and fabricated it onto the silicon wafer by using soft lithography (Annual Report 2021-22, pp. 24). In the reporting year, a prototype was developed using Polydimethylsiloxane (PDMS) and bonded on glass-slides using plasma cleaner. The device was tested for gradient generation using Fluorescein isothiocyanate (FITC) dye. The time taken for the dye to form a gradient was noted. It took approx. 20 min for gradient formation and 30-40 min for the dye to diffuse in the entire device. However, while testing the device using mouse sperm, it was not possible to capture a larger view of the device even at 10X magnification as the device dimensions were much larger. At 4X magnification, whilst a larger view of the device could be captured, it was not possible to track the sperm. So, the device design had to be modified and simulation experiments in different mesh sizes had to be done. The modifications and simulations were done in Comsol Multiphysics 5.5 version (Fig. 1).

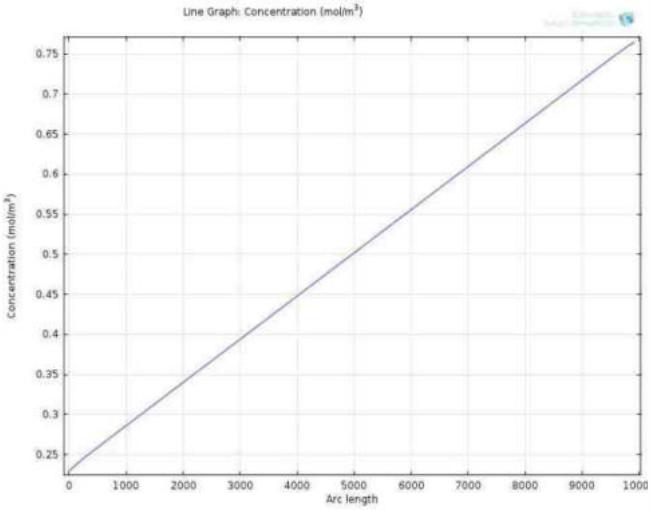


Figure 1: Simulation results of the modified device design. Profile of the gradient generated in the microfluidic device.

After successful simulation, the 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 this was used to cast the device in Polydimethylsiloxane (PDMS). Testing of the modified device is ongoing. In our earlier study, N-formyl L-aspartate (NFA) was identified as a chemoattractant using Holtzman rat sperm. As the proposed devices is to be tested for its ability to sort sperm based on chemotaxis and using mouse sperm, it was necessary to study whether mouse sperm are also chemotactic to NFA. Towards this, sperm of C57BL/6 mice were used. The effect of NFA on mouse sperm was assessed using the microfluidics based chemotaxis assay reported by us earlier. Percent sperm moving towards NFA, sperm VSL in response to different concentration gradients of NFA and distribution profile of sperm VSL. Distribution profile of sperm VSL indicated a higher percentage of sperm with VSL> 140µm/sec in the presence of NFA at all gradient concentrations compared to that seen with media alone. Although the average VSL in response to 0.0005M NFA gradient appeared to be better, percentage of sperm responded to NFA as well as the average VSL was much better with 0.001M gradient of NFA (Fig. 2). Hence the chemotaxis assay will be repeated with 0.001M NFA gradient. Superovulation and oocyte retrieval protocol for mouse was also optimized.

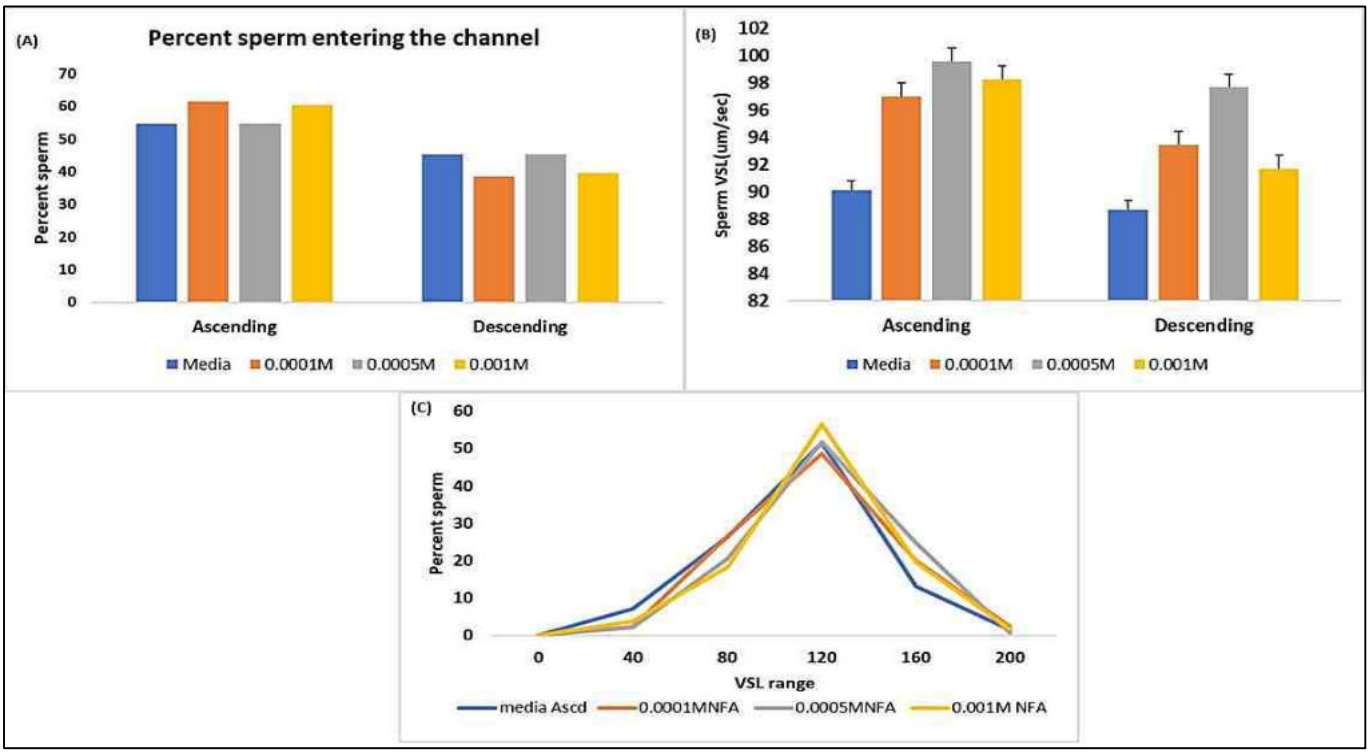


Figure 2: Mouse sperm response to NFA. (A) Percent sperm moving towards NFA (ascending) and away from NFA (descending) at different concentration gradients of NFA; (B) sperm VSL in response to different concentration gradients of NFA tested. Values are Mean ± SEM; (C) Distribution profile of sperm VSL.

2.12 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 the Young Scientist Scheme)*

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

Male infertility can be caused by either acquired or genetic factors. The Y chromosome microdeletions (YCMD) lead to loss of genes involved in spermatogenesis and are the leading genetic cause of male infertility. The gold standard for YCMD screening is testing for six STS markers recommended by the European Academy of Andrology [EAA] in a multiplex PCR format. However, these six markers fail to diagnose ~48% of cases in the Indian sub-continent leading to a high rate of misdiagnosis. We have developed an India-specific STS panel for YCMD diagnosis in a multiplex PCR format. The current project involves in-house and external validation of the assay in independent laboratories located Pan-India and the development of a machine learning-based algorithm to predict clinical outcomes in assisted reproduction technologies in males having YCMD. In-house validation of the developed assay has shown 100% repeatability, reproducibility, and robustness, with lower limit of quantification - 10ng/µl and upper limit of quantification - 500ng/µl. The assay has a shelf life of two years and is

stable for up to 20 freeze thaw cycles. Testing in clinical samples has identified presence of a microdeletion in 14% cases. The most common defect identified was the gr/gr subdeletion in AZFc (8%), complete AZFc deletion (3%), partial deletions of AZFb (2%) and complete AZFa deletion (1%). 72% deletions were identified by testing for Non-EAA STS while only 28% deletions were identified by testing for EAA STS. Thus, the developed assay has 100% sensitivity, specificity, positive predictive value and negative predictive value and 0% false positive and false negative rate. To date, the external centers have tested ~600 cases. The developed assay has been validated and the technology is transferred to APS LifeTech (Pune, India). The algorithm, 'FertilitY Predictor', is copyrighted by the collaborators at Amity University, Noida. Validation of the predictive algorithm in external IVF laboratories is ongoing.

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

Principal Investigator	:	**DVS Sudhakar**
Co Principal Investigator	:	R Gajbhiye
Project Asoociate	:	Dipty Singh
Duration	:	2022-2025

This study is primarily aimed at identification and characterization of genetic causes associated with Multiple Morphological Abnormalities of Sperm Flagella (MMAF) in infertile men from India. A total of 16 infertile men with asthenoteratozoospermia phenotype were recruited and semen analysis was performed. Based on the semen parameters and inclusion criteria of MMAF, nine infertile men were further selected for the study. Semen analysis was performed as per the WHO criteria (WHO manual, 2010) and infertile men samples that showed multiple flagellar defects (coiled, bent, short, and absent flagella) were selected for further studies. Flagellar ultrastructure imaging (using Transition Electron Microscope) has been completed for six samples and the remaining three samples are being processed for imaging. DNA was isolated from the blood samples obtained from six MMAF individuals and was sent for whole exome sequencing. Currently, we are recruiting study participants only from the NIRRCH andrology clinic. We are planning to include a couple of centers to meet the sample numbers (n=100) required for the study.

RTI/ STIs/HIV/ MICROBICIDES

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**
Co-Principal Investigators	:	Anushree Patil, Jayanti Mania-Pramanik
Project Associates	:	S Chauhan, Anushree Patil, Jayanti Mania Pramanik, Kiran Munne, Shahina Begum, Suchitra Surve Padmaja Keskar, Pranita M Tipre, BMC, Mumbai Varsha Tryambake
Collaborators:		Arpita Singh, STI Clinic Municipal ART Centre, Nagpada Shrikala Acharya , MDACS, Wadala Jayanthi Shastri, Sandhya Sawant, BYL Nair Hospital, Mumbai
Duration	:	2019-2023

Mycoplasma genitalium (MG) is an emerging STI/RTI associated with wide spectrum of reproductive morbidities like cervicitis, tubal factor infertility, endometritis and pelvic inflammatory diseases. The literature from Asian populations is very sparse regarding the prevalence and response to treatment for this pathogen. Objectives of the study are i) to detect MG in women attending STI clinic with clinically diagnosed lower genital tract infections cervicitis, cervicovaginitis; ii) to compare its detection rate in endocervical swab, vaginal and urine samples; iii) to assess the clinical and microbiological response to treatment among infected women following treatment with standard management; and iv) to investigate the presence of co-infection of other common RTIs causing organisms with MG. A prospective study was conducted on 341 sexually active women attending Municipal STD clinic and ART Centre with complaints of lower genital tract infection. Endocervical swab, vaginal swab, and urine samples were tested for MG using uniplex and multiplex real-time PCR kits. Gram stain was used for detecting bacterial vaginosis and Candida. Data on HIV, rapid plasma reagin (RPR), treponema pallidum particle agglutination (TPHA), Hepatitis C, and HBsAg was also collected. The mean age was 33.4±5.7 years.

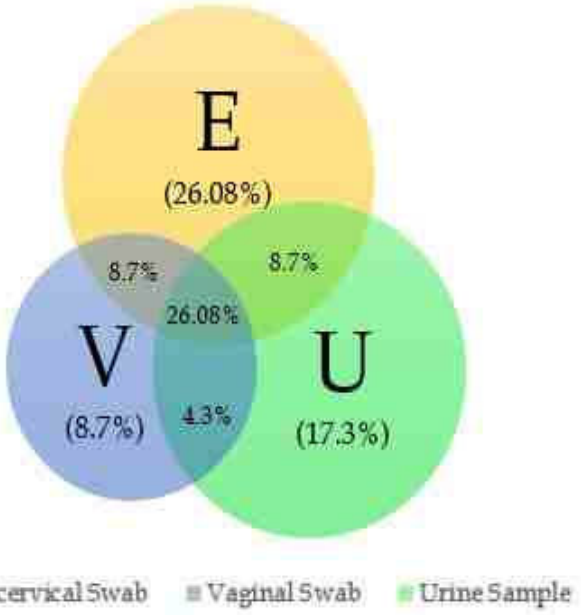


Figure 1: Represent the Venn diagram of percentage positivity for MG in samples from various sites.

Majority of them 88.3% (n=301) were sex workers by occupation and rest 11.7% (n=40) had other occupations. Maximum 95% (n=324) belonged to upper lower socioeconomic class. Majority 64.8% (n=221) used public toilets and only 35.2% (n=120) accessed private toilet facilities. Smoking and alcohol use was reported by 9.7% (n=33) and 27.6% (n=94) respectively and 51% (n=174) had addictions to smokeless tobacco. Condom use was reported by 85.6% (292) women. Majority 88.3% (n=301) of them had exposure to multiple sexual partners in last six months. The prevalence of MG among the women was 6.3% (n=23). As shown in Fig. 1 out of these individually maximum positive samples were detected in only endocervical sample 25% (n=6), followed by 17.3% (n=4) in urine samples. A combination of endocervical, vaginal and 2 hr urine sample also had positivity 26.08% (n=6). There was a concordance of 47.8% among uniplex and multiplex kits as eleven endocervical samples were positive for MG with both kits. *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) was detected in 2.3% (n=8) each, out of which in 0.87% (n=3) both were detected. *Trichomonas vaginalis* 1.5% (n=5), *Ureaplasma urealyticum* 17.3% (n=59), *Ureaplasma parvum* 21.7% (n=74) and *Mycoplasma hominis* (MH) 59.8% (n=204) were also detected. All these three genital mycoplasma were detected in 1.17% (n=4) women. Seropositivity for HIV was seen in 14.7% (n=50), 2.1% (n=7) were positive for syphilis by RPR and 7.03% (n=24) were positive for TPHA, 2.6% (n=9) were seropositive for HBsAg and 3 (0.9%) were seropositive for HCV. Bacterial vaginosis was detected in 22.3% (n=76) whereas vaginal candidiasis was seen in 3.2% (n=11). Maximum co-infections seen with MG were in those women who were positive for HCV, RPR, TPHA, NG, CT, MH and HIV.

Table 1: Associated risk factors for *Mycoplasma genitalium* infection among women

Risk factor		MG positive (Total 23) %(n)	p
Age	< 29	6.8(5)	0.96
	≥ 30	-18	
Occupation	FSW	6.9(21)	0.69
	Others	5.3(2)	
Toilet facility	Private	6.7(8)	0.96
	Common	6.8(15)	
Addictions	Smoking	18.2(6)	0.006*
	Not smoking	5.5(17)	
	Smokeless tobacco	9.8(17)	0.04*
	Non smokeless tobacco	3(5)	
	Alcohol Yes	6.1(15)	0.42
	No	8.5(8)	
Condom use	Yes	6.5(19)	0.67
	No	8.2(4)	
HIV	Reactive	8(4)	0.7
	Non reactive	6.5(19)	
RPR	Positive	28.6(2)	0.02*
	Negative	6.3(21)	
HBsAg	Reactive	0(0)	0.4
	Non reactive	6.9(23)	
HCV	Reactive	33.3(1)	0.06
	Non reactive	6.5(22)	

As shown in Table 1, addiction in form of tobacco use was found to be significantly associated with MG. Higher proportion of female sex workers had MG than their counterparts. Women with HIV, HCV and RPR had higher prevalence of MG infection though statistical significant results were obtained only in RPR positive women. Condom use was protective for MG infection though statistical significant results were not obtained. Out of 23 women, 26.08% (n=6) came for follow up and 73.9% (n=17) were lost in follow up. Out of these, six 66.6% (n=4) were negative for MG and 33.3% (n=2) were positive for MG at the end of three months. Higher prevalence of MG as compared to CT and NG suggests a need to focus on this STI causing pathogen in women with high-risk behavior. Syndromic management continues to play a pivotal role in the management of RTI/STI. However, there is a need of etiological testing to generate evidence to detect non-classical STI.

3.2 Molecular Characterization and Biochemical Properties of Vaginal *Ligilactobacillus Salivarius*

Principal Investigator	:	**Clara Aranha**
Co-Principal Investigator	:	K Chaaithanya
Project Associate	:	Shreya Peddakolmi
Duration	:	2021-2022

Ligilactobacillus salivarius is a part of the indigenous microbiota of the vaginal and gastrointestinal tract (GIT) and oral cavity of humans. There is no data on the genomic variability of the species for identifying strain-specific properties. Eighteen Strains of *L. salivarius* from a collection of vaginal Lactobacillus isolates were explored to evaluate the probiotic utility of diverse *L. salivarius* strains by phenotyping and genotyping. The genomic diversity of *L. salivarius* isolates was characterized by genetic fingerprinting using random amplified polymorphic DNA (RAPD) Genetic fingerprinting of the strains by RAPD identified five different strains among the 18 isolates.

Different phenotypic traits of Lactobacillus strains such as lactic acid production, inhibitory potential towards urogenital pathogens, and biofilm formation were evaluated. 55% of the strains were L lactic acid producers and 45% of strains produced D lactic acid. The postbiotic metabolites from different strains showed greater than 75% inhibition of growth on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and around 50% on *Neisseria gonorrhea* within 24 hrs. Complete inhibition of growth by post metabolites of different Lactobacillus strains was observed on *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* at 72 hrs and 96 hrs. However, the metabolites showed growth of *N. gonorrhoea* with time. Variation in biofilm formation was observed among the different strains. High-biofilm-forming strains (4) produced a dense biofilm, whereas low-biofilm-forming strains (14) produced a thin, sparse biofilm (Fig. 1).

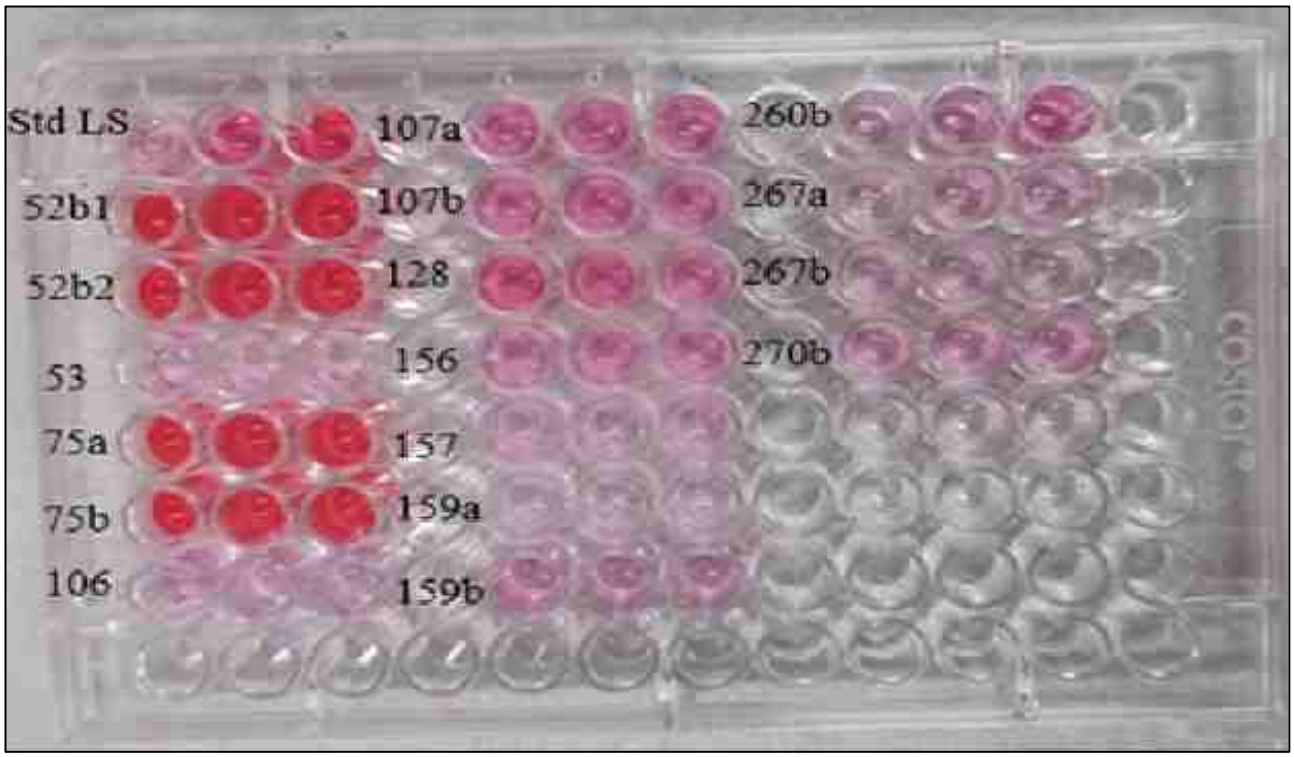


Figure 1: Biofilm formation by different strains of *L. salivarius* determined by safranin staining. The numbers denote the different strains of *L. salivarius*

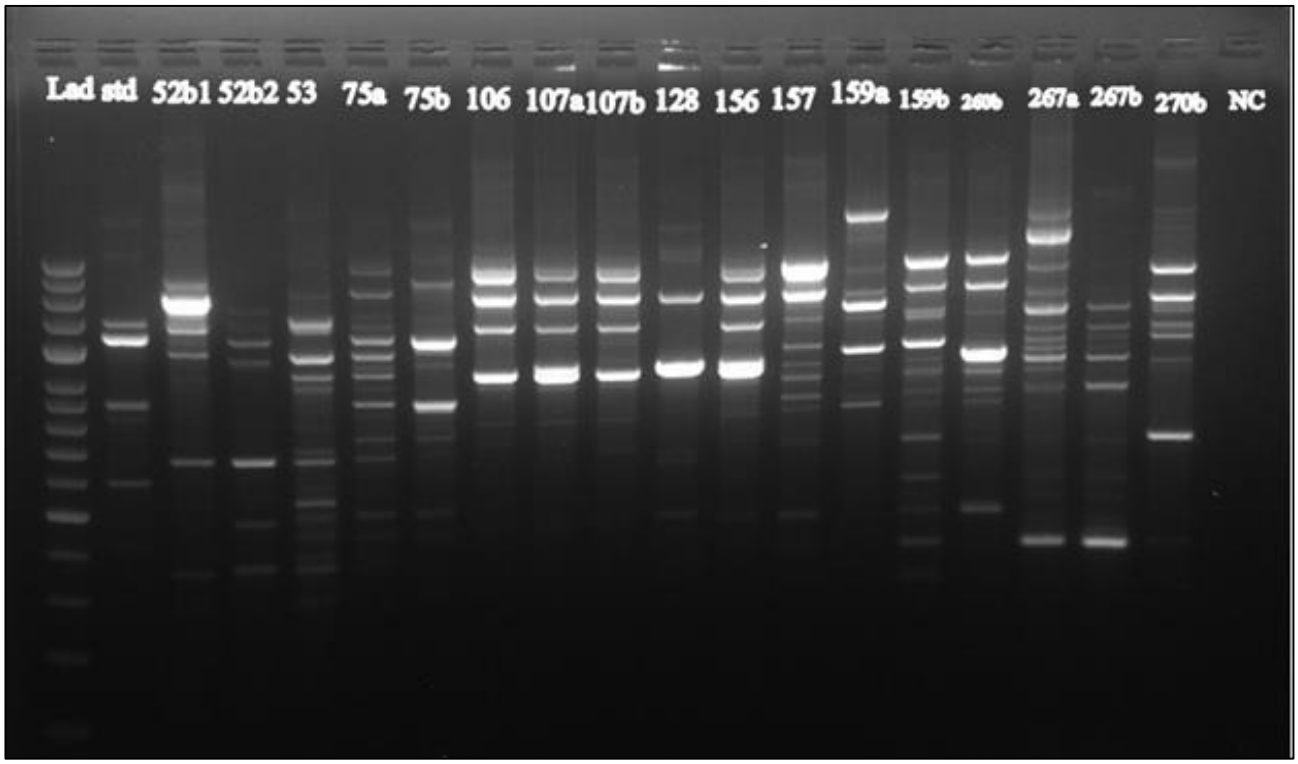


Figure 2: Random Amplification of Polymorphic DNA of different strains of *L. salivarius*. The numbers denote different strains of *L. salivarius*. Lane 1 denotes 100 bp ladder

3.3 Isolation and Characterization of Biosurfactants Produced by Vaginal *Ligilactobacillus Salivarius* and *Limosilactobacillus Reuteri*

Principal Investigator	:	**Clara Aranha**
Co Principal Investigator	:	V Bhor
Project Associates	:	R Shaikh, Manasi Kadam, Haimanti Sarkar
Duration	:	2021-2022

Lactobacilli constitute an important part of the vaginal microbiota and are recognized as potentially useful bacteria through the production of lactic acid, hydrogen peroxide, bacteriocins, exopolysaccharides, and biosurfactants (BS). During the current year, we isolated and characterized biosurfactants (BS) from vaginal isolates *Ligilactobacillus salivarius* and *Limosilactobacillus reuteri*. Further, we investigated the antimicrobial, biofilm-disrupting and antiproliferative activities of the biosurfactants. The biosurfactant molecules secreted into the extracellular medium (excreted biosurfactant-EBS) and that which remain attached to the cell surface (Cell bound biosurfactant-CBBS) of two species *L. reuteri* and *L. salivarius* were extracted by acid precipitation and cell agitation respectively. Cell-bound BS and excreted biosurfactant production were observed in both Lactobacillus isolates. However, the surface activity was greater in cell-bound biosurfactants. The cell-bound biosurfactants from *L. salivarius* had greater inhibitory activity against urogenital bacteria (*E. coli*, *S. aureus*, *P. aeroginosa*) than *L. reuteri*; no activity was seen against fungal pathogens. Both Lactobacillus species inhibited biofilm formation and adhesion of *G. vaginalis*. *L. salivarius* CBBS showed maximum cytotoxic activity against Hela cells (Fig. 1). Vaginal lactobacilli produced biosurfactants that displayed antimicrobial activity against pathogens, disrupted biofilm formation, and exhibited antiproliferative activity in cervical cancer cells. These properties justify further exploration of biosurfactants of indigenous vaginal lactobacilli as biotherapeutics for RTIs and cervical cancer. Further characterization is in progress to reveal the specific composition of the natural biosurfactants.

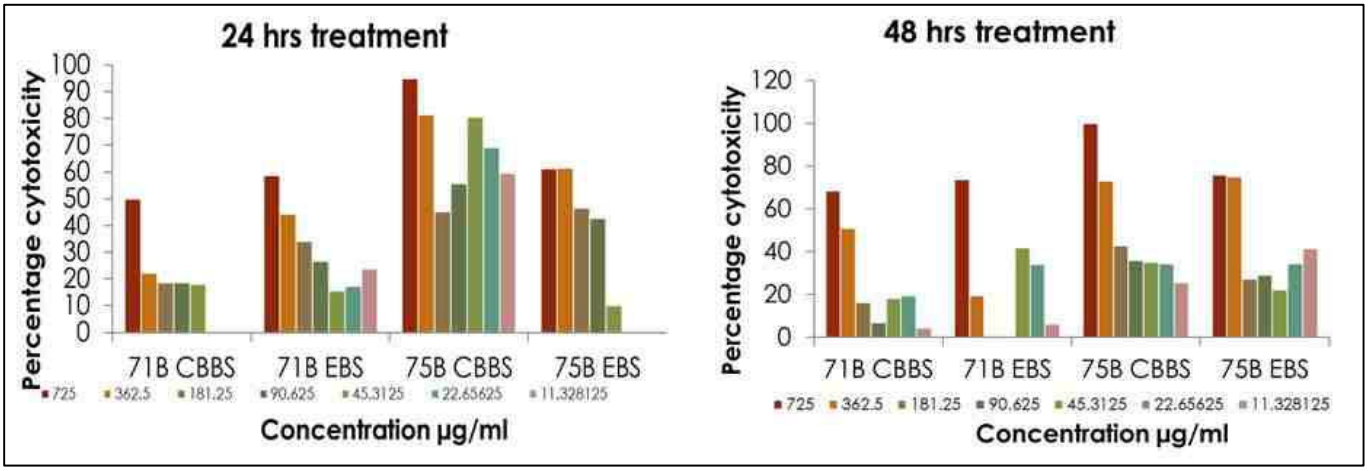


Figure 1: Cytotoxicity of Cell Bound Biosurfactants (CBBS) and excreted biosurfactants (EBS) derived from *Ligilactobacillus salivarius* (75B) and *Limosilactobacillus reuteri* (71B) on Hela cell line post treatment for 24 hrs and 48 hrs.

3.4 Evaluating the Inflammatory, Microbiome Profile and Co-infections in Women Diagnosed with Treatment Failure, Relapse or Recurrent Bacterial Vaginosis *(Partly Funded by Indian Council of Medical Research)*

Principal Investigator	:	**Deepti Tandon**
Co-Principal Investigators	:	V Bhor, Kiran Munne, Clara Aranha
Project Associates	:	Anushree Patil, Shahina Begum, Rachna Dalvi
Collaborator	:	N Mayadeo
Duration	:	2022-2025

Bacterial Vaginosis (BV) is a highly prevalent reproductive tract infection with reported prevalence from India ranging from 21% to 44.8% in different communities and clinical settings. Despite giving standard antimicrobials like metronidazole or secnidazole, short term cure is 30-40% and recurrence rate as greater than 50%. This prospective study in collaboration with KEM hospital was initiated in February 2022 to understand the burden of women suffering from recurrent/relapse and treatment failure BV. It will identify bacterial species in women with recurrent/relapse BV and those experiencing treatment failure by analysing vaginal microbiome. Further correlation was carried out with Nugent score, Amsel's criteria and inflammatory markers.

3.5 Integrated Analyses of Genomic Scale Metabolic Models and Omics Profiles to Capture the Host-Pathogen-Environment Interplay of *Candida sp* *(Partly Funded by Department of Science and Technology - Science and Engineering Research Board)*

Principal Investigator	:	**Susan Thomas**
Co-Principal Investigators	:	Taruna Madan, KV Venkatesh
Project Associates	:	Kshitija Rahate, Amrita Kalathil, Shuvechha Chakraborty
Duration	:	2021-2024

Candida spp. is a leading cause of fungal infections worldwide with emergence of antimicrobial drug resistance due to overdependence on a limited number of antifungal drugs. The study aims to evaluate host-pathogen interactions of *Candida spp*. in environments that replicate different host niches using multi-omics data and genome-scale metabolic models. Preliminary studies to assess the effect of the components of the host microenvironment on the growth and viability of *C. albicans*, *C. tropicalis* in the human vaginal epithelial cell lines, VK2/E6E7 and A-431, have been completed. An in-vitro model of host-pathogen interactions in the vaginal milieu has been generated using cell culture inserts for the co-culture of human vaginal epithelial cell lines with *C. albicans* and *C. tropicalis*. The vaginal environment was simulated by supplementing the growth medium with 700 ng/L estradiol, 7.5 µg/L progesterone and 110 mM lactic acid and incubating the plates under hypoxia. This host-pathogen interaction model is validated based on the elevated levels of Candida virulence factors and the expression of host defence molecules during the course of experiment. The global gene, protein and metabolite profiles of the host and pathogen will be generated using RNA-sequencing and mass spectrometry-based analyses.

In a separate study, of published transcriptomic datasets was analysed to develop context-specific models for understanding host-pathogen interaction. RNA-sequencing datasets from a previous study GSE56091 were overlaid on *C. albicans* metabolic model, iRV781, and the human metabolic model, RECON3D, to make context-specific metabolic models for *C. albicans* (SC5314, WO-1) and host cells (HUVECs, OKF6/TERT-2 cell line). For identification of essential genes in Candida under normal and infected states, series of single gene knockouts were performed in Candida context-specific models. Differences in the expression of essential genes was not observed between infected and uninfected states of Candida with HUVECs at different timepoints (90 min, 5 hr, and 8 hr). It was observed that genes belonging to amino acid metabolism, cofactor and vitamin metabolism were enriched in the infection state with OKF6/TERT-2 cell line (Fig. 1).

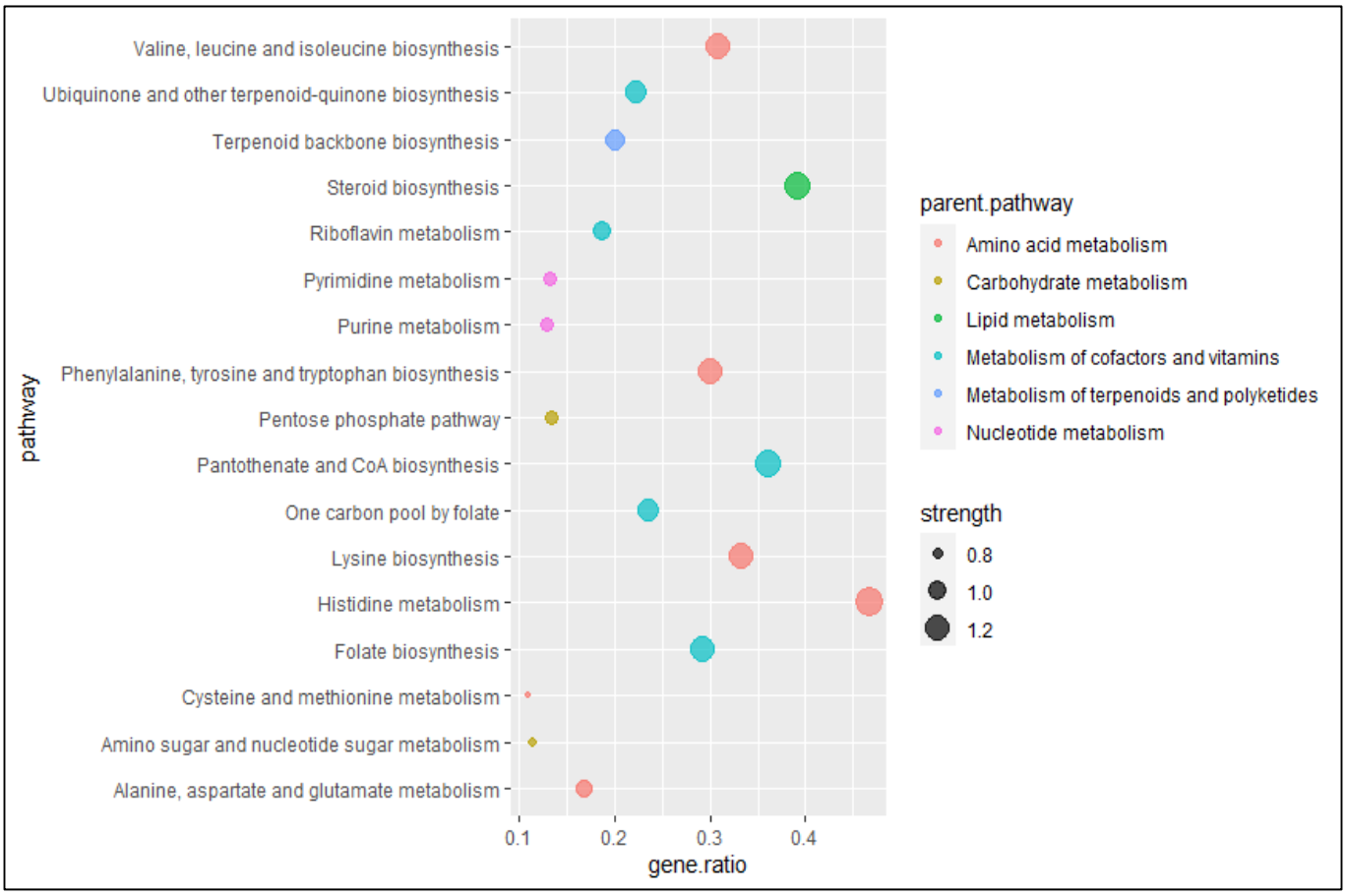


Figure 1: Pathways enriched for essential genes in *C. albicans* with OKF6/TERT-2

Integration of multi-omics profiles with systems biology models is expected to further our understanding of the pathophysiology of candidiasis. The research will reveal new approaches for the discovery of novel drugs and targets for management of candidiasis.

Review of literature revealed that a consolidated resource describing the landscape of candidiasis in India is absent. To address this gap, we developed an online resource named EpiCandIn by manual curation of published literature obtained by searching PubMed and ScienceDirect databases for articles pertaining to Candida infections in Indian population. EpiCandIn contains data available since 1972

from 51 sites in 16 states and 4 union territories of India. This resource is integrated with visualization tools and will be useful for public health researchers and policy makers to gain insights on the emerging trends and management of Candida infections in India. EpiCandIn can be accessed at epicandin.bicnirrh.res.in.

3.6 CD4+ T Regulatory Cells Dysfunction Associated with Latent TB Infection in PLHIV *[Partly Funded by Indo-US Joint Program on Prevention of HIV/AIDS and STDs Collaborative Awards (Indian Council of Medical Research -NIH)]*

Principal Investigator	:	**V Patel**
Co-Principal Investigators	:	V Bhor, Nupur Mukherjee, Kiran Munne, Taruna Madan
Project Associates	:	Shilpa Bhowmick, P Devadiga, AK Singh, S Khan, Nandini Kasarpalkar, Snehal Kaginkar, S Birje, Shilpa Kerkar, A Jogdand
Collaborators	:	Sushma Gaikwad, Sachee Agrawal, Jayanthi Shastri, BYL Nair Hospital, Mumbai Vidya Nagar, Priya Patil, JJ Hospital, Mumbai
Duration	:	2020-2023

The risk of reactivation of Latent TB Infection (LTBI) in otherwise healthy individuals is 10-15% over a lifetime which greatly increases (5-15% annually) following HIV-1 co-infection. During *Mycobacterium tuberculosis* (MTb) infection, T regulatory cells (Tregs) modulate host responses by neutralizing hyperinflammation and thereby prevent damage to the lungs. However, in HIV-TB coinfection this may prove detrimental to the development of efficient pathogen specific responses. Here we evaluated the dynamics of Tregs in PLHIV stratified by their LTBI status following ART initiation. A total of 115 (ART naive HIV-1 infected n=85; seronegative control n=30) individuals were recruited. Ex-vivo immunophenotyping by flow-cytometry of CD4+ Treg cells (CD3+ CD4+ CD25bright CD127low/neg) and their naive/memory status on the basis of CD45RA was carried out. The participants were classified into different groups as (i) HIV-LTBI- (ii) HIV-LTBI+ (iii) HIV-TB+ (iv) HIV+LTBI- (v) HIV+LTBI+ (vi) HIV+TB+ on the basis of Interferon Gamma Release Assay (IGRA) results. Frequency of CD4+ Tregs cell subset was found to be elevated following HIV-1 infection in the absence of ART compared to HIV seronegative individuals. These levels were reduced in HIV+LTBI+ individuals as compared to HIV+ LTBI- individuals while their frequency showed a stark increase in HIV+TB+ individuals. Upon follow-up after 6 months, HIV+LTBI+ individuals showed an increasing trend in the frequency of total Tregs, though subset analysis revealed a decline in the frequency of memory Tregs. A significant decrease in the frequency of circulating Tregs in HIV+LTBI+ individuals was observed in the absence of ART which needs to be evaluated in context of other dysfunctions such as increased T cell activation and exhaustion levels.

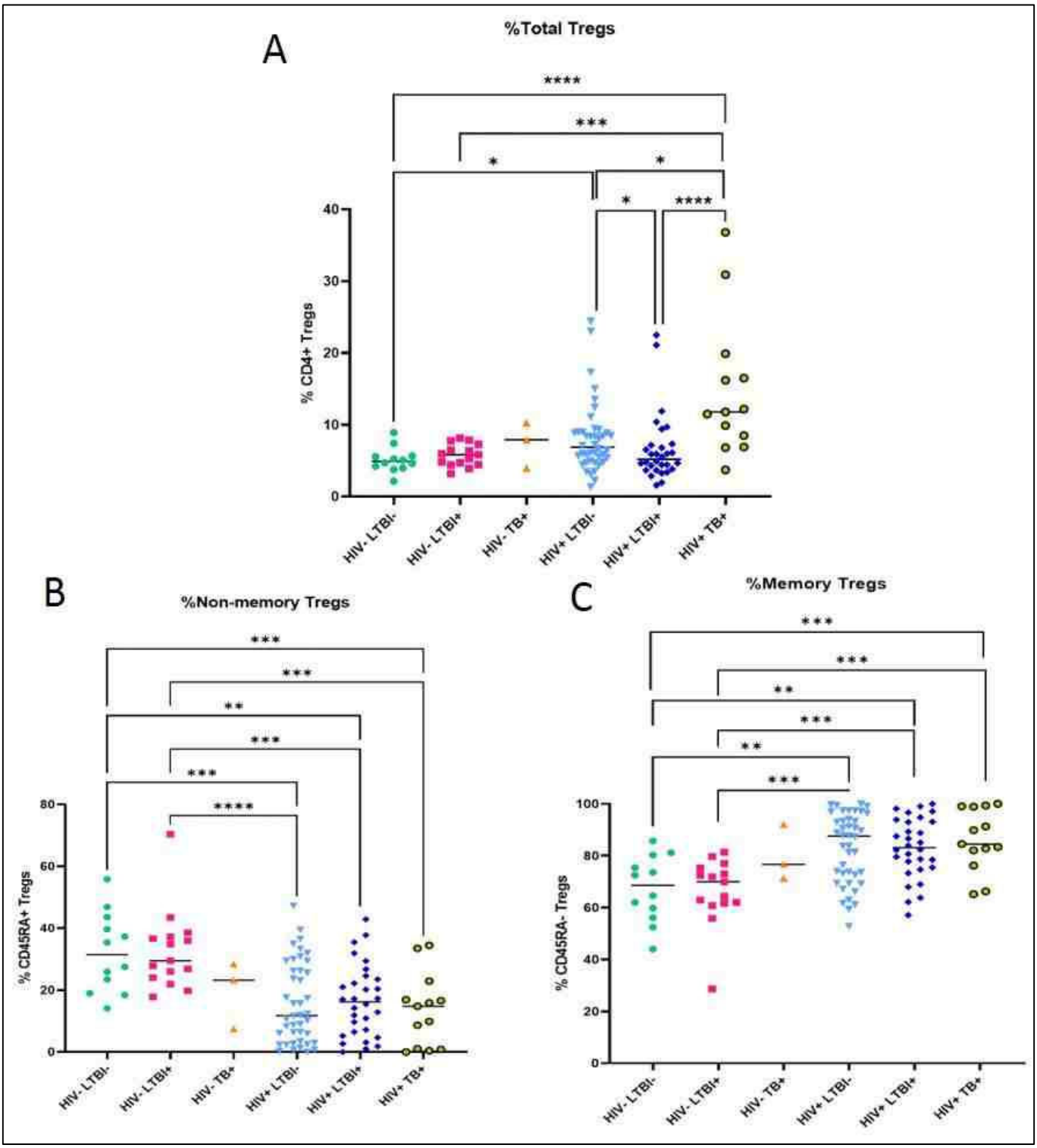


Figure 1: Frequency of T regulatory cells and its subsets. Frequency of (A) CD4+ T Regulatory cells (Tregs) [CD3+ CD4+ CD25high CD127low/neg] (B) Naïve/Non-memory T Regulatory cells [CD3+ CD4+ CD25high CD127low/neg CD45RA+] (C) Memory T Regulatory cells [CD3+ CD4+ CD25high CD127low/neg CD45RA-]. Comparison between groups were calculated by Kruskal-Wallis nonparametric test (*p < 0.05; **p < 0.01; ***p < 0.001; ****p>0.0001).

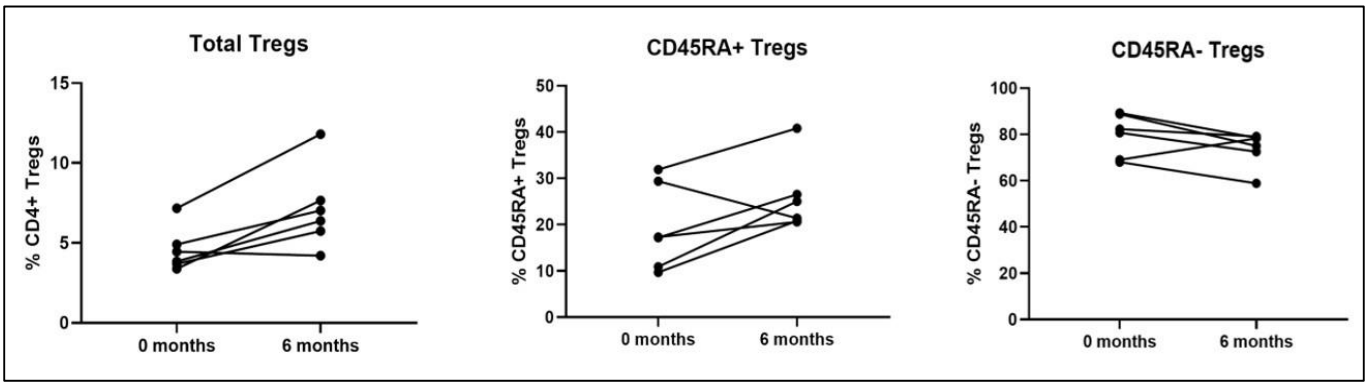


Figure 2: Frequency of T regulatory cells and its subsets at 6 months follow-up: Pairwise comparison between 0 and 6 months of frequency of (A) CD4+ T Regulatory cells (Tregs) [CD3+ CD4+ CD25high CD127low/neg] (B) Naïve/Non-memory T Regulatory cells [CD3+ CD4+ CD25high CD127low/neg CD45RA+] (C) Memory T Regulatory cells [CD3+ CD4+ CD25high CD127low/neg CD45RA-]. Pairwise comparison was calculated by Wilcoxon matched-pairs signed rank test.

3.7 Improving Treatment Literacy and Adherence among People Living with HIV (PLHIV) through Innovative Strategies *[Funded by National AIDS Control Organisation (NACO)]*

Principal Investigator	:	**Kiran Munne**
Project Associates	:	S Chauhan, H Sharma, ARTC Bhopal Namita Parashar, ARTC Jabalpur A Vasantrao Kadam, ICMR NARI, Pune R Sindhu, NACO, M Sebastian, NACO H Kaur, Haryana State AIDS Control Society, Haryana
Duration	:	2020-2022

Treatment literacy is defined as understanding the major issues related to an illness or disease – such as the science, treatment, side – effects, and guidelines so that the patient can be more responsible for their own care and can demand their rights when proper care is not available to them. The objective of the study was to design multimodal interventions for improving treatment literacy and adherence among PLHIV and capacity building of healthcare providers involved in HIV care at ART centre to improve the quality of care. A quasi-experimental cohort study with sequential mixed methods design was conducted in two ART centres namely ARTC Bhopal and ARTC Jabalpur in 2 phases – intervention development and intervention phase with the help of Focus Group Discussions for PLHIVs, In-depth interviews for health care providers and structured questionnaires for pre and post intervention for both. The findings from rapid assessment have been shown in Fig. 1. The key barriers identified were lack of adequate knowledge about disease and treatment, side effects of ART, economic constraints, social stigma and discrimination and gaps at health system level. In addition, some of the ways to overcome these barriers were provision of proper health information, convenient access to ART centres, a technology-assisted recall mechanism about treatment regimen to PLHIV and adequate family and social support, particularly support at workplaces. The overall percentage adherence increased from 82.50% at baseline visit to 95.85% post-intervention. The baseline adherence was significantly higher at Bhopal ARTC than Jabalpur ARTC. In addition, it was more in the participants

from upper middle class as per modified Kuppuswamy scale. Optimal score among PLHIV was found to be significantly associated with optimal adherence and study site. Around 20% improvement in treatment literacy among PLHIV. Post intervention assessment showed significant improvement in knowledge score, adherence and CD4 count (Table 1). Having detectable drug concentration in the assay was highly predictive of viral load suppression. Healthcare providers already had good mean score of pre-intervention questionnaire (8.7/10) which increased post intervention to 9.4/10. Cohen's d is 0.48 suggesting medium effect. The adherence based on adherence scale significantly correlated with self-reported adherence The multimodal interventions pilot tested at two ART centers through this study were found to be effective in improving treatment related knowledge and adherence can be scaled up to other ART centers in India. Intervention-associated improvement in ART adherence, CD4 count and virologic suppression can further reduce opportunistic infections and associated mortality, improve life expectancy, and prevent sexual transmission of HIV. Interventions given to the healthcare providers were helpful in their capacity building as well as the e-learning module on positive living and positive prevention will help them to impart positive attitude towards life in their PLHIV clients. Treatment preparedness/adherence scale and adherence calculation app could be a useful resource for the ART centers reporting lower optimal adherence.

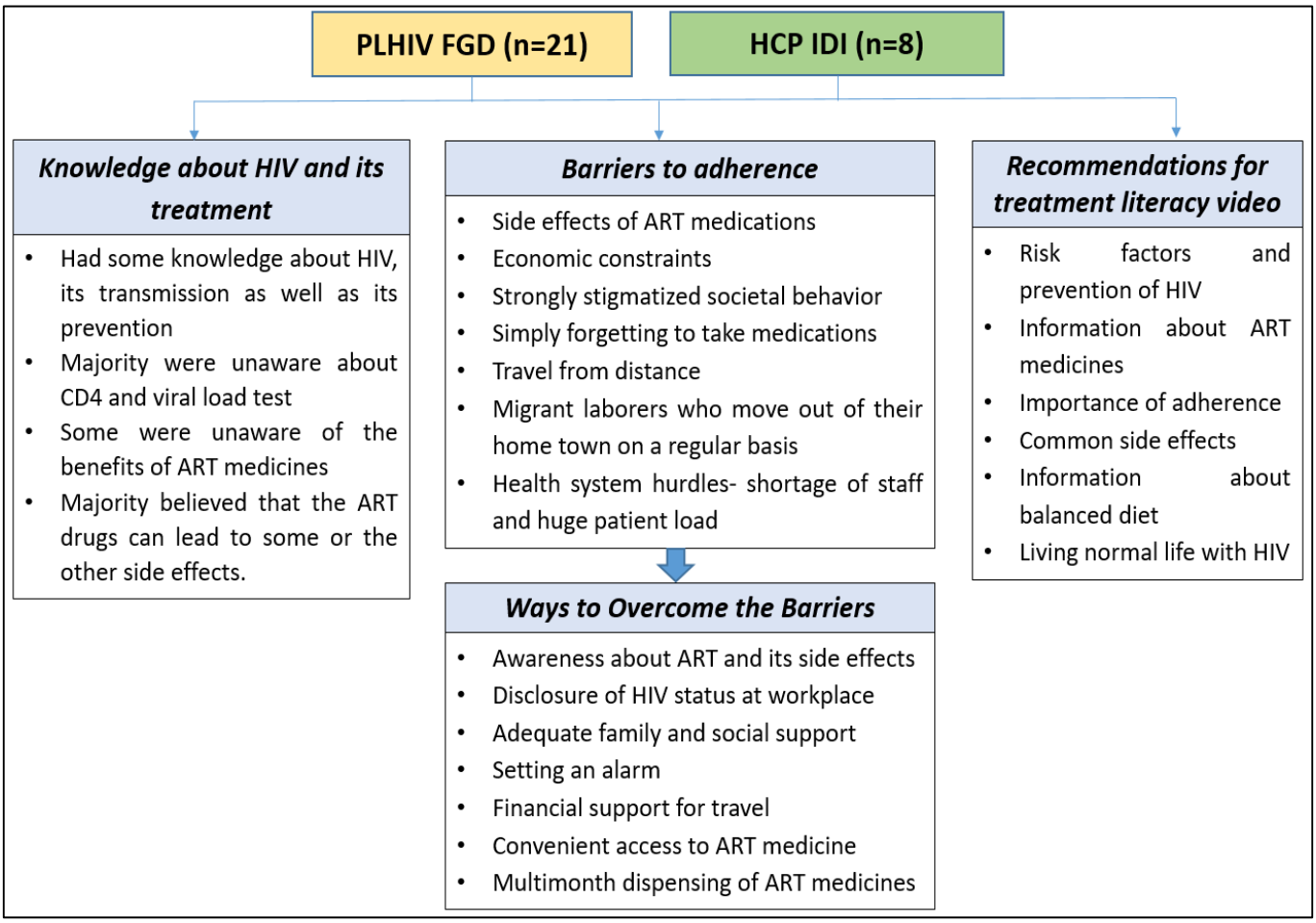


Figure 1: Findings of qualitative study (rapid assessment)

Table 1: Pre and post intervention assessment of the PLHIV study participants

Variables		Pre intervention	Post intervention	p value
Knowledge score (n= 241)	≤10	158 (65.56)	78 (32.36)	<0.001
	>10	83 (34.43)	163 (67.63)	
Adherence % (n=241)	100	176 (73.02)	228 (94.6)	<0.001
	99-95	22 (9.12)	3 (1.24)	
	94-90	15 (6.22)	8 (3.31)	
	< 90	28 (11.61)	2 (0.83)	
CD4 count (n=187)	≤ 200	56 (29.94)	21 (11.22)	<0.001
	>200	131 (70.05)	166 (88.77)	
Viral load (n=62)	<50	46 (74.19)	55 (88.7)	0.08
	50-999	10 (16.12)	4 (6.45)	
	≥1000	6 (9.67)	3 (4.83)	

3.8 Longitudinal Analysis of Integrin $\alpha_4ß_7$ Expressing T Lymphocytes in People Living with HIV

Principal Investigator : **V Bhor**
Project Associates : P Devadiga, Nandini Kasarpalkar, Shilpa Bhowmick, V Patel, Taruna Gupta, Nupur Mukherjee, Kiran Munne, S Birje
Collaborators : Vidya S Nagar, Priya Patil, Grant Medical College and J J Group of Hospitals, Mumbai
Jayanthi Shastri, Sachee Agrawal, TN Medical College and BYL Nair Hospital, Mumbai
Duration : 2021-2024

Depletion of $CD4^+$ T cells is the hallmark of HIV infection. Intestinal $CD4^+$ T lymphocytes are depleted during HIV infection, irrespective of the route of exposure. Integrin $\alpha_4ß_7$ is recognized as the principal gut homing receptor of T lymphocytes. Integrin $\alpha_4ß_7$ expressing lymphocytes are preferentially targeted by HIV in order to facilitate migration of infected cells to the gut associated lymphoid tissue, thereby contributing to pathogenesis. Effector memory T cells which express integrin $\alpha_4ß_7$ upon infection could differentiate into central memory T cells and become latent HIV reservoirs. Further, the detection of integrin $\alpha_4ß_7$ on circulating T cells would provide an estimate of activated, exhausted and memory subset cells trafficking between the systemic circulation and the gut. We previously reported an increased frequency and count of integrin $ß_7^{Hi}$ $CD8^+$ memory T cells in anti-retroviral therapy (ART) naïve HIV infected individuals compared to HIV negative controls. There is limited availability of data globally as well and the absence of data from India on the effect of ART on the proportion of integrin $\alpha_4ß_7$ expressing T cell subsets in HIV infected individuals. In view of this, we performed a longitudinal study on ART naïve HIV infected individuals (n=14) recruited at Integrated Counselling and Testing Centres (ICTC) and ART centres at JJ Hospital and Nair Hospital, Mumbai and followed up after completion of 6 months of ART. The results revealed an overall trend towards decrease in integrin $\alpha_4ß_7$ expressing

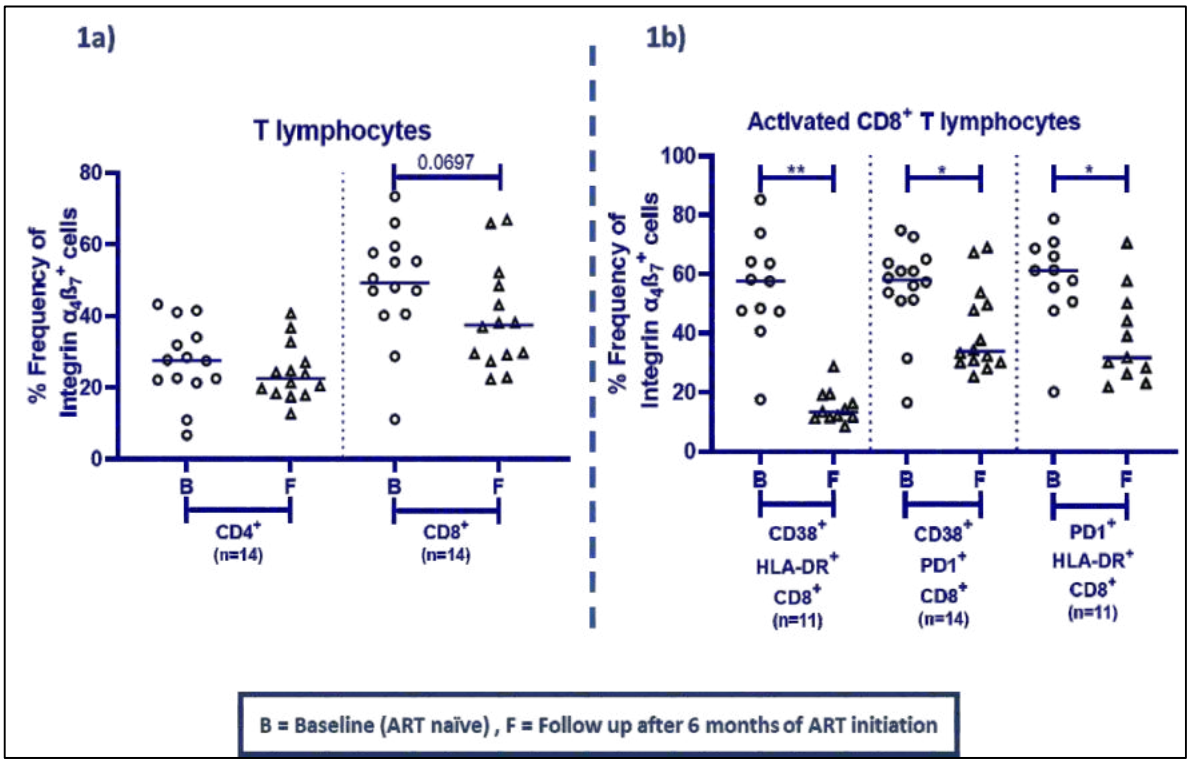


Figure 1: a) Frequency of integrin ß7 expressing CD4+ and CD8+ T cells. b) Frequency of integrin ß7 expressing activated CD8+ T cells. Statistical significance was calculated by paired Wilcoxon Test; *, p < 0.05; **, p < 0.01.

T cells after 6 month of ART (Fig. 1a). This was in contrast to a corresponding increase in CD4+ T cell count following 6 months of ART. The frequency of integrin α4ß7 expressing activated CD8+ T cells (CD38+ and HLADR+) decreased after ART initiation (Fig. 1b), suggesting an overall reduction in immune activation in response to the therapy. Furthermore, an increase in integrin ß7+ transitional memory CD4+ T cells but not CD4+ T cells was observed following ART (Fig. 2a and 2b). The increase in integrin ß7+ CD4+ transitional memory cells indicates an increase in gut homing potential of these cells post 6 months of ART and thereby suggests that these cells are likely to be infected and become latent HIV reservoirs. The alterations in the proportion of integrin α4ß7 expressing T cell subsets observed following ART indicates that it could serve as a potential maker for disease progression and response to treatment.

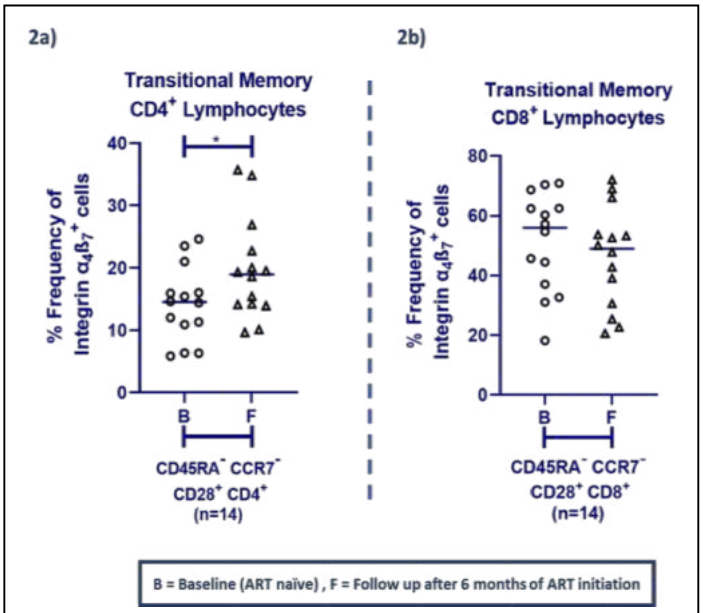


Figure 2: a) Frequency of Integrin ß7 expressing transitional memory CD4+ T cells. b) Frequency of Integrin ß7 expressing transitional memory CD8+ T cells. Statistical significance was calculated by paired Wilcoxon Test; *, p<0.05.

FERTILITY REGULATION

4. FERTILITY REGULATION

4.1 Health Systems Analysis and Evaluations of the Barriers to Availability, Utilization, and Readiness of Family Planning and Contraceptive Services in COVID-19 Affected Areas of Maharashtra, India *(Partly Funded by WHO Geneva)*

Principal Investigator	:	**Beena Joshi**
Co-Principal Investigators	:	Deepti Tandon, R Prusty, Bhavya MK
Project Associates	:	Shabana Khan, P Sanap, I Mashal, Shraddha Chalwadi, Pranali Ahir, Mayuri Ghawat, S Gaikwad, Kanchan Unawane
Collaborator	:	Government of Maharashtra
Duration	:	2022-2023

A multi-country qualitative research study was undertaken by WHO in India, Tanzania and Nigeria to understand and explore the impact of COVID-19 on Family Planning services, which are generally not prioritized compared to other clinical services. In India, the study was undertaken in Thane district of Maharashtra, one of the most COVID affected districts. Two blocks in the district were randomly selected to represent urban and rural health facilities. Health System's preparedness and response in providing Family planning services during the COVID pandemic was assessed along with the challenges or barriers faced by the 'clients i.e. young couples' and 'health care providers' (HCP) regarding provision and access to FP services. The trends in the uptake of contraceptive services during COVID in comparison with pre- and post-COVID situations were studied using HMIS data. It was observed that MOHFW had issued an advisory in early April 2020 to provide FP services under the essential service category. Household distribution of spacing methods and use of telemedicine were suggested strategies with suspension of sterilization camps. During the first wave, health care providers were overwhelmed with additional challenge of COVID duties while they tried to provide routine services. Additional manpower support and commodities to prevent and manage COVID was available more evidently in urban than in rural areas. COVID vaccination program received good support in both rural and urban areas. E-consultation was mainly for COVID than for other services. Stigma, discrimination and psychological stress were faced by nearly all HCPs. Spacing methods were well distributed through ASHA workers who were a critical link in maintaining the supply of contraceptives. Both men and women from rural and urban settings reported using condoms and Oral Contraceptive (OC) pills as their preferred methods for family planning. Some couples also implied that since all services were closed due to the lockdown, FP Services were also unavailable. This suggests that health system did not communicate about what services were available with the community. Therefore, people depended on private pharmacies for availing contraceptives. Few men reported feeling comfortable accessing services directly from female ASHA workers. Unintended pregnancies and shortage of injectable contraceptives were also reported. Health care providers endorsed that FP and abortion services should be categorized under essential services during future pandemics. Telemedicine and prompt communication on the available services were identified as essential strategies that needed focus. Couples suggested few innovative initiatives like installing Condom box or vending machines and identifying contraceptive depo holders and including male health workers to facilitate access to contraceptives during future pandemics.

4.2 Study of Adverse Event Profiles of Copper containing Intrauterine Contraceptive Devices TCu380A and TCu200B

Principal Investigator	:	**Lalita Savardekar**
Co-Principal Investigator	:	Anuradha Majumdar, IPAMSB's Bombay College of Pharmacy, Mumbai
Project Associates:	:	Varsha Vaze, IPAMSB's Bombay College of Pharmacy, Mumbai Neera Mehta
Duration	:	2020- 2023

Three-decade data records of married women of reproductive age who were inserted copper containing intrauterine contraceptive devices (IUCD) TCu200B, TCu380A in the Woman's Health Clinic of ICMR- NIRRCH, Mumbai were considered for the study. Data records of 696 Copper T insertions among 438 women with either Copper T 200B (42.2%) or Copper T 380A (57.8%) were included. Number of devices (696 devices) that were inserted by women ranged from 1 (n = 284), 2 (n= 88) 3(n= 40) , 4 (n= 20), 5 (n=8), 6 (n=1). The frequently reported adverse events at removal of the device for TCu200 (65 of 297) vs TCu380A (144 of 399) were as follows: accidental pregnancy (1.0% vs 0%), complete expulsion (1.0% vs 0.3%), partial expulsion (3.7% vs 12.8%), low placement (1.4% vs 2.8%), menorrhagia (5.7% vs 8.5%), irregular bleeding /spotting (1.0% vs 3.3%), abdominal pain/backache (4.1% vs 4.8%), infection (2.7% vs 1.5%), difficult removal (0.7% vs 1.8%) and pricking sensation to husband (0.3% vs 0.3%). The reasons for difficult removal of the device (n=9) were as follows: thread broken while removing device (n=2), on examination Copper T thread was missing and was broken (n=6), cervical perforation of the posterior lip (n-1). The devices were removed in the clinic/referral hospital by IUD removal hook or by artery forceps. None of the cases needed hysteroscopic removal or surgical intervention. Among the adverse events reported with use of TCu380A and TCu 200B respectively, of concern were pregnancy (0% vs 1.01%), partial expulsion (3.6% vs 1.7%), bleeding irregularities (4.0% vs 1.3%), menorrhagia (8.0% vs 5.7%). Other reported events like pricking sensation to self/spouse (1.3% vs 1.7%) and those reported earlier were unacceptable to couple and may deter a woman from using Copper T.

4.3 Longitudinal Cohort Study to Evaluate the Effect of Various Contraception Methods on the Composition and Diversity of the Vaginal Microbiota *(Partly Funded by Indian Council of Medical Research)*

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

As per 2017 estimates, 63% of the married women of reproductive age worldwide use some form of modern or traditional contraception. There have been conflicting evidence regarding the effect of available contraception methods on the vaginal health of women. This longitudinal cohort study was planned among women using various contraceptive methods over a period of six months. Changes in their vaginal health using various parameters like vaginal pH, Nugents Score, Amsel's criteria, culture, vaginal inflammatory profile were analyzed. Species-level composition of the vaginal microbial ecosystem in these women over a period of 24 weeks was determined using 16 SrRNA gene sequencing. This study will help to map 'normal or healthy' vaginal microbiome of healthy women living in the urban belt of Maharashtra as well as gain insights into the effect of various contraceptive methods on the vaginal health of women. A prospective study was conducted among healthy, sexually active, non-pregnant women aged 18 to 45 years (n=40) from a Mumbai community clinic seeking contraception services. Four groups were formed, of those initiating contraception with intrauterine copper T 380A IUCD, condoms, Injectable DMPA, and a control group of tubal ligated women not using any active contraception. The choice of contraception was as per cafeteria approach. The women were followed up longitudinally at 90 and 180 days, and the vaginal milieu was evaluated at 0, 90, and 180 days using Gram stain, vaginal cytokine analysis, and vaginal microbiome analysis. Women who did not complete the follow-ups were excluded from the analysis.

The women recruited had a mean age and BMI of 33.3±6.02 years and 24.98±4.95 Kg/m2, respectively. The women majorly belonged to the lower-middle and upper-lower socio-demographic classes. They all reported having a single sexual partner and a mean coital frequency of 4±2.3 per month. Deep sequencing of the 16S rRNA (V3-V4) amplicon of vaginal samples yielded a total of 20,660,069 reads, with an average of 172,167.24 reads per sample. Variations in the abundance of specific vaginal microbiome species were observed in response to different contraceptive methods. The most prevalent species observed in the cohort was *Lactobacillus iners* (43.56%) followed by *Lactobacillus crispatus* (12.45%), *Lactobacillus gasseri* (8.17%). *Gardnerella vaginalis* was the most abundant pathogenic anaerobe (7.83%). Women using Copper T IUCD showed an increase in the overall relative abundance of *Lactobacillus* from 0 days (58.59%) to 180 days (66.62%), with a rise from 0 to 90 days and a slight decline between 90 and 180 days in the abundance of *Lactobacillus crispatus* (0.87% to 7.07% to 5.30%) and *Lactobacillus iners* (41.57% to 56.42% to 43.75%). While there was no significant change in the abundance of *Gardnerella vaginalis* from 0-180 days, there was an increase in the relative abundance of *Gardnerella vaginalis* 1.95% to 6.34% and *Atopobium vaginae* (1.46% to 5.90%) from 90-180 days. Persistent use of barrier contraceptives resulted in a significant decrease in *Atopobium vaginae* (1.66% to 0.03%) from 0 to 90 days. The relative abundance of *Gardnerella vaginalis* also decreased significantly from 13.36% at 0 days to 4.59% at 180 days. From 0 to 180 days, there was a significant increase in the relative abundance of *Lactobacillus* species from 60.54% to 79.88%. Women taking injectable DMPA had a decrease in the relative abundance of *Lactobacillus* from 0 to 90 days (64.72% to 57.63%), followed by an increase from 90 to 180 days (57.63% to 62.38%). Women who did not use any contraception methods showed a decrease in the cumulative relative abundance of *Lactobacillus* species from 0 to 180 days (75.98% to 59.66%). From 0 to 180 days, the pathogen *Atopobium vaginae* increased 0.615% to 6.67%. This study provides important insights into the impact of different contraceptive methods on the vaginal milieu of women and highlights the need for appropriate contraceptive selection and regular monitoring of vaginal health.

MATERNAL HEALTH

5. MATERNAL HEALTH

5.1 Factors Associated with the Intimate Partner Violence (IPV): Insights from National Family Health Survey

Principal Investigator	:	**Shahina Begum**
Co-Principal Investigator	:	RK Prusty
Duration	:	2022-2023

The public health corollaries of marital violence are well documented and include injury, mental health concerns and physical health consequences for women. Identifying the period during which violence is initiated against women in their marital life is an important step towards developing interventions to prevent violence against violence. The objective of the study was to determine the initiation of violence against women of reproductive age over marital duration using data from population based National Family Health Survey, 2019-21. The prevalence of any physical and/or sexual violence by their husbands was found to be 29%. About 28% women suffered physical violence. Of this, 11% were severe physical in nature like punching, kicking, choking, burning of women or threatening using weapons such as gun, knife or any other harmful weapon (Fig. 1).

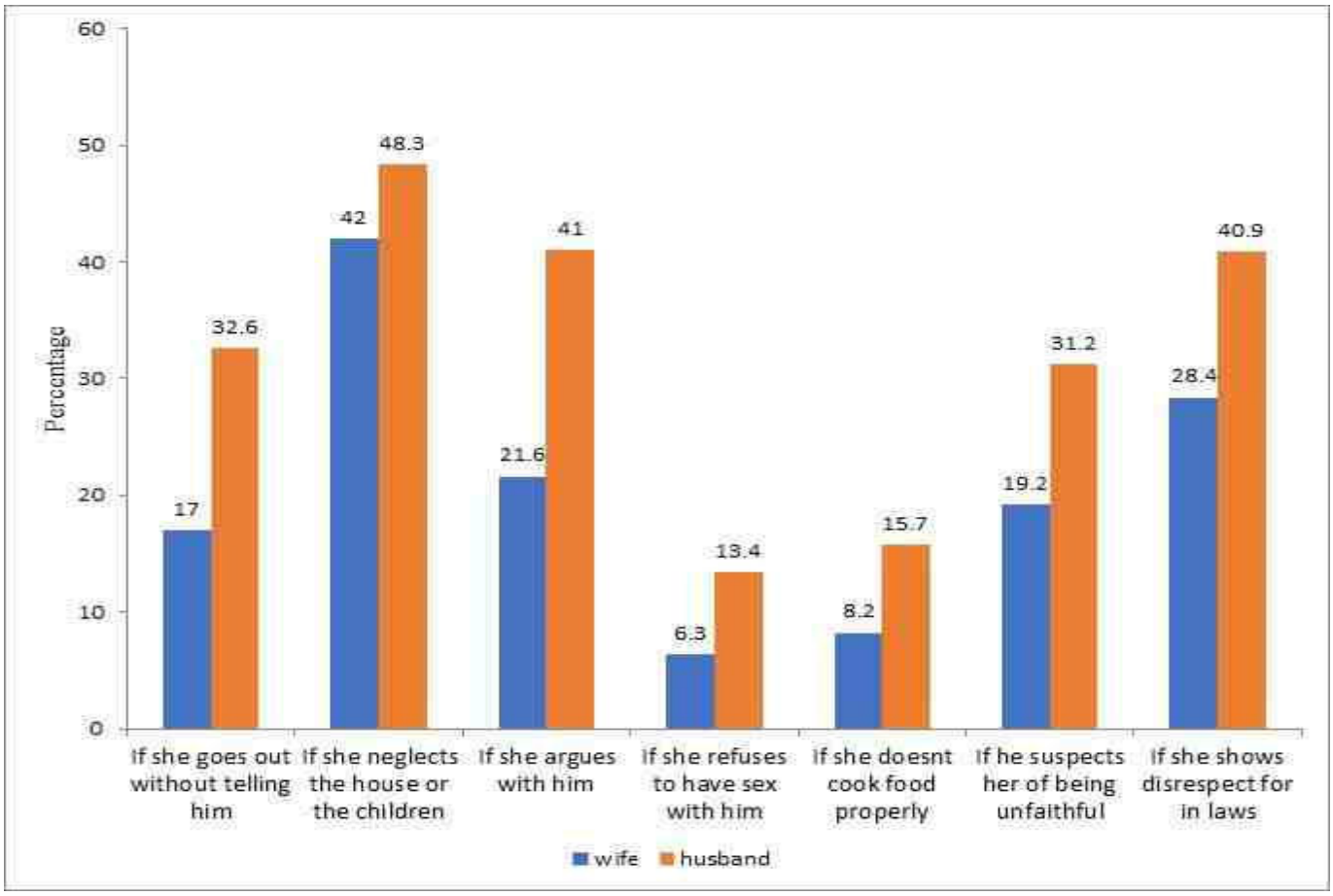


Figure 1: Percentage of husbands and wives justifying wife beating

Around 6% had ever experienced sexual violence in any form. Higher proportion of IPV was reported in early-married life. Around 90% of the IPV were initiated within the first 8 years of marriage (Fig. 2).

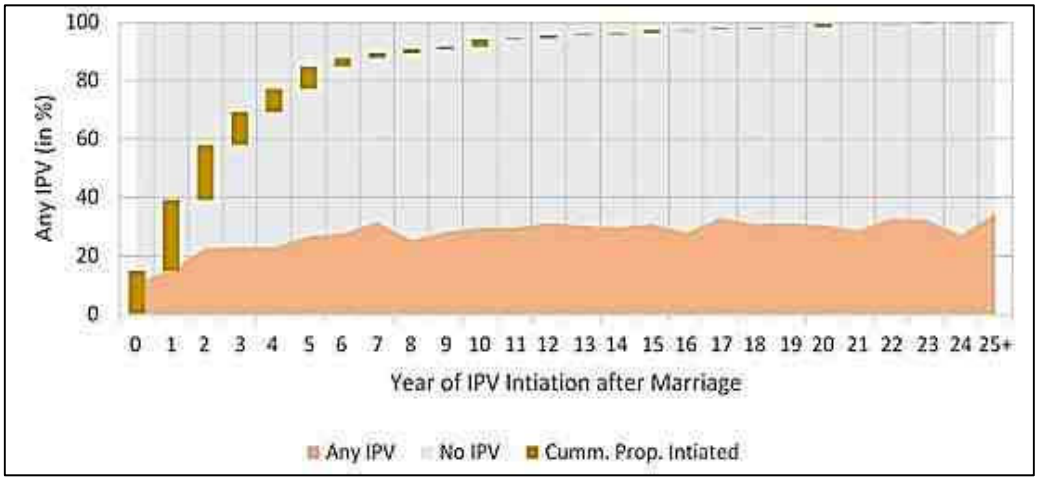


Figure 2: Prevalence of IPV and year of initiation of IPV after marriage among ever-married women aged 18-49 years in India, 2019-21

5.2 Trends, Pattern and Determinants of Pregnancy and Birth Outcomes in India: Evidence from 5 Rounds of National Family Health Surveys (1991-2021)

Principal Investigator : **R K Prusty**
Duration : 2022-2023

Sustainable development goal 3 targets reducing the maternal mortality ratio by two-thirds by 2030 across all the countries. Despite the decline in MMR, adverse pregnancy outcomes (abortion, miscarriages and stillbirths) are on higher side and increasing. There are no studies focusing on the trends, patterns and determinants of birth outcomes in India. With further increase in the use of assisted reproductive technology, there may be a change in the pattern of multiple births in India, which is also not examined. The five rounds of National Family Health Survey data provide a unique opportunity to analyse such trends, patterns and determinants. The NFHS data show a decline in live births from 91% during NFHS (2015-16) to 89% in NFHS (2019-21). The number of abortions decreased (3.4% to 2.9%) over the time period. Nearly half of the abortions were due to unplanned pregnancies (47.6%). Reducing unintended pregnancies will contribute to lower induced abortion and thus improve women`s health. The project is ongoing.

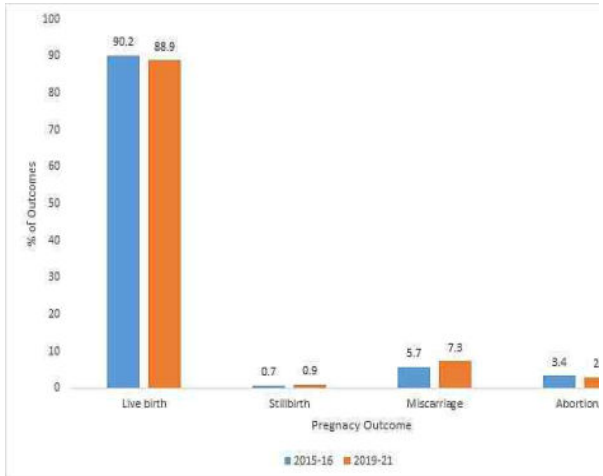


Figure 1: Change in pregnancy outcomes in India during 2015-21

5.3 Understanding the Progress of Maternal Health and Outcomes among Indian Women: Findings from the NFHS

Principal Investigator : **P Kuppusamy**
Project Associates : RK Prusty, KC Itta, RK Gajbhiye, Geetanjali Sachdeva
Duration : 2022-2023

India has achieved significant progress in improving maternal health and outcomes over the last two decades by improving age at first birth, birth spacing interval, reducing number of children, institutional deliveries, and live births. The factors like early marriage, early childbearing, and lack of family planning lead to a shorter time between marriage and childbirth, which results in poor maternal and neonatal outcomes. Pregnancy outcome is an important health indicator of the quality of maternal health. Adverse pregnancy outcome is a major public health problem, which can lead to poor maternal and neonatal outcomes. The fifth round of National Family Health Survey (NFHS-5) data show that the number of women in age 20-24 years age-group who were married before 18 years have reduced marginally from 27% in 2015-16 to 23% in 2019-21. However, every fifth girl is still likely to get married before attaining 18 years of age in India. Teenage pregnancy is more prone to have adverse outcomes including miscarriages, abortions, and stillbirth, and also women are at a higher risk for unplanned pregnancies which lead to undergoing self-abortions due to physical, social, and economic challenges. Our study shows that frequent abortions carry a higher risk of health complications and medical risk. The present study addresses various indicators associated with adverse maternal health and outcomes in Indian women. Data from the fourth (2015-16) (n=195,470 women) and fifth (2019-21) (n=255,549 women) rounds of NFHS were extracted and the absolute and relative changes were calculated for the birth outcomes of the last pregnancy during the five years preceding the surveys.

The study shows that livebirth decreased by 1.3 points from 90.2% in 2015-16 to 88.9% in 2019-21, and nearly half of the Indian states/UTs (n = 17/36) had lower than the national average of livebirths (88.9%) reported during 2019-21. Among the Indian states/UTs, the lowest prevalence of livebirth (78.9% to 76.8%) and highest pregnancy loss (21.2% to 23.1%) was reported in Manipur. The prevalence of miscarriage among Indian women was 7.3% and higher in Manipur (12.3%) in 2019-21. A higher proportion of pregnancy loss was noted, particularly number of miscarriages increased in both urban (6.4% vs. 8.5%) and rural areas (5.3% vs. 6.9%), and stillbirth increased by 28.6% (0.7% vs. 0.9%). Among different age groups, there was a higher prevalence of stillbirth noted among 15-19 year old women in Madhya Pradesh (2.0%). The number of abortions decreased (3.4% vs. 2.9%) among Indian women. Nearly half of the abortions were due to unplanned pregnancies (47.6%) and more than one-fourth (26.9%) of abortions were self-performed. Abortions among adolescent women in Telangana was eleven times higher during 2019-21 as compared to 2015-16 (8.0% vs. 0.7%). More abortion cases were reported in women in urban setting than in rural setting (5.0% vs 2.5%) during 2019-21. This study concludes that an increased prevalence of miscarriage in both urban and rural areas is a matter of concern. Increased stillbirth rates, and abortion rates due to unplanned pregnancies lead to higher burden of psychosocial and economic cost to the family as well as to the country. This study emphasizes having a need of region-specific, comprehensive and good quality maternal health programs for improving livebirths in India.

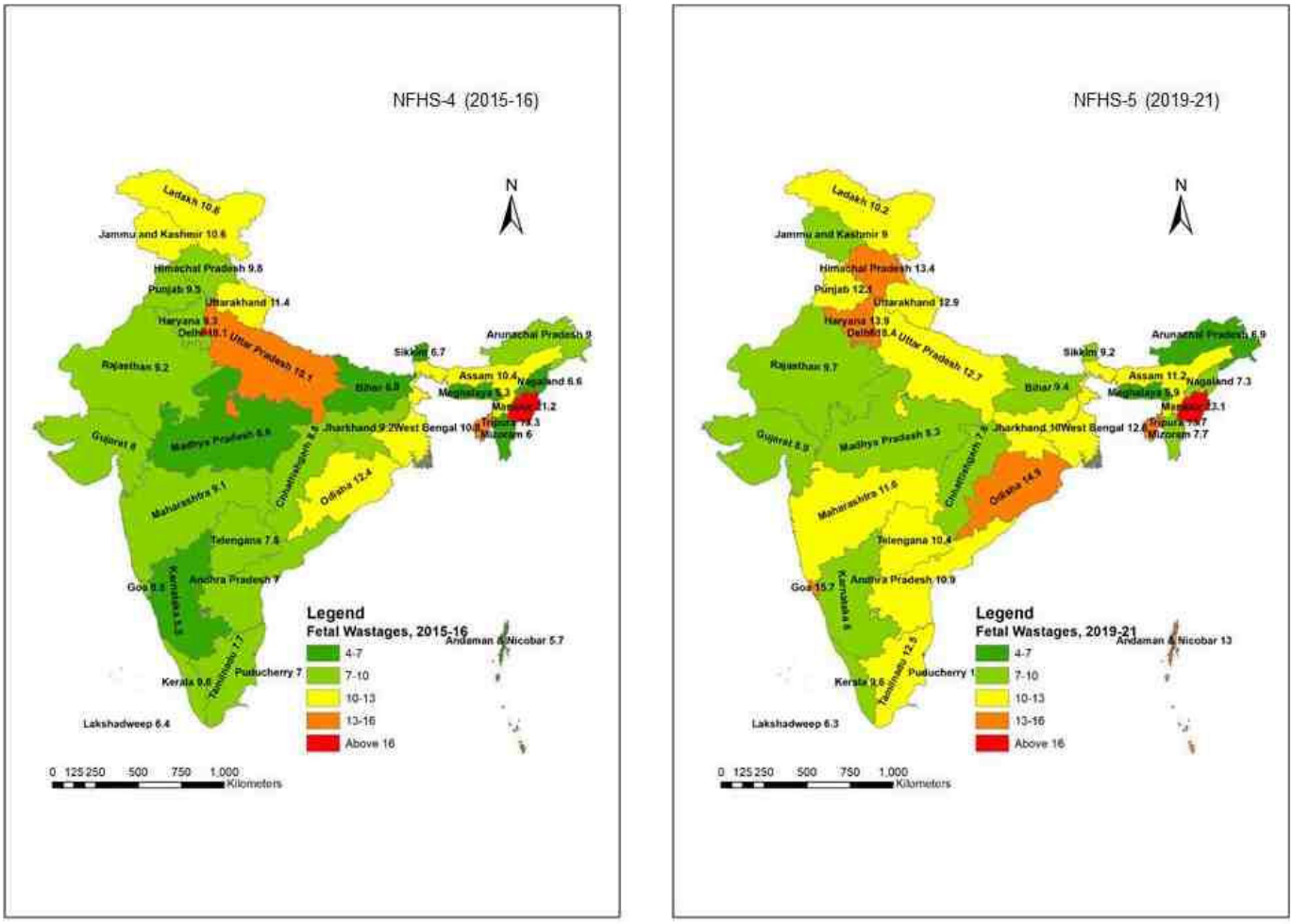


Figure 1: Prevalence of pregnancy loss among Indian women during 2015-21

5.4 Transgenerational Effects of Paternal Hypertension on Fertility and Pregnancy Outcome: An Epigenetic Approach

Principal Investigator	:	**Kumari Nishi**
Co-Principal Investigator	:	Dipty Singh
Project Associates	:	Reshma Gaonkar, Suryakant Mandavkar
Duration	:	2022-2025

The prevalence of hypertension in men within 15-54 year age group is increasing every year. Though observational studies report sexual dysfunction in hypertensive males and effect of paternal hypertension on pregnancy complications and fetal outcomes. However, sufficient experimental evidence are lacking. Towards this, an animal model of Nω-nitro-l-arginine methyl ester (L-NAME) induced male hypertension was developed. L-NAME is a non-specific inhibitor of nitric oxide synthase, responsible for the production of nitric oxide. Male Wistar rats (9-12 weeks) were used for hypertension induction. To select a non-toxic dose of L-NAME, male rats were divided into 5 groups (n=3 each). Groups I, II, III, IV and V were orally administered with L-NAME at 40mg/kg bw, 30mg/kg

bw, 20mg/kg bw, 10mg/kg bw and 0mg/kg bw per day for 3 successive weeks. As compared to control, visibly reduced forelimb locomotor activity was observed in animals of 40 mg/kg/day and 30 mg/kg bw treatment groups. The animals from 20 mg/kg bw and 10 mg/kg bw groups were observed to be active. Animals were sacrificed after 2 days of dose termination. The histological analysis of liver and kidney tissues from the treated groups showed abnormal histology as compared to controls (Fig. 1). Testicular histological analysis showed the presence of amyloid plaques, seminiferous tubule necrosis, loss of interstitial cells and germinal epithelium atrophy in treated animals (Fig. 1).

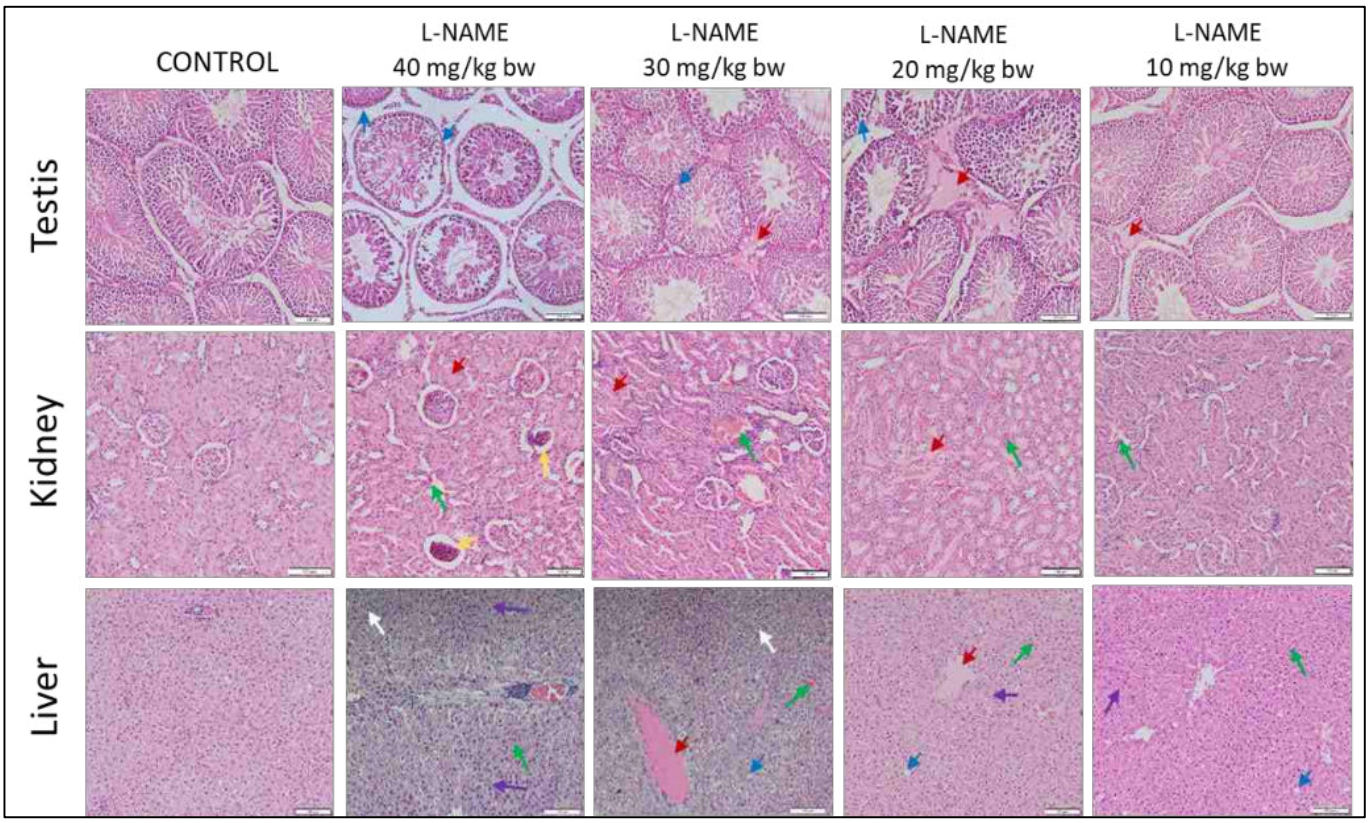


Figure 1: Testicular, kidney and liver histology (H&E; magnification 100X, bar 100µm) of the control and L-NAME treated groups. The testicular sections from treated groups showed increased vacuolization (blue arrow), amyloid plaques in the interstitium (red arrow) as well as inflammation as compared to control rats. The kidney sections also indicated increased inflammatory cell infiltration (green arrow), amyloid deposition (red arrow) and signs of glomerulopathy (yellow arrow) in treated animals as compared to control animals. The liver sections showed inflammatory cell infiltration (RBC infilteration; dotted arrow), amyloid deposition (red arrow), vacuolization (blue arrow), increase in glycogen content (white arrow), hepatocyte hypertrophy (purple arrow).

Based on these observations, the L-NAME dose of 20 mg/kg bw and 10 mg/kg bw was selected for further experimentation. Two new groups of animals were dosed with 20 mg/kg bw (n=7) and 10 mg/kg bw (n=7) L-NAME for three weeks. After five days of termination of L-NAME treatment, two animals from 20 mg/kg bw group had impaired locomotion in front limbs and died eventually. The animals from both L-NAME 20 mg/kg bw and 10 mg/kg bw groups were monitored further for blood pressure changes. Blood pressure was monitored through Automated Non-Invasive Blood Pressure Volume Pressure Recording Tail-Cuff system. The duration of spermatogenesis in rats is ~54 days.

Therefore, to minimize the effect of L-NAME on testicular cell types, animals were monitored for 60 days' post L-NAME treatment. Blood pressure in both 10 mg/kg bw and 20 mg/kg bw groups was monitored regularly during and after completion of treatment. Significant increase in both systolic and diastolic blood pressure was observed in both the treatment groups as compared to control (Fig. 2). The blood pressure was slightly reduced after 60 days in both the treatment groups but the animals were still hypertensive as compared to controls (Fig. 2). The L-NAME dose of 10 mg/kg bw for 3-weeks was found to be the least toxic to induce hypertension in male Wistar rats. The animals were hypertensive after 60 days of L-NAME treatment and found suitable for transgenerational studies to determine the effects of paternal hypertension on fertility and underlying epigenetic mechanisms. Further experimentation to study fertility in these hypertensive male rats are ongoing.

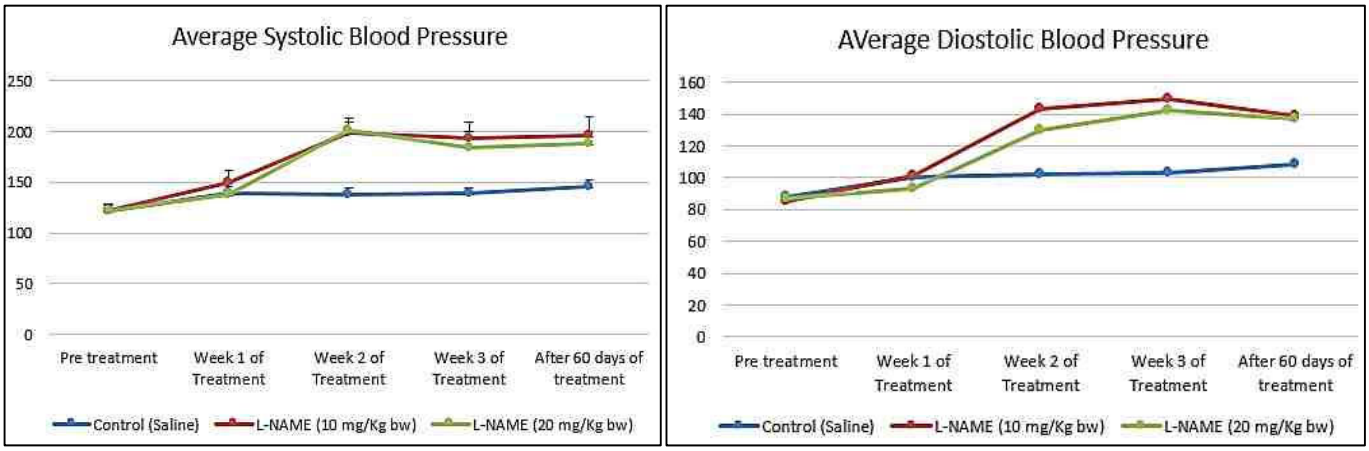


Figure 2: Average systolic and diastolic blood pressure in animals during and after L-NAME treatment. There was an increase in the systolic and diastolic blood pressure in L-NAME treatment groups (hypertensive animals). The blood pressure was high in the hypertensive animals after 60 days of the termination of L-NAME treatment as compared to control.

5.5 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 Departmen of Health Research)*

Principal Investigator	:	**Beena Joshi**
Co-Principal Investigator	:	Deepti Tandon
Coinvestigators	:	Dr. Ragini Kulkarni, Dr. Shahina Begum
Project Associates	:	Kiran Sangwan, Ashwini Padvale, Yogita Avtar, ANM
Collaborators	:	B Hengne, SDH, Dahanu K Bhoye, Jawhar Cottage Hospital
Duration	:	2022-2023

The study was initiated in January 2022 at two subdistrict hospitals in Palghar district – Jawhar and Dahanu. Inclusion criteria were: i) pregnant women in the age group of 18-40 years; ii) gestational age of pregnant women - 22 to 32 weeks; iii) diagnosed with iron-deficiency anemia (IDA) with hemoglobin levels between 5-9.9 gm/dl; and iv) willing to sign an informed consent, deliver at the same site and

willing for follow up. In the reporting period, 300 pregnant anaemic women were screened for iron deficiency anaemia and 200 participants were enrolled in the study and randomly assigned to IV Iron Sucrose (ISC) or IV FCM group. Among them, 168 completed 4-week follow-up and 112 completed postpartum follow-up after 6 weeks. Three women delivered before 4-week follow-up. Interim analysis revealed that the mean rise in hemoglobin post 4-week of drug administration is better in the ISC group (1.8 gm/dl) compared to IV FCM (1.14 gm/dl). Mean rise of hemoglobin after 6 weeks postpartum was 2.34 gm/dl in ISC group compared to 2.11 gm/dl in IV FCM group. However, mean rise in serum ferritin level in FCM group at 4-week follow up was better (113.07 mcg/L) than in ISC group (97.22 mcg/L). The same is true even after 6 weeks postpartum, i.e. mean rise in IV FCM group is 149.05 mcg/L compared to 109.18 mcg/L in ISC group. Out of 200 enrolled pregnant women, low vitamin B12 levels were found in 22% of cases and Inj Vitcofol (intramuscular) was additionally administered to these women. Mild reaction to IV Iron Sucrose in form of nausea and giddiness was observed among 3 women. One woman who received IV FCM had an intra-uterine foetal death (IUFD) before 4-week follow up which was unrelated to the intervention drug as the cause was preeclampsia. All these adverse events were reported to the Ethics Committee. The health care providers found IV FCM a safe and easy-to-use drug compared to IV Iron Sucrose, which needs 3-4 visits by women, and a test dose is needed unlike FCM. The out of pocket expenditure incurred by women receiving IV FCM is Rs 223/- compared to Rs 863/- for ISC. The health related quality of life was calculated using EuroQol tool and Indian tariffs. The score was 0.950 among women receiving IV FCM compared to 0.940 receiving ISC. These parameters will be used along with health system costing parameters to calculate incremental cost-effectiveness ratios to assess if IV FCM is a cost-effective drug for its introduction into programs.

5.6 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**
Co-Principal Investigator	:	RS Barai
Project Associates	:	Ulka Gawde, C Kumar, Shuvechha Chakraborty
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. Antimicrobial peptides (AMPs) are oligopeptides that play a crucial role in the innate immunity of the host. AMPs have shown activity against bacteria, fungi, viruses, protozoa, and cancerous cells. Since AMPs have varied targets, it is difficult for microbes to gain resistance against them. For these reasons, AMPs are gaining importance as anti-infective agents. CAMPR4 database provides manually curated information on natural and synthetic AMPs such as sequence, protein definition, accession numbers, activity, source organism, target organisms, protein family descriptions, N and C terminal modifications and links to databases for the benefit of users (Fig. 1).

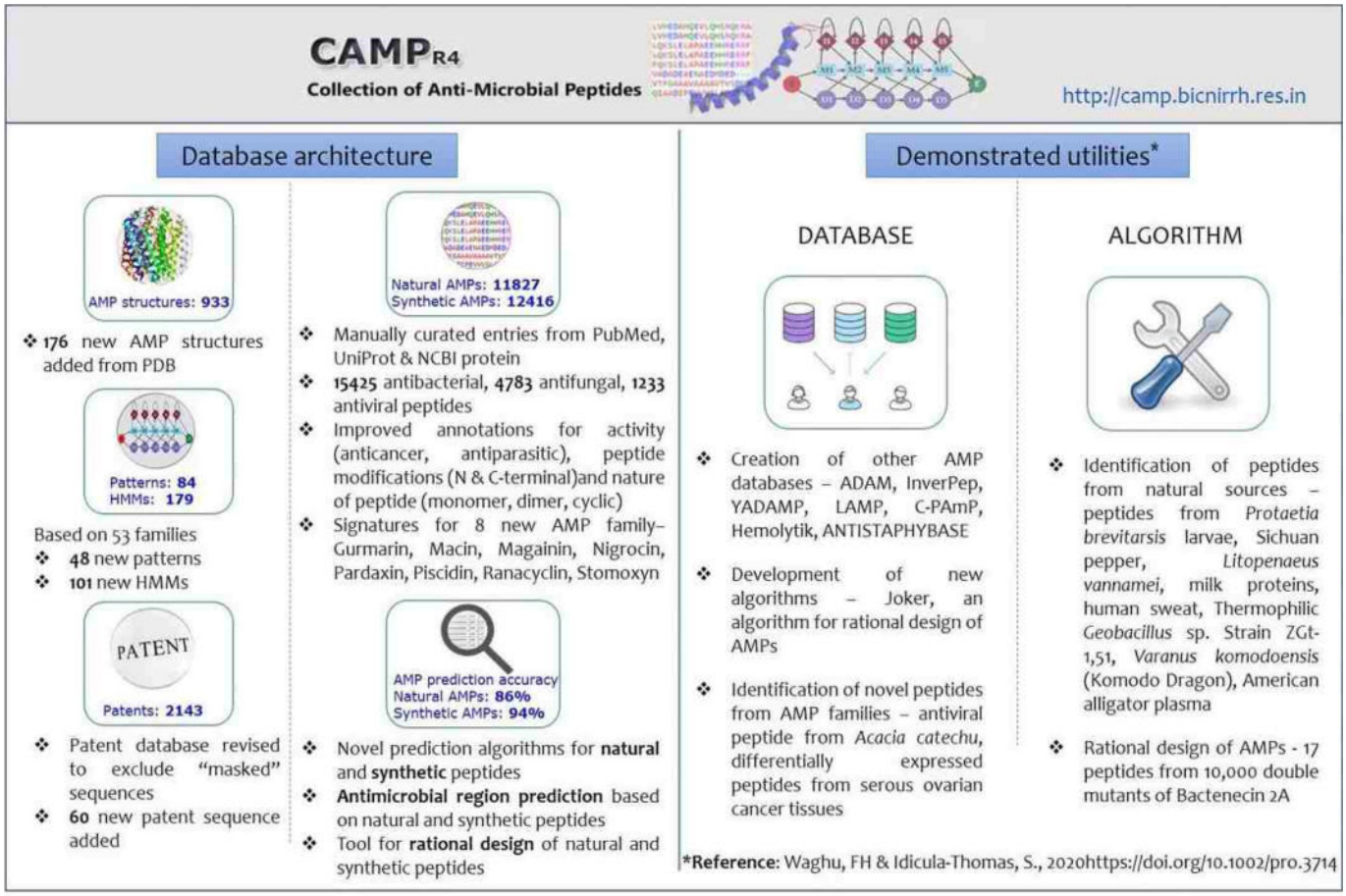


Figure 1: Overview of CAMPR4 database

It holds 24243 AMP sequences, 933 structures, 2143 patents and 263 AMP family signatures (179 HMMs and 84 patterns). In this updated version of the CAMP database, there are separate algorithms for prediction and rational design of natural and synthetic AMPs. The prediction accuracy for natural and synthetic AMPs is 86.5% and 94.3% respectively. CAMP can be freely accessible at http://camp.bicnirrh.res.in/.

Follicle-stimulating hormone receptor (FSHR) plays a vital role in reproduction, cancer progression and osteoporosis. With this therapeutic importance, several small molecule modulators of FSHR have been identified, however the binding sites of these modulators and structural changes that accompany FSHR activity remain elusive.We identified binding sites of both agonist and antagonist FSHR modulators at the same P1 pocket in transmembrane domain (TMD). Agonist and antagonist molecules were found to interact with few distinct residues. TMD residues Ile522, Ala595, Ile602 and Val604 were found to interact only with agonists. Distinctly prominent domain motions and conformational changes in TM helices 3, 4 and 6 for agonist bound FSHR structure were observed. These structural changes were found to be an important and well-conserved mechanism of glycoprotein hormone receptor (GPHR) activation that would help to design potent modulators in the future (Fig. 2).

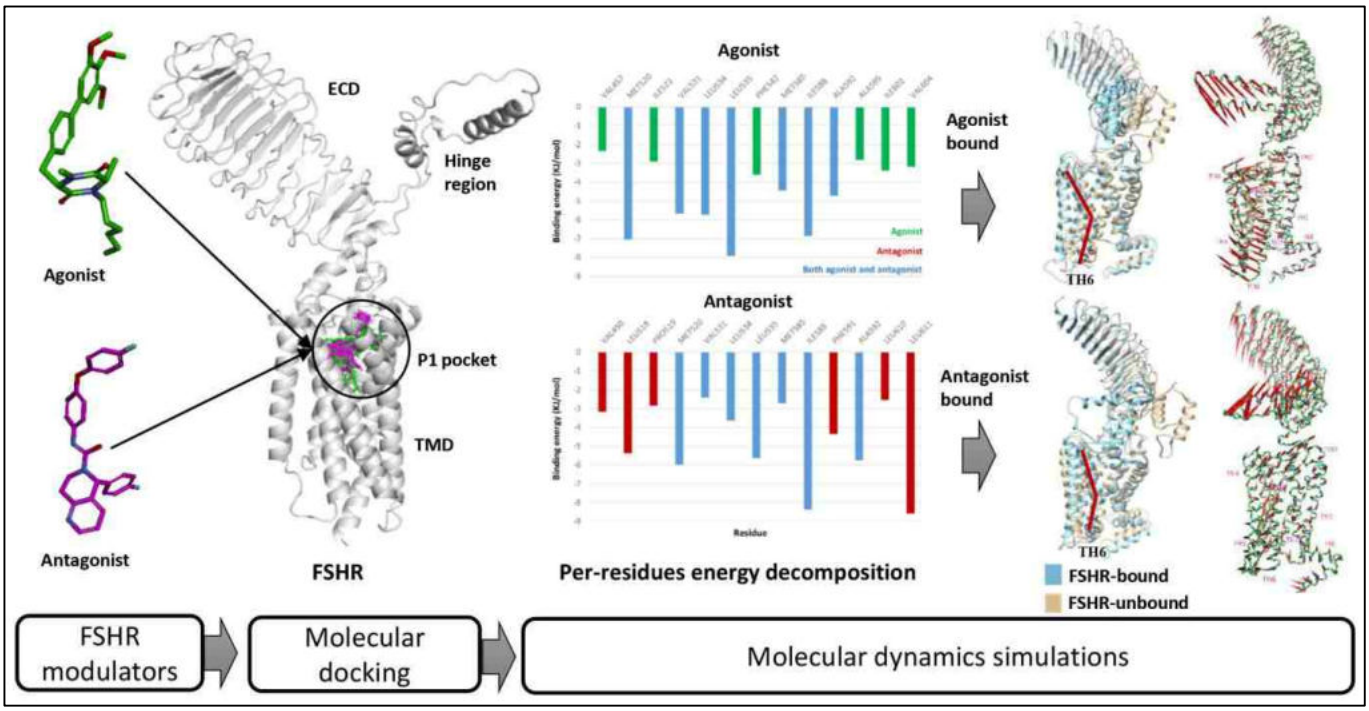


Figure 2: Computational workflow adopted for identification of modulator binding pockets, interacting residues and subsequent domain motions in FSHR

5.7 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**
Co-Principal Investigators	:	Taruna Madan, DK Das
Project Associates	:	Aishwarya Rao, Uma Shinde, Tejashree Sontakke
Collaborators	:	N Mayadeo, Seth GS Medical College & KEM Hospital, Mumbai Vandana Bansal, Geeta Balsarkar, Nowrosjee Wadia Maternity Hospital, Mumbai
Duration	:	2020-2023

Preeclampsia (PE) is a disorder of defective placental development. Early onset PE (EOPE) generally occurring by 34 weeks of gestation, is associated with higher feto-maternal morbidity and mortality. Early prediction of EOPE may aid in planning preventive interventions. Placental exosomes are small vesicles containing proteins and nucleic acids of trophoblast origin in circulation. They appear in the serum by 6 weeks of gestation. Their composition is altered in placental disorders. Due to inaccessibility of placental tissue, placental exosome-derived factors could be better predictors of EOPE. Comparative OMICS analysis of placental exosomes would highlight the potential of exosomes for early prediction of EOPE. Previously (Annual report 2021-2022, pp. 69-71), 39 EOPE cases were identified out of total 891 participants recruited. Isolated circulating placental exosomes depicted characteristic "exosomal"

features with spherical shape via transmission electron microscopic analysis and ~50-150 nm size range via NTA analysis. Preliminary findings of miRNA transcriptomics revealed 15 dysregulated miRNAs (6 upregulated and 9 downregulated) in EOPE-derived circulating placental exosomes compared to controls. A total of 90 miRNAs were found to be present only in EOPE exosomes and 215 miRNA only in controls. Most of these miRNAs were associated with metabolic, immune and inflammatory pathways, which are known to be dysregulated in preeclampsia. Till date, 45 EOPE cases have been identified out of total 1055 participants. Additionally, the proteome analysis of EOPE-derived circulating placental exosomes at term stage of pregnancy identified total 208 proteins, out of which 26 were differential compared to healthy controls. Gene Ontology enrichment analysis of the total identified proteins revealed significantly enriched biological processes such as complement and coagulation cascade, hemostasis, lipid transport and metabolism along with involvement of innate immune system (Fig. 1). These processes are known to be associated with EOPE. Further, Genome-wide DNA methylation analysis of DNA derived from circulating exosomes at term revealed 154 differentially methylated CpGs (DMCs). Out of these 154, 23 DMCs were hypermethylated and 131 were hypomethylated. Preliminary analysis showed that most of these DMCs belong to mitochondrial genome.

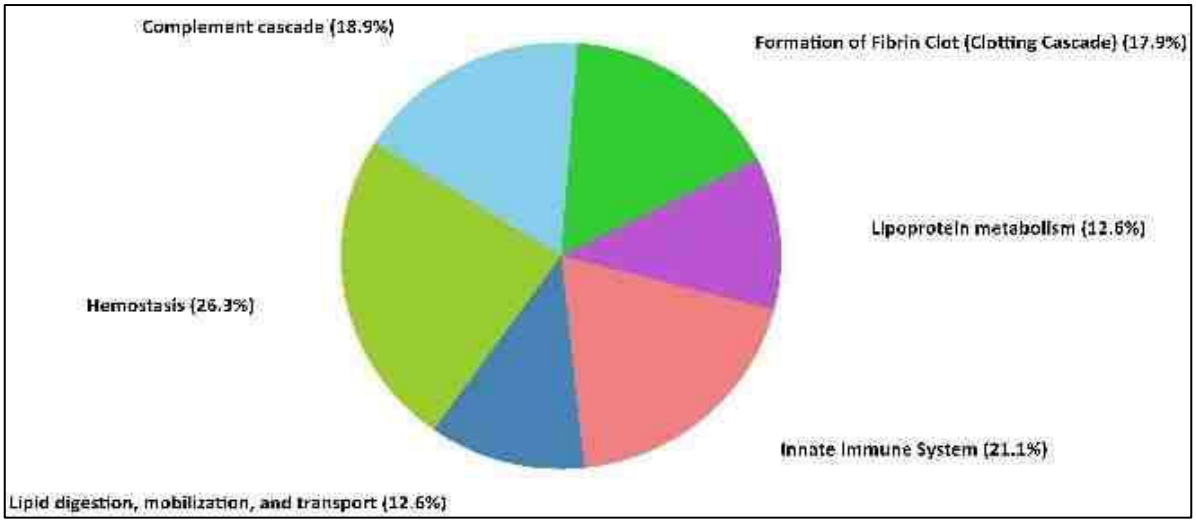


Figure 1: Gene ontology enrichment analysis for proteins identified in EOPE- derived placental exosomes

5.8 Aberrant Paternal Imprinting: a Risk Factor for Preeclampsia *(Partly Funded by Lady Tata Memorial Trust)*

Principal Investigator	:	**Nafisa H Balasinor**
Co-Prinicipal 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 pregnancy-specific disorder with the symptoms of hypertension and proteinuria. In the absence of proteinuria, it is detected by elevated liver enzyme levels and end-organ damage. It occurs in about 2-18% pregnancies in the developing world. The major underlying cause of preeclampsia is shallow placentation due to inadequate trophoblast invasion and incomplete spiral artery remodelling. The paternal genome plays an important role in the development of the placenta. Experiments in mice showing the non-equivalence of parental genomes in terms of contribution towards embryonic and placental development unravelled the phenomenon of genomic imprinting. Imprinted genes are those genes, which are expressed in a parent-of-origin specific manner due to differential methylation of DNA. Imprinted genes play important roles in placental growth and development. Several imprinted genes have been found to show aberrant methylation and expression in the placentae of preeclamptic women. However, the association of sperm epigenetic status with preeclampsia has not been investigated till date. Hence, the objectives of the present study were to measure the global methylation levels in the spermatozoa of partners of women experiencing preeclampsia; to measure the methylation levels of imprinted genes and the imprinted chromosome 19 miRNA cluster (C19MC) in the spermatozoa and; to quantify the expression levels of the imprinted genes and the C19MC miRNAs in the placental villi.

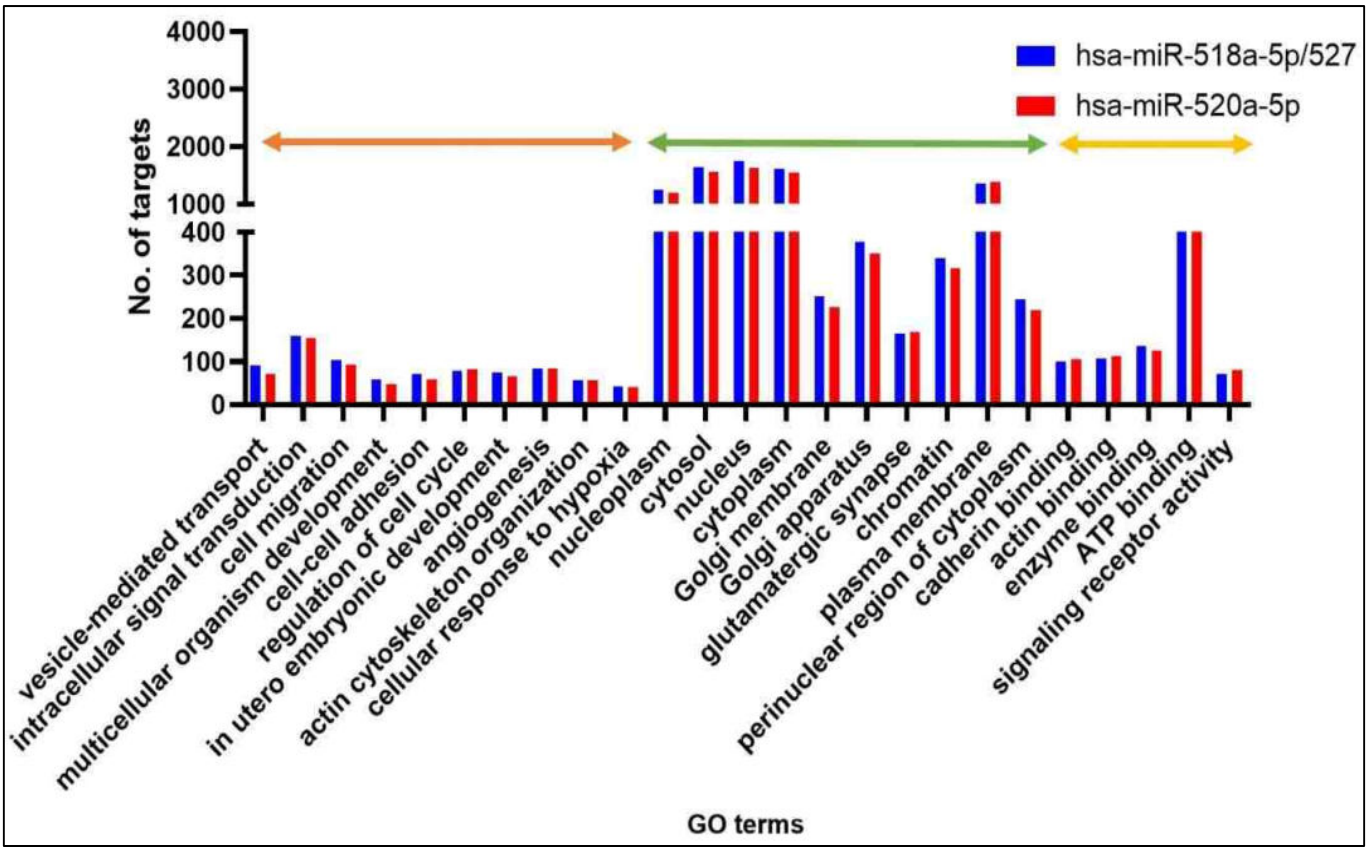


Figure 1: Significantly enriched gene ontology terms of targets of hsa-miR-518a-5p/527 and hsa-miR-520a-5p (p value ≤0.05). Orange, green and yellow arrows depict biological process, cellular component and molecular function, respectively.

Participants were recruited into two groups: Case group, comprising couples in which the female partner is experiencing early-onset preeclampsia (preeclampsia onset at ≤ 34 weeks of gestation) and control group, consisting of couples in which the female partners have normal pregnancy with no medical issues. The number of couples recruited till date, are 25 and 14 in the control and case group, respectively. The sperm concentration, motility, morphology and chromatin compaction were not significantly different between the two groups. The gestational age, placental weight and birth weight were significantly lower while the systolic and diastolic blood pressure values were significantly higher in the case group. This group also had four women whose babies died in utero and three women whose babies suffered from intra-uterine growth restriction. Methylation analysis of differentially methylated regions (DMRs) of imprinted genes was carried out by pyrosequencing (Annual report 2021-2022, pp. 68-69). In the case group, a significant upregulation in the expression of DLK1, PHLDA2, CDKN1C, PEG3 and IGF2 was observed in the placental villi. As revealed by small RNA transcriptomic study, all three differentially expressed miRNAs (DEMs) namely, hsa-miR-520a-5p, hsa-miR-518a-5p and hsa-miR-527 from C19MC were upregulated in the placental villi of cases. Their mRNA targets were enriched for biological processes such as transport, signal transduction, migration, adhesion and molecular functions such as cadherin binding, enzyme binding and signalling receptor activity (Fig. 1).Analysis using KEGG database revealed the pathways associated with some of the important target genes (Table 1). Validation of the DEMs and their targets by qPCR is underway. The study will help in understanding whether sperm DNA methylation is associated with preeclampsia.

Table 1: Associated pathways of targets of hsa-miR-518a-5p/527 and hsa-miR-520a-5p. *hsa-miR-527 shares the same targets as hsa-miR-518a-5p

DEM	Target	Pathway
hsa-miR-518a-5p/527*	*CALD1*	Vascular smooth muscle contraction
	DAB2IP	Apoptosis, TNF signaling pathway
	AGTR1	Vascular smooth muscle contraction, Renin-angiotensin system
	HGF	MAPK signaling pathway
	ACAA2	Fatty acid metabolism
hsa-miR-520a-5p	*ITGA4*	PI3K-Akt signaling pathway, Focal adhesion, ECM-receptor interaction
	CADM3	Cell adhesion molecules
	DAGLA	Retrograde endocannabinoid signaling, Aldosterone synthesis and secretion
common between hsa-miR-518a-5p and hsa-miR-520a-5p	*LIMD1*	Hippo signaling pathway
	VCAM1	NF-kappa B signaling pathway, Cell adhesion molecules
	CDH5	Cell adhesion molecules, Leukocyte transendothelial migration
	CDH1	Hippo signaling pathway, Cell adhesion molecules
	PTGS2	VEGF signaling pathway
	RXRA	PPAR signaling pathway, PI3K-Akt signaling pathway
	CUL3	Hedgehog signaling pathway
	CNR1	Retrograde endocannabinoid signaling
	MGLL	Retrograde endocannabinoid signaling
	EGLN3	HIF-1 signaling pathway

5.9 Developing an Immunochromatography Based Strip Test for Analysing P_LGF Concentration for Prediction of Risk for Developing Preeclampsia

Principal Investigator	:	**Bhakti R Pathak**
Project Associates	:	Ananya Breed, Shahina Begum
Collaborators	:	K R Damania, A Pawar, Nowrosjee Wadia Maternity Hospital, Mumbai
Duration	:	2017-2024

Placental Growth Factor (P_LGF) is a proangiogenic marker. Imbalance of pro and anti-angiogenic factors leads to complications during pregnancy like preeclampsia (PE) and fetal growth restriction (FGR). Low levels of P_LGF in circulation have been shown to predict the onset of PE by multiple studies. Detection of markers in the urine is a non-invasive method and hence is preferable. Urinary and circulatory P_LGF levels in pregnant women are reported to be comparable. P_LGF levels rise and reach maximum at 28-30 weeks of gestation and decline thereafter. Our preliminary data comparing urinary P_LGF in healthy pregnant women (n=21) and in PE cases (n=11), showed lower levels in PE as expected (Annual report 2019-2020, p. 78). Further, amongst the healthy pregnant (HP) women, those who had lower urinary P_LGF delivered low birth weight babies.

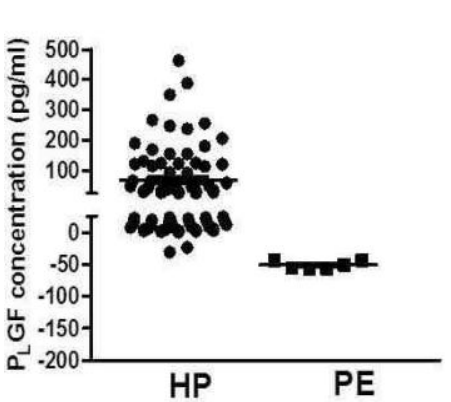


Figure 1: Comparison of P_LGF levels in HP and PE cases at early (28-32 weeks) third trimester

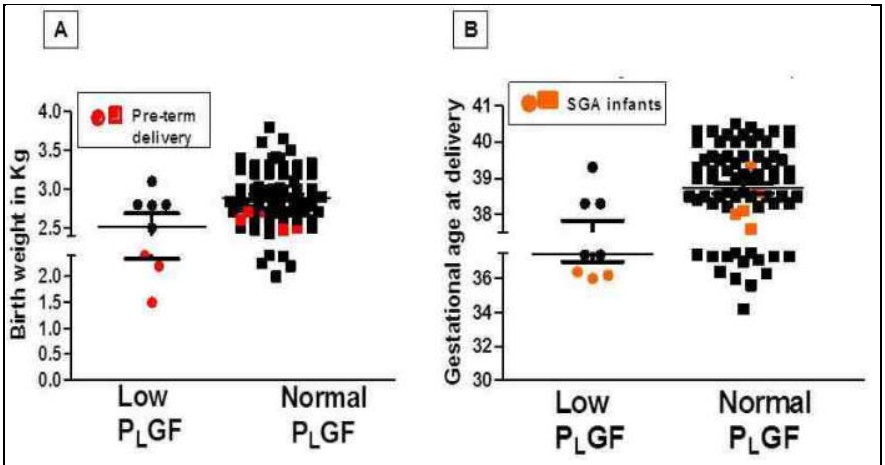


Figure 2: Association of P_LGF levels at 10th percentile with (A) birth weight of babies and (B) gestational age at the time of delivery

During the reporting period, more participants were recruited. Random urine samples were collected from 81 HP and 6 women with PE in early third trimester (28-32 weeks of gestation). Urinary P_LGF levels were determined by ELISA. It was observed that urinary levels of P_LGF in HP women ranged from 0-388 pg/ml as reported for the circulatory levels of P_LGF at gestational age of 28 to 32 weeks. Recent studies have reported lower circulatory P_LGF levels in women who delivered small for gestational age (SGA) infants. Our results showed that there is a difference in P_LGF levels of HP and PE cases (Fig. 1) and they matched with the published reports. Therefore, in order to evaluate the association of urinary P_LGF levels on pregnancy outcomes, a cut off $<10^{th}$ percentile was applied. In low P_LGF group ($<10^{th}$ percentile: 3.242 pg/ml), 3 out of 8 women (37.5%) delivered pre-term (<37

week) and all 3 delivered SGA infants whereas in normal P_LGF group, 5 out of 73 women (6.84%) delivered pre-term but their baby birth weight was appropriate. There are 5 SGA infants in normal P_LGF group but these women delivered full term. Our study indicates that low urinary P_LGF levels are associated with pre-term delivery and SGA infants (Fig. 2). Infants with weight below 10th percentile of our study population were considered as SGA infant. Our data strongly suggest low urinary P_LGF in early third trimester to be indicative of preterm delivery with SGA infant and warrants further studies with larger sample size.

5.10 Evaluation of Synergistic Impact of Nano-curcumin and Alpha-Linolenic Acid on Pathophysiology of Pre-eclampsia *(Partly Funded by Indian Council of Medical Research)*

Principal Investigator	:	**V Dighe**
Co-Principal Investigator	:	T Madan
Project Associates	:	A Tiwari, Shruti Desai, 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 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 have been investigated individually for their effects on pre-eclampsia. The present study aims to investigate the synergistic impact of ALA and Nanocurcumin (NC) in a Lipopolysaccharide (LPS) induced Preeclamptic rat model as well as in normotensive pregnant wistar rats. In the reporting year, reproductive toxicity of Curcumin, NC, ALA, and a combination of NC plus ALA was assessed in female Wistar rats. Five groups (n=12) of rats were inducted as follows:- Group-I: vehicle control (0.5% CMC); Group-II: Curcumin (400 mg/kg BW) treated; Group-III: NC (400 mg/kg BW) treated; Group-IV: ALA (150 mg/kg BW) treated; Group-V: ALA (150 mg/kg BW) with NC (400 mg/kg BW). Doses were given from 14 days preconception and continued till Post-Natal Day (PND) 13. Half of the animals from each group (n=6) were sacrificed at GD20 to assess pre- and post-implantation loss (PIL and POL). There was no significant PIL and POL observed in the treatment groups compared to the control. There were no treatment-related changes observed in vital organs. The remaining half of the pregnant rats (n=6) were allowed to deliver normally. Pups' parameters for weight and anogenital distance (AGD) was noted on day 0, 4, and 13 Vaginal Opening (VO) and Testicular Descent (TD) day of F1 pups were also noted. All the pup parameters were found to be normal in the treated groups compared to the control. Pups were further followed up till post-natal day 75 for sexual maturation and F1 offsprings were sacrificed on PND30, PND45, and PND75 to assess any visceral abnormality and blood parameters. No visceral abnormality was found in any group and also blood parameters were found to be within range compared to the control. To ascertain the transgenerational effect, six males and females of the F1 generation were mated with naïve females and males respectively to assess fertility parameters. No significant change was found in fertility parameters amongst the study groups. Our preliminary results demonstrate that Curcumin, NC, ALA and ALA+NC do not cause an adverse effect in pregnancy and on the reproduction and development of F1 and F2 generation. This advocates their safety for use as a

nutritional supplement in pregnancy and lactation without adverse outcomes in reproductive functions and the development of F1 and F2 generation.

Table 1: Copulation Index of F0 female rats, *Values are expressed as Mean ± SD. Statistical analysis was carried out by one-way ANOVA followed by a student's t-test.

	Vehicle Control	Curcumin	Nano-curcumin (NC)	Alpha-Linolenic Acid(ALA)	ALA+NC
No. of mated rats (n)	12	12	12	12	12
No. of pregnant rats (n)	11	11	12	12	12
Mating index (%)	91.66	91.66	100	100	100
Body weight gain (grams)	84.000±18.037	71.777±18.532	75.555±17.770	72.285±13.732	70±18.154
Gravid uterus weight (grams)	64.118±14.599	65.087±16.765	63.094±14.213	71.056±14.386	57.800±14.237
Rate of visceral abnormality (%)	0	0	0	0	0
Rate of Abortion (%)	0	0	0	0	0
Corpora lutea/ dam (n)	14.500±0.577	14.200±1.923	13.250±0.957	13.000±1.000	13.000±1.826
Implantation sites/ dam, (n)	13.750±0.957	13.400±2.702	12.500±0.577	12.000±1.414	12.250±1.500
Pre implantation loss (%)	**3.571±1.123**	**6.300±1.882**	**2.857±0.912**	**3.846±0.692**	**5.535±0.756**
Resorbed foetuses/ dam, (n)	0	0	0	0	0
Dead foetuses/dam, (n)	0	0	0	0	0
Post implantation loss (%)	**0**	**0**	**0**	**0**	**0**
Live foetuses/dam, n	13.750±0.957	13.400±2.702	12.500±0.577	12.000±1.414	12.250±1.500
Placental weight (grams)	7.246±0.685	6.628±1.688	6.442±0.451	6.158±0.669	6.383±0.455

5.11 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 Associates	:	Apoorva S Pawar, Antara Banerjee, D Modi, Ananya Breed, Meghali Borkortoky
Collaborators	:	Pooja Bandekar, Nowrosjee Wadia Maternity Hospital, Mumbai
Duration	:	2021-2026

Trop1 (commonly referred to as EpCAM- Epithelial Cell Adhesion Molecule) and Trop2 (Trophoblast Protein 2), initially identified as trophoblast cell surface antigens, were later studied for their role in cancer cell proliferation and invasion. However, their placental expression pattern across gestation and specific role in placental physiology remain largely unknown. EpCAM and Trop2, are known to undergo proteolytic processing by a membrane serine protease, matriptase. The regulated activity of matriptase towards its targets is essential for normal mouse placental development. The present study aims to determine the expression and proteolytic processing of Trop1 and Trop2 in a developing rat placenta in normal and pathological placental conditions. We previously reported differential mRNA expression levels of EpCAM, Trop2 and matriptase in the rat placenta across gestation (Annual report 2021-2022, pp. 78-79). Further, in the reporting year, we aimed to evaluate their expression patterns in the morphologically and functionally distinct rat placental zones at late gestation (GD 19.5). For the zone-wise collection of rat placental tissue, microdissection procedure reported for the mouse placenta at late gestation by Qu *et al.* (2014) was followed. The total proteins isolated from these tissues were tested by western blotting. EpCAM was present in all three zones, however, Trop2, and matriptase

were predominantly expressed in the labyrinth zone of the GD19.5 rat placenta (Fig. 1). Proteolytically cleaved forms of EpCAM (34kDa) and Trop2 (43kDa) were predominantly present in the labyrinth zone where there is a maximal expression of matriptase suggesting that the regulation of these molecules by matriptase is required in the labyrinth zone. Expression of known trophoblast-specific markers cytokeratin-7 and E- cadherin was seen in both the fetal compartments- labyrinth and the junctional zone of rat placenta which composed of different trophoblast subtypes. Vimentin (a known stromal marker) was found to be predominantly expressed in decidua zone of the placenta as depicted in Fig. 1. Further, we also attempted to evaluate the effect of oxidative stress on the expression patterns of EpCAM, Trop2 and matriptase. For this, rat placental tissue explants (GD 15.5) were treated with 3mM H_2O_2 for 24hrs. The expression of EpCAM, Trop2, as well as matriptase, was found to be reduced upon H_2O_2 treatment (Fig. 2). Whereas, expression of cytokeratin-7 and vimentin was unaltered under stress conditions suggesting that the levels of EpCAM, Trop2, and matriptase are regulated by oxidative stress. The results also hint towards the possible contribution of these proteins to placental pathologies like pre-eclampsia.

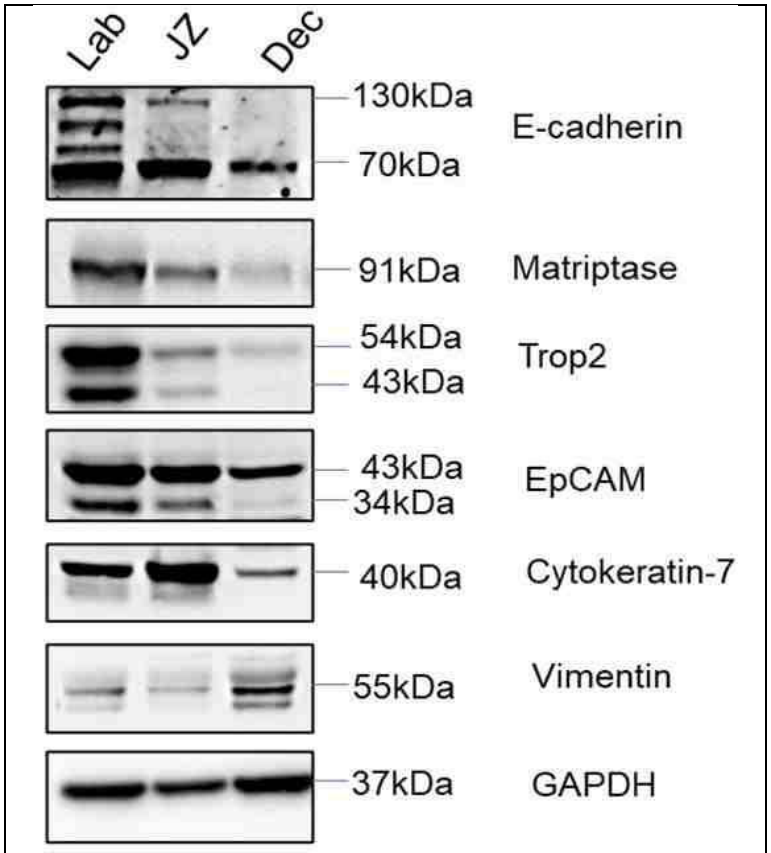


Figure 1: Expression of the proteins of interest in the cell lysates of the dissected zones of rat placenta at late gestation (GD19.5). Molecular weight of the indicated proteins is shown on the right. The expression of GAPDH in the three different zones serves as the loading control (Lab- Labyrinth, JZ- Junctional Zone, Dec- Decidua)

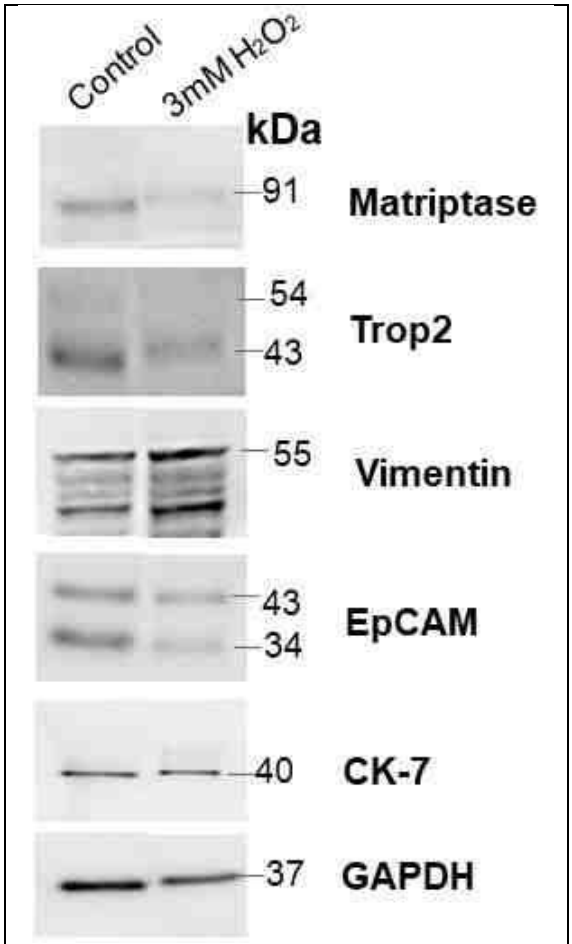


Figure 2: Detection of the proteins of interest in rat placental explants (GD15.5) treated with or without 3mM H_2O_2.

5.12 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 Balasinor, Anushree Patil, Deepti Tandon
Collaborators	:	Vandana Bansal, Nowrosjee Wadia Maternity Hospital, Mumbai Padmaja Samant, KEM Hospital and GS Medical College, Mumbai
Duration	:	2018-2023

The condition of loss of two or more consecutive clinical pregnancies before the 20th week of gestation is termed as Recurrent Pregnancy Loss (RPL). Several maternal factors are known to contribute to the pathology, yet 50% cases remain idiopathic (iRPL). This study aims to explore the possible association with paternal exposure to endocrine disruptors and sperm epigenetic modifications in male partners of iRPL couples. This is a case-control study, including apparently healthy fertile couples and couples experiencing first trimester iRPL. A total of 50 fertile couples and 63 iRPL couples have been recruited in the study. Higher prevalence of sperm DNA fragmentation and lower sperm 5-methylcytosine (5-mC) levels have been previously reported in men whose partners are experiencing iRPL. This highlights the importance of the male gamete in maintenance of a successful pregnancy. In the reporting year, sperm DNA fragmentation and 5-mC were correlated with semen parameters and steroid hormone levels in male partners of fertile and iRPL couples. Semen and blood samples were collected from 36 from fertile control and 45 from iRPL case men.

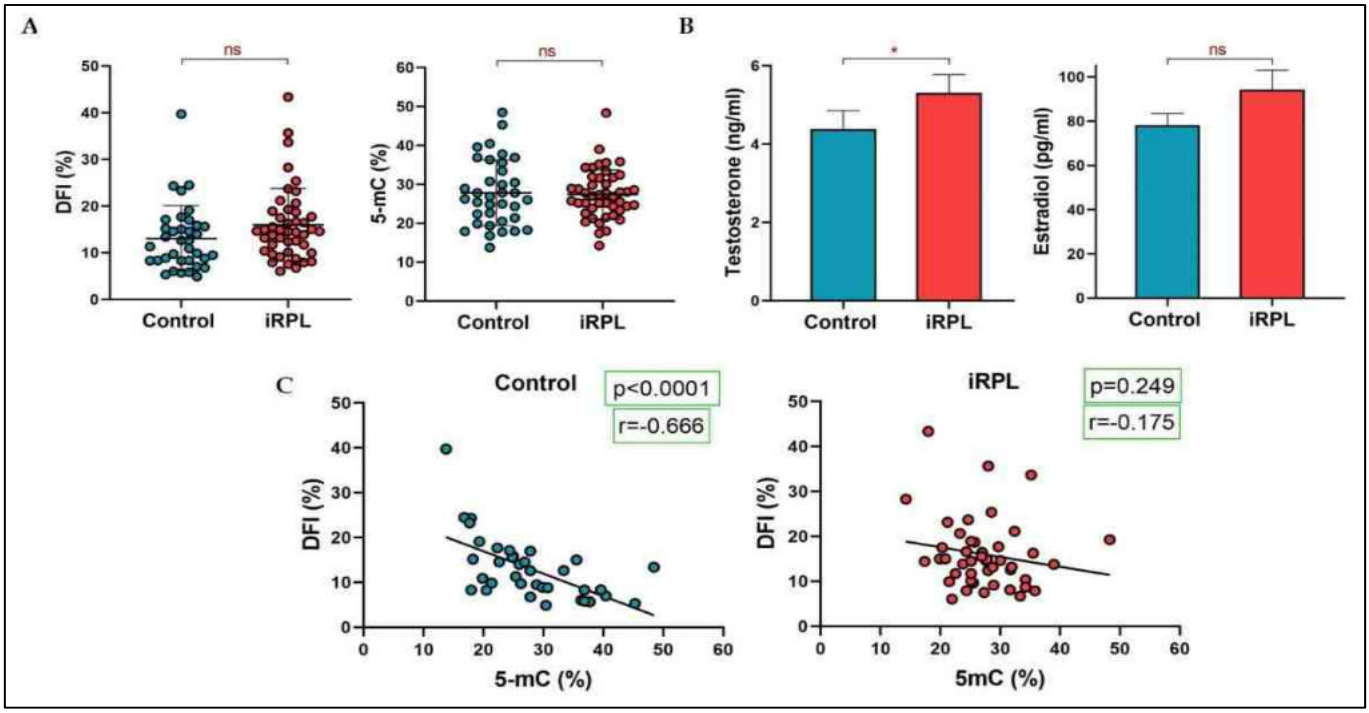


Figure 1: (A) Percentage of DNA fragmentation Index (DFI) and genomic 5′methyl cytosine (5-mC); (B) Serum levels of Testosterone and Estradiol; (C) Correlation between sperm 5-mC (%) and DFI (%) of Control and iRPL groups. Individual data points and linear regression line are shown. Statistical significance: $p \leq 0.05$.

Correlation analysis was done by calculating two-tailed non-parametric Spearman's co-efficient. Incidence of sperm DNA fragmentation was higher, though non-significant in the iRPL group. No differences in the sperm 5-mC values were noted (Fig. 1A). Significantly higher serum testosterone levels were noted in the iRPL group. No significant difference in the serum estradiol levels was observed (Fig. 1B). A strong significant negative correlation of sperm DFI and 5-mC in the control group but no significant correlation was observed was observed in the iRPL group (Figure 1C). No significant correlations of semen parameters with sperm DFI and 5-mC were noted in both groups. A non-significant negative correlation of serum testosterone levels and sperm DFI was observed in both groups (Fig. 2A). Serum estradiol showed no correlation in with sperm DFI (Fig. 2B). No correlation patterns were noted between serum testosterone and estradiol with sperm 5-mC (Fig. 2C&D). With lower 5-mC levels in sperm genome, there is a higher incidence of sperm DFI in fertile men. However, this trend is not significant in men of iRPL group and this could be due to other underlying DNA methylation alterations in genomic regions probably unsusceptible to fragmentation. Serum testosterone may have a protective function in lowering chances of sperm DFI.

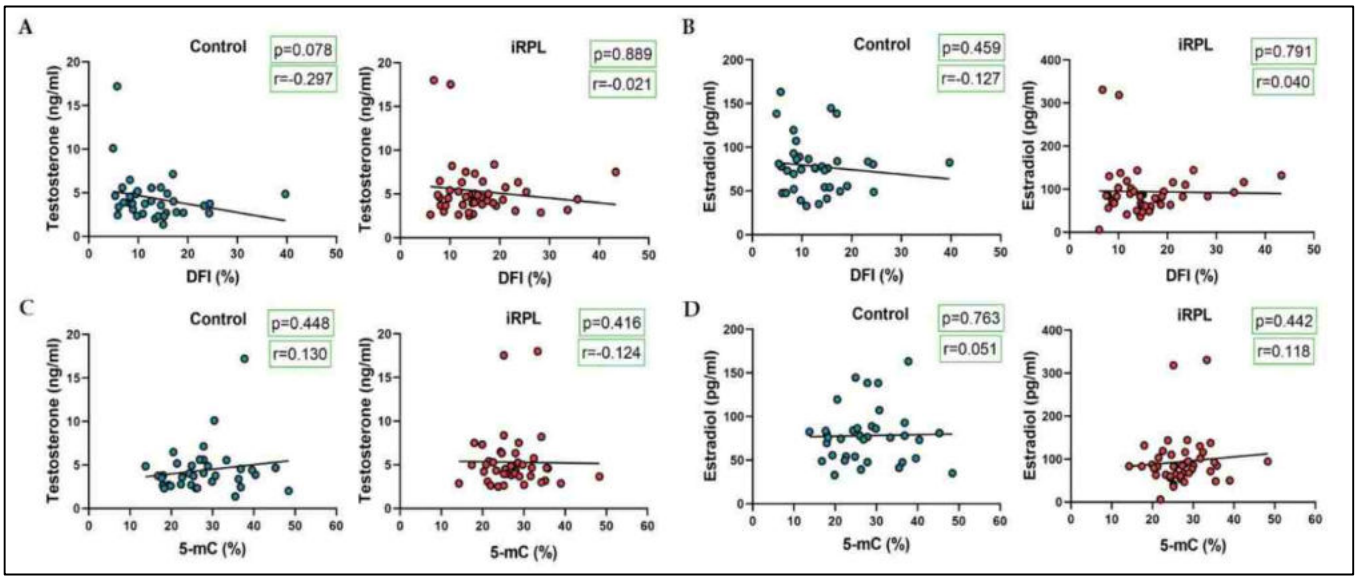


Figure 2: Correlation between DFI (%) and (A) Serum Testosterone, (B) Serum Estradiol; Correlation between 5-mC (%) and (C) Serum Testosterone, (D) Serum Estradiol of control and iRPL groups. Individual data points and linear regression line are shown. Statistical significance: $p \leq 0.05$.

5.13 Delineating Immune Correlates of HCMV Congenital Transmission *(Partly Funded by Department of Biotechnology and India Alliance Department of Biotechnology Wellcome Trust)*

Principal Investigator	:	**V Patel**
Co-Principal Investigator	:	V Bhor
Project Associates	:	Halrsha Palav, Gauri Bhonde, Varsha Padwal, Shilpa Velhal, Jacinta Pereira, AK Singh, S Ghosh, Kalyani Karandikar
Collaborator	:	Purnima Satoskar, Nowrosjee Wadia Maternity Hospital, Mumbai
Duration	:	2020-2025

Human Cytomegalovirus (HCMV) infection is associated with bad obstetric history (BOH) and adverse pregnancy outcomes (APO). Here, we characterized antiviral humoral profiles, systemic and virus specific cellular immune responses concurrently in pregnant women (n=67) with complications including BOH and associated these signatures with pregnancy outcomes. Infection status was determined using nested blood PCR, seropositivity and IgG avidity by ELISA. Systemic and HCMV specific (pp65) cellular immune responses were evaluated by flow cytometry.

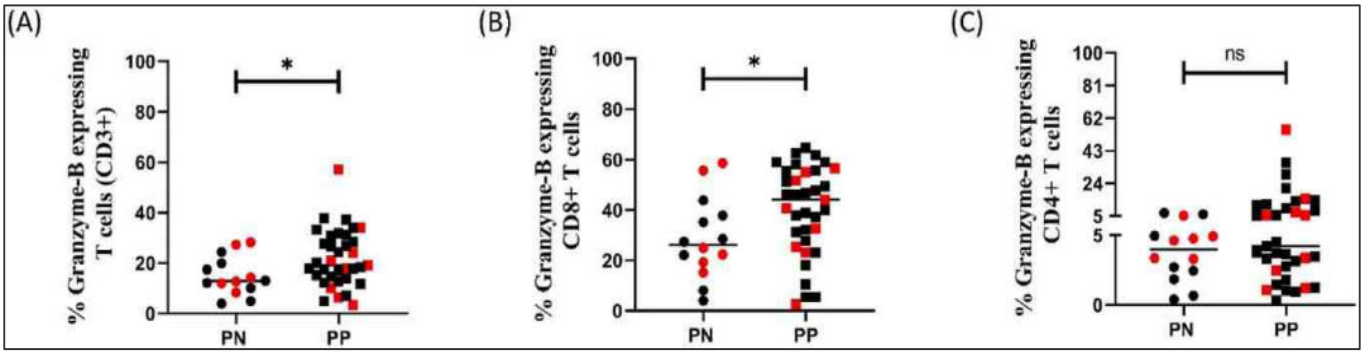


Figure 1: Increased cytotoxic potential associated with HCMV bloodPCR positivity: Cytotoxic potential in HCMV PCR Negative (n= 4) and HCMV PCR positive (n=35) pregnant women with BOH and PC. (A) % Granzyme-B expressing T cells (CD3+) (B) % Granzyme-B expressing CD8+ T cells (C) % Granzyme-B expressing CD4+ T cells. Data points in red indicate samples for which intracellular cytokine staining assay was performed. Comparisons between groups were evaluated by non-parametric Mann–Whitney U test (*$p<0.05$). $p<0.05$ was considered significant.

Table 1: Parameters of pregnant women with BOH and PC. IgG avidity - L: low; I: intermediate; H: high. T: Toxoplasma, R: Rubella, C: HCMV, H: HSV 1&2. Comparisons between groups were evaluated by Fisher's exact test. $p<0.05$ was considered significant.

Pregnancy Outcome (n= 33)	Adverse Pregnancy outcome (n=14)	Normal outcome (n=19)	*p* value
HCMV Blood PCR Positive	14/14	10/19	0.0039
HCMV Blood PCR Negative	0/14	9/19	
HCMV IgG Negative	1	3	
L / I	4	6	>0.9999
H	9	10	
HCMV IgM Positive	5	1	0.0616
HCM IgM Negative	9	18	
TRCH IgG Positive	4	0	0.0245
TRCH Ig Negative	10	19	

Seropositivity was determined for other TORCH pathogens (n=33) on samples with recorded pregnancy outcomes. This approach was more sensitive in detecting HCMV infection. Blood PCR positive participants, irrespective of their IgG avidity status, had higher cytotoxic potential in circulating CD8+ T cells ($p<0.05$) suggesting that infection associated cellular dysfunction was uncoupled with avidity maturation of antiviral humoral responses. Also, impaired anamnestic degranulation of HCMV-pp65-specific T cells compared to HCMV blood PCR negative participants ($p<0.05$) was observed. APO correlated with HCMV blood PCR positivity but not serostatus ($p=0.0039$). Most HCMV IgM positive participants (5/6) were HCMV blood PCR positive with APO. None were

found to be IgM positive for other TORCH pathogens. Multiple TORCH seropositivity, however was significantly enriched in the APO group (p=0.024). Generation of HCMV specific high avidity IgG antibodies had no bearing on APO (p=0.9999). Our study highlights the utility of an integrated screening approach for antenatal HCMV infection in the context of BOH, where infection is associated with systemic and virus specific cellular immune dysfunction as well as APO.

5.14 Molecular Analysis of HLA-G in Pregnant Tribal Women and its Role in Infectious Etiologies Modulating Intrauterine Inflammation - A Prospective Cohort Study *(Partly Funded by Indian Council of Medical Research)*

Principal Investigator	:	**KC Itta**
Co Principal Investigator	:	VM Bhor
Project Associates	:	Anushree Patil, Clara Aranha, Ragini Kulkarni, SK Mishra
Collaborators	:	B Hengne, PN Dhodi, A Gadag, Smita Bari
Duration	:	2023-2026

The underlying mechanism of the differential role of HLA-G in the host, viz. protecting the fetus from the mother's immune response and a concurrently minimalizing effect on the mother's protective nature against pathogens, is still being explored. Identifying the genotype of HLA-G is crucial in understanding its dual effect in pregnant women. The study aims to determine the molecular analysis of HLA-G in pregnant tribal women (18-35 Years) and associated infections in a cohort of pregnant women and its influence on pregnancy outcomes. A total of 14 study participants were recruited (1st to 31st March 2023). Blood samples were processed for infectious aetiologies (viruses, parasites, and bacteria). Two samples were positive for Hepatitis C virus and one each for cytomegalovirus and toxoplasmosis. Analysis of soluble HLA-G levels (sHLA-G), and HLA-G genotyping of the samples are under investigation.

CHILD HEALTH RESEARCH

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 Department of Health Research)*

Principal Investigator	:	**S Pande**
Co-Principal Investigator	:	D Das
Project Associates	:	Shaini Joseph, V Bhanothu, Neha Minde, H Gawade, Shiny Babu, Seema Kadam
Collaborators	:	Vandana Bansal, Nowrosjee Wadia Maternity Hospital, Mumbai
Duration	:	2021-2024

This study aims to identify genetic mutations associated with fetal malformations in an Indian cohort, evaluate the efficacy of available genetic platforms and use the data generated for effective genetic diagnosis and possible management options. Anomalies detected in the fetus are a major reason for most couples opting for elective abortions. Most of the congenital anomalies detected in early gestation are known to have an underlying genetic etiology. Assessing Products of Conception (POC) for genetic anomalies both at the chromosomal and molecular level will aid in effective genetic counseling and management of these couples in future pregnancies. Forty-five POC samples were evaluated to delineate a genetic cause for observed fetal anomalies. Pathogenic genetic defects were identified in close to 30% of POCs. The interim results of this study suggest genetic defects as the major causes of fetal anomalies and advocate for a need to evaluate the POCs for genetic defects to enable appropriate counseling and management in future pregnancies. Almost 40 pre and post-test counseling sessions were arranged for study participants.

6.2 Identification and Evaluation of Novel Metabolites with the Potential of Prenatal Diagnosis of Fetal Congenital Heart Diseases (CHD) - A Pilot Study *(Partly Funded by Department of Science and Technology - Science and Engineering Research Board)*

Principal Investigator	:	**V Bhanothu**
Collaborators	:	Jayashree Mishra, Bai Jerbai Wadia Hospital for Children, Mumbai Vandana Bansal, Nowrosjee Wadia Maternity Hospital, Mumbai
Duration	:	2022-2025

This project aims to identify and assess the potential of novel metabolites in the early diagnosis of fetal congenital heart diseases. Blood and Urine samples were collected from 44 healthy pregnant women between 11 to 24 weeks of gestation and 4 pregnant women with fetal CHDs. As per our preliminary data, lower fetal heart rate, maternal pulse rate, and body weight in women with fetal CHD and higher gestational age, nasal bone length, nuchal translucency, and parental age compared to controls were noted. The samples have been sent for NMR analysis and LC-MS/MS analysis to IISER, Pune, and NCL, Pune respectively. The study may provide novel insights into the metabolomics of fetal CHD, as the Indian context varies with unique genetic makeup and geoethnic risk factors.

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	:	**Suchitra Surve**
Co-Principal Investigator	:	Ragini Kulkarni
Project Associates	:	Priyanka Gawai, U Pachalkar
Collaborators	:	S Bodade, D Suryawanshi, M Chavan, P Pagi
Duration	:	2020 – 2023

India has large number of newborns with birth defects with a prevalence of 61 to 69.9/ 1000 live births. However, there is a dearth of database on birth defects in India. Presently very few individual groups are conducting surveillance, most of which are hospital-based surveillance where participation is voluntary and depends upon the motivation of hospitals. With this background, present population-based birth defect surveillance has been initiated in November 2020 at MRHRU Dahanu in 7 rural blocks of Palghar district, Maharashta to estimate prevalence of birth defects i.e. live births and still births detected upto 6 weeks of age. This study is one of its kind to link 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. screening at public health facilities by MOs, ANMs and community-based newborn screening by ASHAs during *Home Based New Born Care* (HBNC) visits. The project staff visited all the public health facilities and screened records for birth defects quarterly.

Figure 1: Data collection at PHCs

Figure 2: Meeting at PHC and distribution of IEC Material

During reporting year, two rounds of field visits for data collection purpose were conducted between April 2022 to June 2022 and between October 2022 till December 2022 in 38 PHCs, 7 rural hospitals and 2 sub district hospitals. Third and final visit of data collection is ongoing since March 2023. Data were

collected from facilities and communities through ASHAs and verified through RBSK monthly reporting to avoid missing data. 138 birth defect cases were identified at facility level, 68 cases were identified through RBSK and 25 cases through ASHAs. After removal of duplicate cases, a total of 166 birth defect cases have been identified. Blockwise distribution of birth defects shows highest number of cases in Dahanu (38) and Jawhar (37) followed by Palghar (35), Vikramgadh (22), Wada (13), Talasari (11) and Mokhada (10). Among major structural defects, cases of CHDs (24) and club foot (24) were highest, followed by cleft lip and or palate (20), multiple birth defect with atleast one major congenital anomalies (15), spina bifida (8), hydrocephalous (7) imperforate anus (5), limb deformities (6), Down syndrome (1), ambiguous genitalia (2). Out of 156 live births, 126 cases were referred for further management/ treatment and 14 of the 126 cases were surgically operated.

Table 1: Frequency distribution of birth defects in rural blocks of Palghar district

Type of birth defects	April 2022 to March 2023	April 2021 to March 2023
Congenital heart diseases (CHDs)	24	54
Club Foot	24	53
Cleft lip and Palate	20	49
Miscellaneous (Umbilical /diaphragmatic hernia, siremonelia, Nail patella syndrome, etc)	11	28
Multiple birth defects (more than one defect, with atleast one major congential anomaly)	15	25
Male genital deformities (hydrocele, hypospadias)	13	24
Hydrocephalous	7	19
Polydactyl and Syndactyl	10	19
Ear and eye deformity	12	17
Imperforate Anus	5	16
Neural tube defects	8	13
Upper and lower limb deformity	6	14
Colloidan skin	4	11
Down syndrome	1	7
Omphalocele and Gastrochisis	4	6
Ambiguous genetalia	2	6
Total	**166**	**361**

6.4 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

(Partly Funded by Indian Council of Medical Research)

Study Coordinating Center: ICMR-National Institute of Immunohaematology

Principal Investigator : **Suchitra Surve**
Co-Principal Investigator : Ragini Kulkarni
Project Associates : Yugali V Kore, Aarati Patil, Jidnyasa Kore, Shweta Dube, A Gawad, S Solanki
Collaborators : S Bodade, Palghar District
D Suryavanshi, District Health Officer

B Hengne, SDH Dahanu
P Dhodi, SDH Kasa

Duration : 2019 - 2024

The current study is being undertaken to establish a newborn screening program for Sickle cell disease in tribal populations of India for early detection, to understand the magnitude of the problem and to understand the barriers for undertaking such programme and to measure the benefit of early comprehensive care of affected babies. The study also aims to evaluate the genotypic and phenotypic correlation to understand role of genetic modifiers for disease severity. The study being implemented at Dahanu block of Palghar District (Subdistrict Hospital Dahanu (SDH) and Kasa, PHCs) through Model Research Health Unit (MRHRU), Dahanu is part of the multi centric study, implemented through 7 centers across the country [Maharashtra (2 centers), Gujrat, Tamil Nadu, Odisha, Madhya Pradesh and Rajasthan]. 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. Enrollment of participants and newborn sample collection commenced from December 2, 2020 at SDH Dahanu. New-borns who were suspicious of sickle cell trait and disease were to be confirmed after 6 weeks at MRHRU Dahanu. Awareness camp about Sickle cell disease was conducted for ASHA workers at Ganjad PHC in December 2022. ASHA workers were explained briefly about Sickle cell disease, its presentation in tribal population, clinical symptoms, importance of investigations and timely treatment. During the reporting period, 2145 newborns were screened for sickle cell Disease.

Table 1 depicts pattern of haemoglobinopathies amongst them. Fifty babies had haemoglobinopathies of undermined significance and 49 babies had undetermined result (High P3=32, Ao>F=14). High P3 are due to older samples i.e. degenerated haemoglobin. In newborns, unclear correlation with Ao>F could be due to heel prick and potential maternal contamination in cord blood, contributing to variability. Since the initiation of the project, 6275 new-borns are screened for sickle cell disease. A total of 33 babies were sickle cell homozygous (Sickle Cell Disease), 532 babies were Sickle Cell Heterozygous (sickle cell trait).

Table 1: Newborn screening data Dahanu and Kasa from April-2022 to March-2023

Total number of Deliveries	April 2022-March 2023	Total
Normal	2145	5383
HbS heterozygous	231	532
HbS homozygous	16	33
Other hemoglobinopathies (including undetermined result)	99	327
Total live births undergone newborn screening by HPLC	2491	6275
Other Hemoglobinopathies-50 (S window with raise A2=13 , Unknown window=26 , D window=02 , C window=03 , Raise A2=02, S window with unknown window=01, S window with C window=02, S window with Ao>F=1)		
Undetermined / unknown significance results with: 49 (High P3=32 , Technical Error = 03 , Ao>F = 14)		

The babies are being followed up at 6 weeks for confirmation of diagnosis as per protocol. Screening of family members including parents and siblings is being done at 6 weeks, in case of babies with trait or disease followed by genetic counselling. Sickle cell homozygous babies are being started on

prophylactic antibiotics and Folic acid from 3 months as per the standard of care and followed up to 3 years of life. Hydroxyurea is also started for babies at optimal age. Follow-up of babies is facilitated at SDH Dahanu in coordination with Indian Academy of Pediatrics and haematologists at Municipal Cooperation of Greater Mumbai.

Figure 1: Awareness of Anganwadi workers

Figure 2: PHC visit for follow-up of babies

6.5 Molecular Profiling of Common Clinical Phenotypes Associated with Congenital Hypothyroidism - a Pilot Study *(Partly Funded by Indian Council of Medical Research)*

Principal Investigator	:	**V Bhanothu**
Co-Principal Investigator	:	S Pande
Collaborators	:	Sudha Rao, Bai Jerbai Wadia Hospital for Children, Mumbai
Duration	:	2023-2026

This project focusses on identifying pathogenic genetic variants in children with thyroid dyshormonogenesis (TDH) presenting different clinical phenotypes. Blood samples from 15 index cases (Age: 0-12 yrs), their parents and also from 2 cases and their fathers were collected. 11 of the 17 children with TDH were females and 6 were males. The samples are being processed for whole exome sequencing. Exome sequencing for two samples has been carried out and an analysis in progress. This study may aid in developing a cost-effective molecular diagnostic panel, which will be of help to clinicians for the early detection of congenital hypothyroidism.

6.6 Mission Program on Paediatric Rare Genetic Disorders (Mumbai Chapter) *(Partly Funded by Department of Biotechnology)*

Principal Investigator	:	**S Pande**
Co-Principal Investigator	:	V Bhanothu
Project Associates	:	Shaini Joseph, DVS Sudhakar, Suchitra Surve, Neha Minde, H Gawde, Shiny Babu
Duration	:	2021-2026

This study aims to investigate the primary causes of pediatric rare genetic disorders and understand the genetic status of undiagnosed rare pediatric cases and their parents/siblings using Next Generation Sequencing (NGS) and functional assays. All approval (IEC and SRC) have been received and planning is in process for conducting awareness sessions in the community, schools, and colleges. Patient recruitment has been initiated. Identification of the genetic etiology of rare genetic disorders using a comprehensive genetic evaluation is undergoing.

6.7 Gut Microbiome-Immune Axis Influencing Pathology in HCMV Infected Infants with Neonatal Cholestasis

Principal Investigators	:	**V Bhor**, **V Patel**
Project Associates	:	Kalyani Karandikar, Gauri Bhonde, Harsha Palav, Varsha Padwal, Shilpa Velhal, Jacinta Pereira, Himali Meshram, A Goel
Collaborator	:	Ira Shah, Bai Jerbai Wadia Hospital for Children, Mumbai
Duration	:	2016-2023

Human cytomegalovirus (HCMV) is a leading cause of congenital infections worldwide. However, only a small subset of the infected infants exhibit sequelae such liver, central nervous system (CNS), hearing, and ophthalmologic defects. Therefore, there is a need to understand the underlying host factors that may influence both, the individual susceptibility to the infection as well as the progress of the infection. Gut microbiota, a non-genetic host factor, in particular, has been shown to influence the immunity to a spectrum of disorders including several viral infections. While the role of gut dysbiosis in liver pathology of the pediatric population including neonatal/infantile cholestasis has been described, no study has concurrently explored the influence of the gut microbiome dysbiosis and active HCMV infection on disease severity in infants with neonatal cholestasis.

In view of this, the present study evaluated gut microbiome profiles by 16S rRNA deep sequencing and immune profiles by multi-parametric flow cytometry in a cohort of HCMV infected cholestatic infants (IgM positive, n=21; IgM negative, n=25) from BJ Wadia Hospital for Children and compared these profiles with those obtained from age matched healthy infants (n=10). HCMV infected IgM-positive individuals exhibited increased clinical severity in terms of liver dysfunction, altered $CD4^+$: $CD8^+$ ratio, and elevated Granzyme B levels in cellular immune subsets. Gut microbiome analysis revealed distinct and differential diversity and composition within infected groups aligned with clinical severity reflected through the increased abundance of Gammaproteobacteria, reduced Bifidobacteria, and a unique signature mapping to the HCMV infected IgM negative group (Fig. 1).

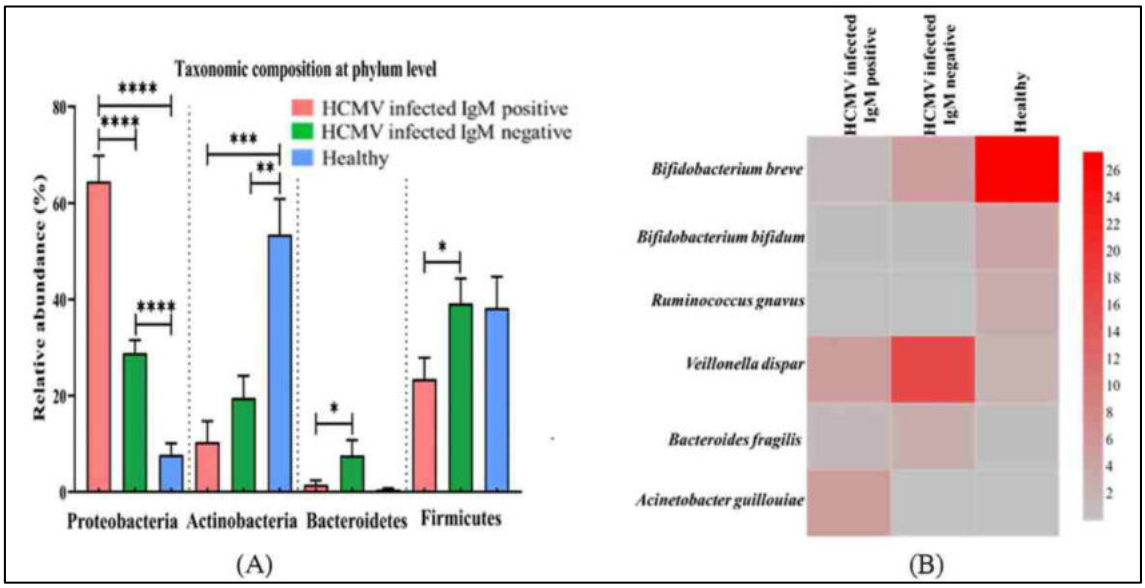


Figure 1: Taxonomic profile of HCMV-infected infants with neonatal cholestasis with healthy controls; (A) Relative abundance of the predominant phyla present in HCMV infected infants namely HCMV infected IgM-negative (n=15) and HCMV infected IgM-positive (n=15) with neonatal cholestasis compared to healthy controls (n=10). (B) Differential abundance analysis carried out by LefSe at the phylum level. Taxa above LDA score>3, p=0.1, FDR adjusted are depicted. Data represented as mean±SEM. Statistical significance was calculated by Mann-Whitney U-test; *, $p < 0.05$; **, $p < 0.01$, ***, $p<0.001$; and ****, $p<0.001$.

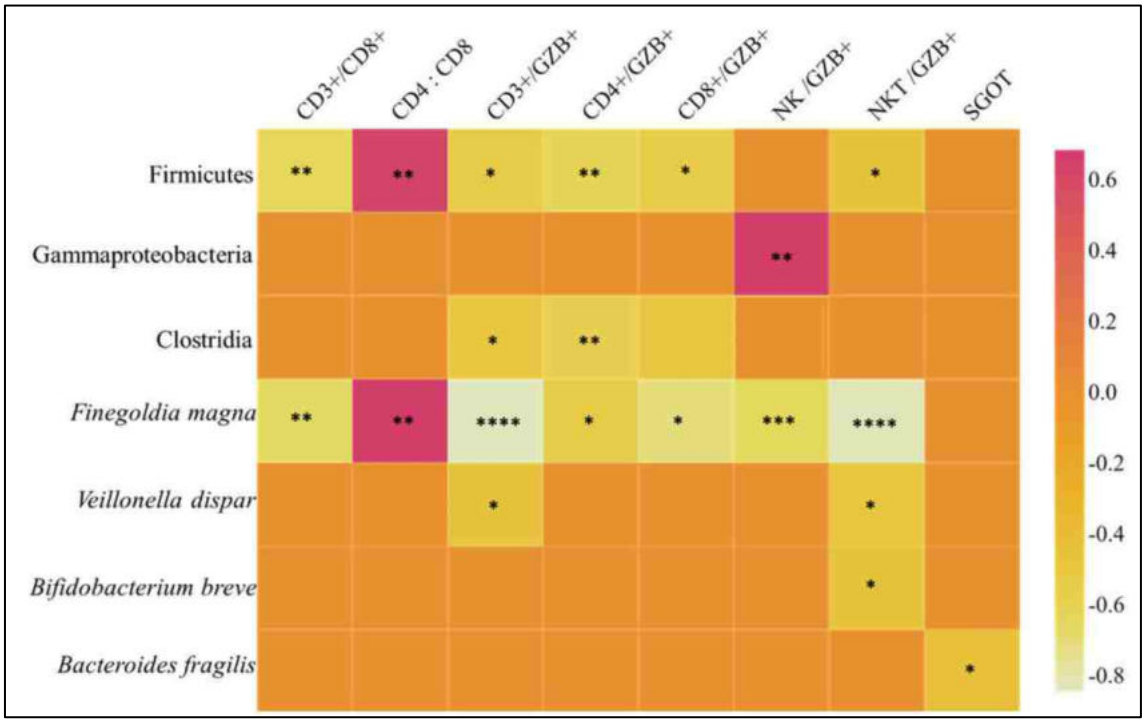


Figure 2: Microbiome-Immune axis- Correlation matrix of frequency of immune cells with the relative abundance of bacterial taxa in HCMV infected infants with cholestasis. The strength of the correlation is indicated from the shade of the colour. Only the significant correlations have been plotted. Statistical significance; *, $p < 0.05$; **, $p < 0.01$; ***, $p<0.001$; and ****, $p<0.001$.

The overrepresentation of Gammaproteobacteria in the IgM-positive group is suggestive of an increased inflammatory environment within these individuals, corroborated by the systemically elevated cytotoxic potential (Granzyme B positivity) observed across lymphoid subsets including $CD8^+$ T cells and NK cells. Correlation analyses revealed associations between *Bifidobacterium breve*, Gammaproteobacteria, Firmicutes, Clostridia, *Finegoldia magna, Veillonella dispar,* and Granzyme B expressing immune cell subsets (Fig 2). Thus, the results of the study strongly indicate the occurrence of a novel gut microbiome-immune axis, in the context of active HCMV infection that governs pathology in HCMV infected infants with neonatal cholestasis. The gut microbiome and cellular immune signatures identified herein could serve as additional putative therapeutic targets using probiotic and immunomodulatory strategies for the management of infants with HCMV infection associated neonatal cholestasis.

6.8 Community based Screening and Management of Latent TB among Under-Five Children from Urban Slums in Mumbai: Phase II – Screening of TB Contacts of Index Case in Age Group of 5-12 Years *(Funded by Department of Health Research)*

Principal Investigator	: **Suchitra Surve**
Co-Principal 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	: Mangala Gomare, MCGM, Mumbai
Duration	: 2019-2023

Children account for approximately 11% of all tuberculosis (TB) patients worldwide, with over 1.1 million children infected with TB each year. In India, an estimated 3.33 lakh children in the 0–14 year age group are affected with TB each year (28% of global childhood TB burden). By 2030, the WHO`s End TB Strategy seeks to reduce TB mortality by 90% and TB incidence rates by 80%. An essential pillar in this regard is the diagnosis and treatment of latent tuberculosis infection (LTBI). The National TB Elimination Programme (NTEP) does not presently include routine screening for LTBI. Few studies have demonstrated prevalence of 40-48% of LTBI with limited information in under-five age group. The present study is a community-based study in urban slums in collaboration with a tertiary hospital, aiming to screen under-five children (Phase I) and 5–12-year age group (Phase II) at risk for LTBI as per the WHO guidelines using Interferon Gamma Release Assay (IGRA-Quantiferon Gold Plus). The study also aims to assess adherence to treatment and progression of disease during follow up. Total 64 children have been recruited from March 2022 to April 2023 and thus the total number of children recruited (from phase I and II) are 433. For patients, total IGRA positivity was found to be 31.25% (n=20) whereas total TST positivity was found to be 40.62% (n=26). Children suspected for LTBI on basis of IGRA and TST were evaluated at BJ Wadia Hospital-TB Clinic and were ruled out for active tuberculosis by X ray Chest and Gene Xpert. Only one child had active tuberculosis infection. Children confirmed of having LTBI have been referred to health posts and started on INH prophylaxis as per the NTEP guidelines.

Table 1: Bifurcation of IGRA and / or TST positive patients

Bifurcation	August 2022 to April 2023 n= 64 (100%)	Total (Phase 1 + Phase 2) n=369+64=433 (100%)
IGRA Positive + TST Positive	17 (26.5%)	56 (12.9%)
IGRA Positive + TST Negative	3 (4.6%)	10 (2.3%)
IGRA Negative + TST Positive	8 (12.5%)	48 (11.0%)
IGRA Negative + TST Negative	36 (56.2%)	319 (7.3.66%)

6.9 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, Bai Jerbai Wadia Hospital for Children, Mumbai
Duration	:	2021-2026

Oxidative phosphorylation (OXPHOS) disorders, also known as mitochondrial disorders, are a heterogeneous group of disorders caused due to mutations in the mitochondrial DNA (mtDNA) or in nuclear genes encoding the mitochondrial subunits. The prevalence of mitochondrial disorders is difficult to establish, predominantly because of their clinical and genetic heterogeneity. Various phenotypic variations arise due to rearrangements and point mutations in the mtDNA. The prevalence of individual mutations is much higher, approaching 1 in 200 live births. Hence, OXPHOS diseases should also be ruled out during differential diagnosis of familial diseases. The objectives of the present study are to classify mitochondrial disorders into biochemical and histopathological phenotypes and further identify genetic variations in mitochondrial and nuclear genomes.

During the reporting year, 15 patients suspected with mitochondrial disorders were enrolled. Muscle samples were obtained from 4 patients (P2, P3, P10 and P15), due to various underlying clinical symptoms such as hypotonia. Estimation of biochemical enzyme activity in muscle tissue homogenates revealed that P2 and P3 were deficient for mitochondrial Complex II and Complex III with a residual enzyme activity of 6.1% and 7.7% respectively in P2 and 26.9% and 4.9% respectively in P3. Multi complex deficiency of Complex I and IV was observed in P10 with residual enzyme activity of 8.2% of Complex I and 4.3% of Complex IV. Similarly, Complex III (11.2%) and Complex IV (8.2%) were found to be deficient in P15 (Fig. 1).

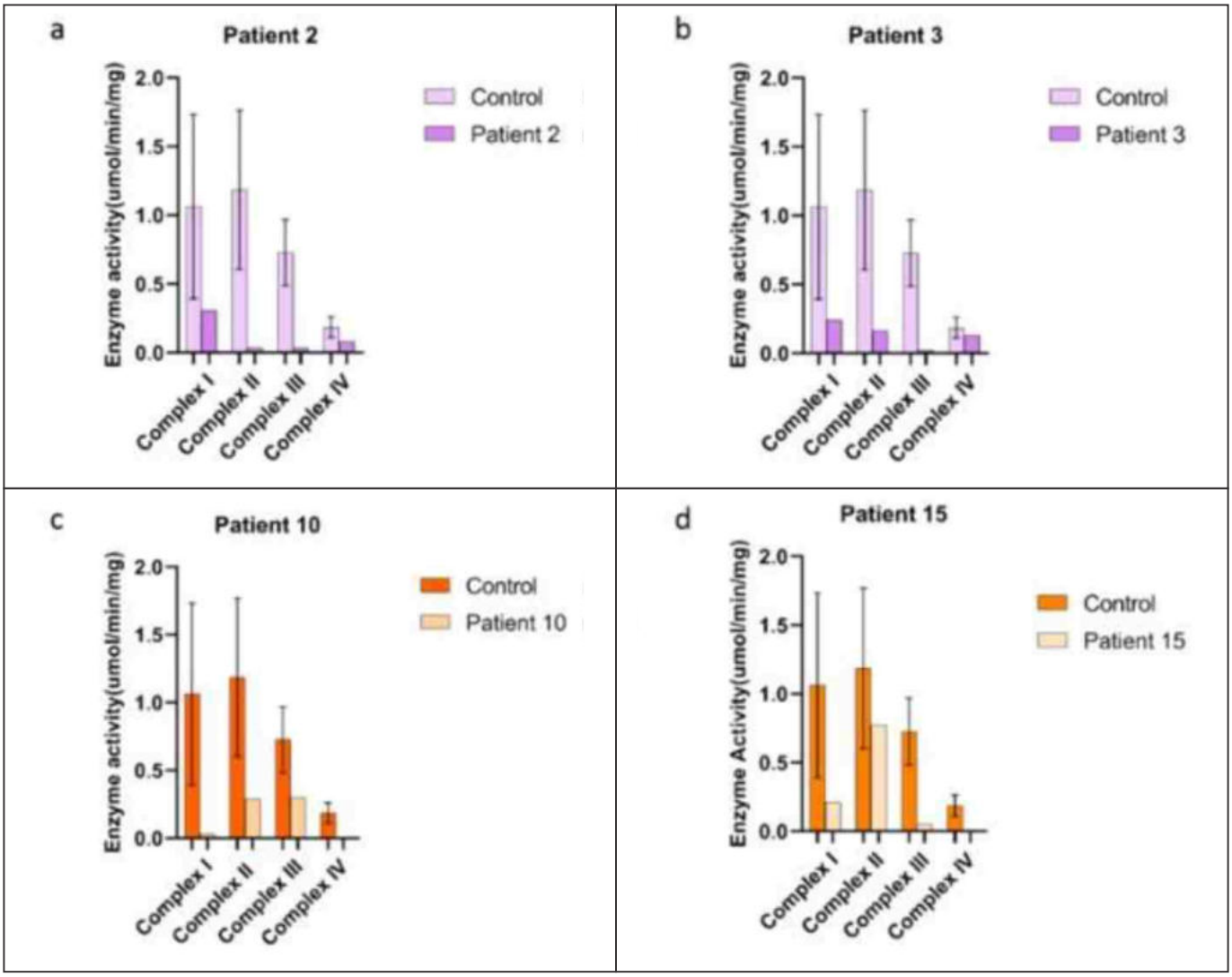


Figure 1: Mitochondrial enzyme activity of Complex I, II, III and IV. Comparative enzyme activity of a) P2, b) P3; c) P10; and d) P15

Genetic analysis and mitochondrial DNA sequencing were carried out. Multiple deletions were observed in Long range PCR in P10 and P15 (Fig. 2a&b). Further, Whole Exome Sequencing (WES) was carried out for all the patients. Amongst the 15 patients, two patients had mutations in POLG gene. Patient P10 harbored a compound heterozygous variant consisting of a novel mutation in POLG (NP_002684.1:p.Trp261X) along with another known missense variant (NP_002684.1:p.Leu304Arg) (Fig. 2c&d). A novel variant (NP_002684.1:p.Trp261X) caused premature termination and hence, it was considered to be a pathogenic variant. *In-silico* analysis of the missense variant NP_002684.1:p.Leu304Arg was found to be 'deleterious' using SIFT (score: 0.03) and 'probably damaging' with polyPhen-2 (score: 1.00) prediction tool. The other patient, P14 had a novel homozygous variant NP_002684.1:p.Phe750Val in POLG gene (Fig. 2e). *In-silico* analysis of this variant was found to be probably damaging by PolyPhen-2 (score: 0.974). All the genetic variants identified in WES were validated using Sanger sequencing.

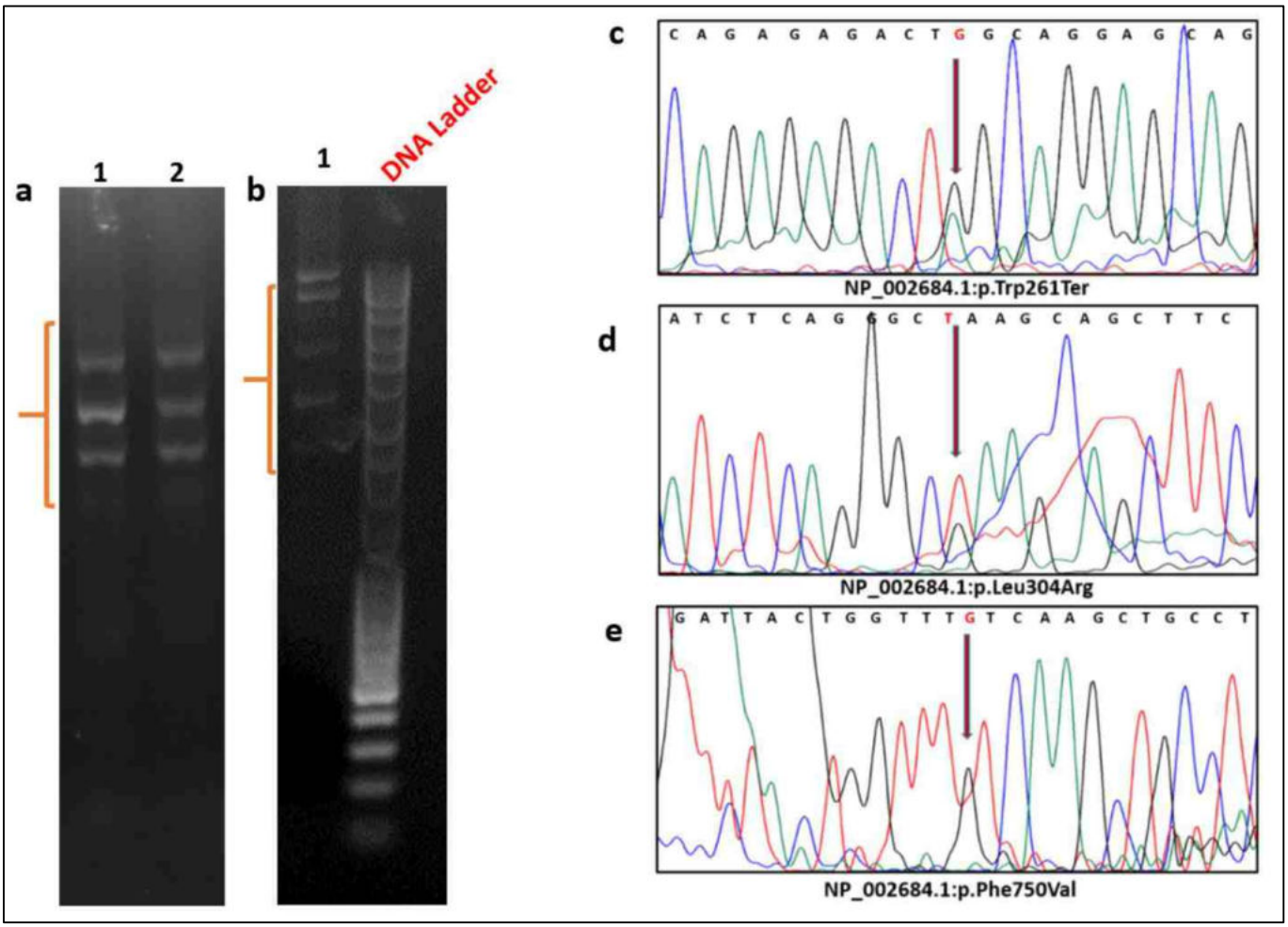


Figure 2: Long range PCR of patient P10, lane 1-2 showing multiple deletions in mitochondrial DNA (a); Long range PCR of P15 (b), Multiple deletions on mitochondrial DNA are marked by bracket; DNA sequencing chromatograms of NP_002684.1:p.Trp261Ter in P10 (c); NP_002684.1:p.Leu304Arg in P10 (d); NP_002684.1:p.Phe750Val in P14 (e).

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

(Funded By Indian Council of Medical Research)

Principal Investigator	:	**Suchitra Surve**
Co-Principal Investigators	:	Antara Banerjee, Sudha Rao, Bai Jerbai 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, Baisalee Khargharia
Duration	:	2021-2024

Precocious Puberty (PP) is premature reactivation of the hypothalamic-pituitary-gonadal axis leading to GnRH secretion. It may be Central (CPP or ICPP), Peripheral or Incomplete Precocious Puberty. It is considered as onset of breast stage II development before the age of 8 years in girls and genital stage 2 development before 9 years in boys. 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. 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. Biomarkers such as Kisspeptin, Neurokinin B and Neuropeptide Y biomarkers are reported for their potential in diagnosing ICPP and early puberty.

The present study is planned to explore the clinical and therapeutic relevance of above-mentioned markers. This study is being carried out at a tertiary hospital in Mumbai and community clinic. Girls in the age group of 6 -9 years with probable diagnosis of ICPP and those with Incomplete variants of PP and age matched controls are included. From Abhyudaya Nagar Clinic of ICMR-NIRRCH, 40 heathy age-matched controls who met the inclusion criteria were recruited after screening of 250 girls. From collaborating hospital (BJ Wadia Hospital for Children) after screening 81 girls, 39 girls who met inclusion criteria for probable precocious puberty cases, recruited as PP cases. Out of 39 cases, 18 were confirmed ICPP cases and 11 were confirmed incomplete puberty (PT) cases, remaining cases are yet to be classified. A significantly higher level of Kisspeptin was observed in ICPP cases and PT cases as compared to control participants (n=40) but there was no significant difference between ICPP and PT cases (Fig. 1).

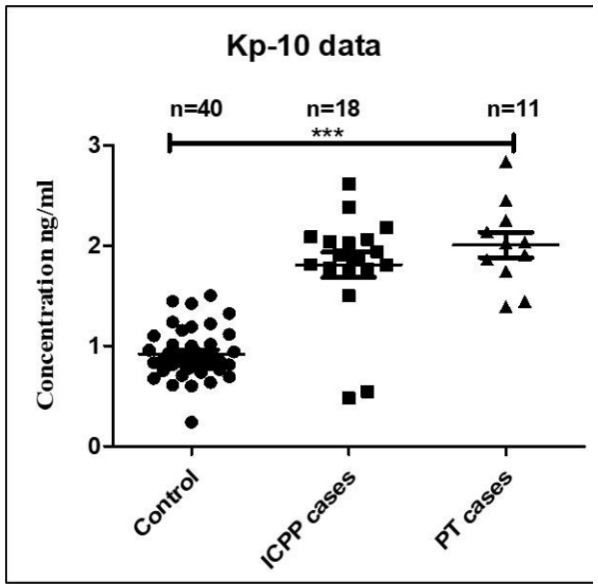
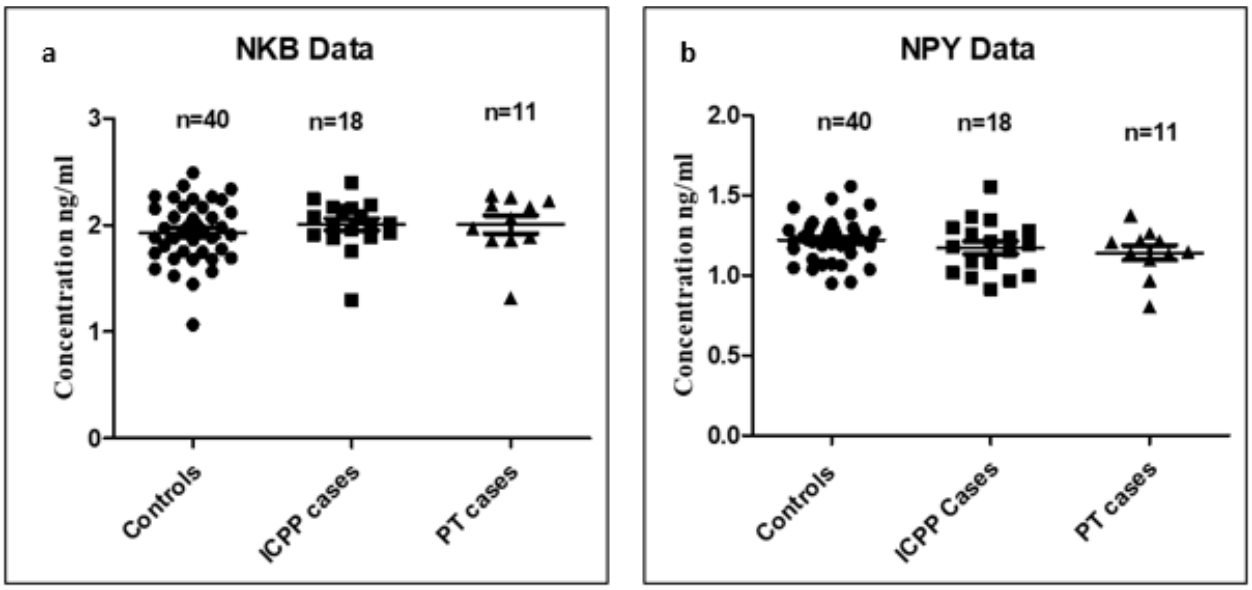


Figure 1: Estimation of circulating levels of Kisspeptin-10 by ELISA in control participants (n=40), ICPP cases (n=18) and PT cases (n=11) respresented by circles, squares and triangles respectively. Graph represents mean±SEM value for each participant assayed in duplicates.

Statistical significance was determined by 1 way ANOVA with Tukey's multiple comparison post-hoc test (p=0.0008). In case of Neurokinin B (Fig. 2a) and Neuropeptide Y (Fig. 2b) levels, no significant difference was observed among control participants, ICPP cases and PT cases.



Figures 2: Estimation of Neurokinin B and Neuropetide Y levels by ELISA.

6.11 Role of Kisspeptin Mediated Signalling in Onset of Puberty

Principal Investigator	:	**Antara Banerjee**
Co-Principal Investigator	:	Suchitra Surve, Bhakti Pathak
Project Associate	:	Shital Bhanarkar
Clinical Collaborators	:	Sudha Rao, Bai Jerbai Wadia Hospital for Children, Mumbai
Duration	:	2021-2024

The signaling of a kisspeptin-10neuropeptide through its cognate G-protein coupled receptor, the kisspeptin receptor (KISS1R), heralds the onset of puberty. Secretion of gonadotropin releasing hormone is triggered downstream to kisspeptin-10 signaling through KISS1R, which activates the hypothalamo-pituitary-gonadal axis, resulting in sexual maturation. Consequently, mutations in this hormone-receptor pair lead to pubertal disorders. Activating mutations in kisspeptin-10 or KISS1R are implicated in idiopathic central precocious puberty. Functional studies on such naturally occurring activating mutations can therefore provide insights into the pathophysiology of idiopathic central precocious puberty (ICPP). In the reporting year, determination of genetic variants in a girl with idiopathic central precocious puberty was carried out by whole exome sequencing approach. Sequencing results revealed the presence of the H364 variant in KISS1R in homozygous condition, which has been previously reported in other ethnic populations for its association with central precocious puberty. Fig. 1A depicts the position of the residue at 364 position situated in the C-terminal tail of human KISS1R. Targeted DNA sequencing using primers scanning exon 5 of human KISS1R was carried out by Sanger method, for validation of the results of exome sequencing. The DNA electropherograms of L364 KISS1R (encoded by CTC codon) and that of H364 KISS1R (encoded by CAC codon) which is present in the patient is shown in Fig. 1B. The variant sequence is deposited in the dbSNP database (rs350132) and reported in ClinVar (Accession: VCV000447660.14). Analysis by

bioinformatics tools such as Polyphen-2 and SIFT predicted the substitution to be 'neutral' and 'tolerated' respectively, whereas MAPP and MutationTaster predicted it to be 'deleterious' and 'disease causing' respectively. The proband had an apparently normal karyotype (46,XX). Targeted DNA sequencing of her parents' DNA samples was also carried and results revealed that her father was homozygous for the H364 variant in KISS1R whereas her mother was heterozygous (L364/H364 KISS1R). Molecular models of L364 KISS1R and H364 KISS1R (Fig. 2A) were generated on the C-I-TASSER server followed by their visualisation on PyMol. Thereafter, the two structures were superimposed on each other using the 'Align' tool in PyMol. H364 KISS1R showed a root mean square deviation (RMSD) value of 0.533 as compared to L364 KISS1R (Fig. 2B). The binding energy of H364 KISS1R (-13706.1 KJ/mol) was slightly higher than that of L364 KISS1R (-14234.8 KJ/mol) as determined using the Swiss-PdbViewer tool (Fig. 1B). The RMSD and binding energy values indicate deviation in KISS1R structure owing to the H364 substitution that could bring about conformational changes in the receptor structure. Further, *in-vitro* studies are being carried out with the L364 KISS1R and H364 KISS1R constructs, in order to determine the effect of this substitution on the receptor function.

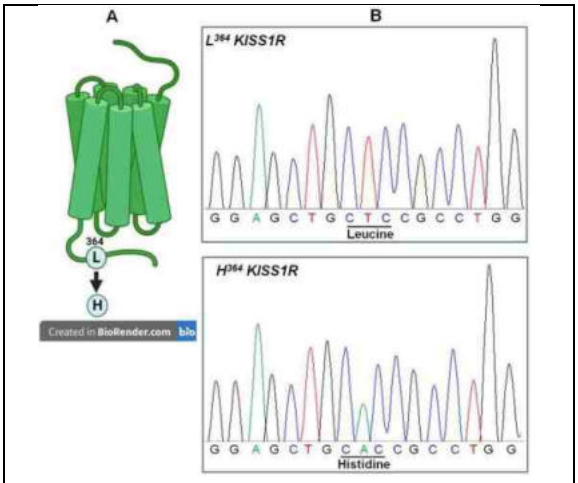
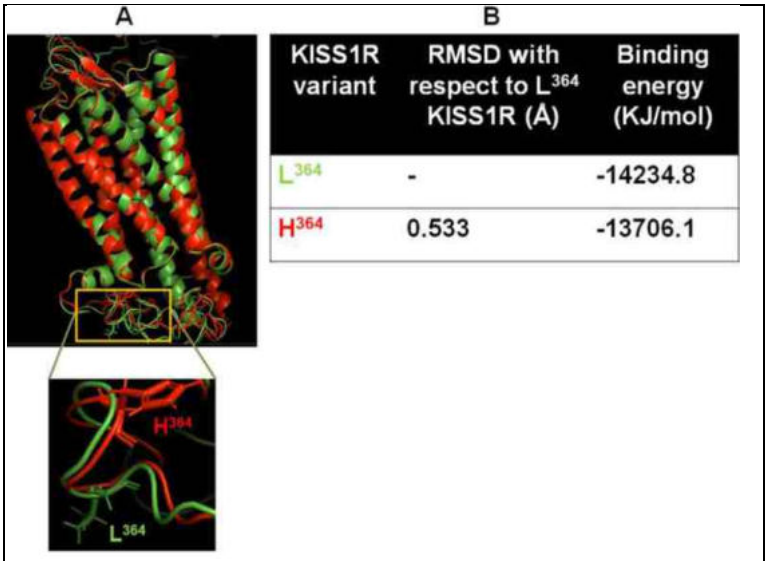


Figure 1: (A) Position of the L364H variant in the C-terminal tail of human KISS1R (Image created using BioRender.com) (B) DNA electropherograms illustrating the homozygous substitution of Leucine (encoded by CTC codon) with Histidine (encoded by CAC codon) in the patient with ICPP.



KISS1R variant	RMSD with respect to L^{364} KISS1R (Å)	Binding energy (KJ/mol)
L^{364}	-	-14234.8
H^{364}	0.533	-13706.1

Figure 2: (A) Homology models of L364 KISS1R (green) and H364 KISS1R (red) superimposed on each other, as visualized using PyMol. (B) Root mean square deviation (RMSD) and binding energy of H364 KISS1R as compared to L364 KISS1R.

6.12 Delineation of the Role of Isoforms of Kisspeptin in Mammalian Reproduction *(Partly Funded by Science and Engineering Research Board, Department of Science and Technology)*

Principal Investigator	:	**Antara Banerjee**
Project Associates	:	Aishwarya Chakraborty, B Kulkarni, Dhanashree Jagtap, Bhakti Pathak
Duration	:	2022-2024

Kisspeptins are neuropeptides that play a central role in mammalian reproduction by inducing puberty and regulating fertility. These neuropeptides are synthesized initially as pro-peptides that undergo cleavages to form four isoforms in humans. The kisspeptin isoforms in humans are kisspeptin-54, kisspeptin-14, kisspeptin-13 and kisspeptin-10. All four kisspeptin isoforms have a common decapeptide sequence towards the C-terminal and the smallest isoform, Kisspeptin-10 can completely activate the Kisspeptin receptor (KISS1R) independently like the others. Literature shows multiple studies carried out to elucidate the role of Kisspeptin-54 and Kisspeptin-10, but lacks data about kisspeptin-13 and kisspeptin-14 in reproduction. We aim to carry out structural and functional characterization of the kisspeptin isoforms in this study. In the reporting year, Kisspeptin-14 (Kp-14), Kisspeptin-13 (Kp-13) and Kisspeptin-10 (Kp-10) were synthesized by F-moc Chemistry (0.25mMol) based Solid Phase Peptide Synthesis. The crude peptides were purified by Analytical RP-HPLC and outsourced for MALDI-TOF analysis to validate the molecular weights of the synthesized peptides. Results revealed that all the three synthesized peptides have the desired molecular weights, which were comparable to their theoretical molecular weights.

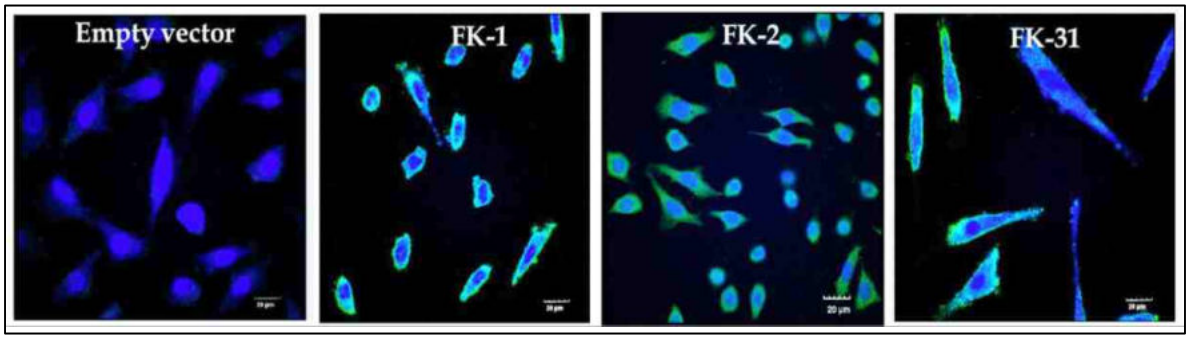


Figure 1: Cell surface expression of WT Flag KISS1R stable clones in CHO cells as visualized by indirect immunofluorescence. Confocal microscopy images (Scale bar=20µM) demonstrates KISS1R (green) with nuclei stained with DAPI (blue).

To study the signaling response of kisspeptin isoforms, stable clones expressing an N-terminal Flag tagged wild type KISS1R (Flag KR) was generated in CHO cells. Clones exhibiting moderate or high levels of KISS1R were further screened for cell surface KISS1R expression by indirect immunofluorescence and flow cytometry using an anti-Flag antibody raised in rabbit followed by staining with an anti-rabbit FITC conjugated secondary antibody. The clones FK-1, FK-2 and FK-31 exhibited cell surface KISS1R expression as seen by confocal microscopy (Fig. 1) and flow cytometry (Fig. 2). The empty vector transfected stable cell line served as a negative control. Currently, kinome profiling and signaling cascades estimating endpoints like arachidonic acid, diacylglycerol and inositol phosphate production are being studied in response to different kisspeptin isoforms.

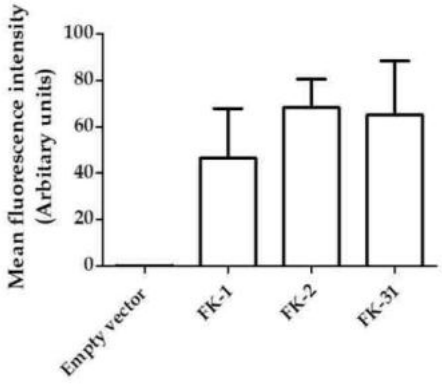


Figure 2: Flow Cytometry analysis to determine cell surface expression of stable clones expressing Flag KISS1R in CHO cells. Graph represents mean±SEM of three independent experiments.

6.13 Effect of Maternal Gestational Micronutrient Deficiency on Offspring's Fertility and its Underlying Epigenetic Mechanisms in Germline

Principal Investigator	:	**Dipty Singh**
Co-Investigator	:	D Modi
Project Associates	:	Anushruti Singh, Nafisa Balasinor, Kumari Nishi
Duration	:	2021-2026

The developmental origin of health and disease (DOHaD) hypothesis suggests that metabolic disorders such as obesity, type 2 diabetes and hypertension may take root during early development. However, developmental of origin of reproductive disorders are not well studied. The maternal nutritional status during early development may have important implications on offspring's reproductive health in later life. This study aims to unravel the effects of maternal methyl donors (vitamin B12, folic acid and methionine) deficiency during gestation on offspring's reproductive development and fertility. For this, pregnant female B6 CBA tg OCT4-GFP mice were fed on AIN 76A control chow diet (CCD), 40% methyl donors' deficient diet (MDD) and 60% MDD. The window period of maternal deficiency was from gestation day (GD) 5 to GD20. F1 offspring were fed on CDD after weaning. The developmental and molecular effects were studied on gestation GD13, GD18, post-natal day (PND) 22 and PND60. At GD13 and GD18 global DNA methylation in fetal gonads and liver were studied (methylation marks erased at GD13 and re-established at GD18). Serum homocysteine levels in dams were evaluated at GD18. At GD13 global DNA methylation levels were found to be reduced in gonads of methyl donor deficient groups, but remains constant in all the groups of somatic tissue (liver) (Fig. 1A). Also, the global DNA methylation percentage in gonads was found to be more at GD18 as compared to GD13 indicating re-establishment of methylation (Fig. 1A). Homocysteine levels were increased in 40% MDD but decreased in 60% MDD (Fig. 1B).

Histopathology studies at GD18, PND22 and PND60 shows testicular atrophy, sloughing and vacuolization in seminiferous tubules of methyl donor deficient groups (Fig. 2A). Furthermore, immunofluorescence studies showed reduced expression of VASA (spermatogonia and spermatocyte marker) and ITGA6 (Spermatogonia marker) in seminiferous tubules of methyl donor deficient groups (Fig. 2B). Quantitative histomorphometry by Aperio ImageScope software indicates reduced number of seminiferous tubules, increased percentage of testicular atrophy and sloughing in seminiferous tubules (Fig. 2C). The present findings suggest that gestational methyl donor deficiency may have detrimental effects on offsprings' reproductive development. Further studies are underway to investigate its effect on fertility of F1 offspring, epigenetic modifications in primordial germ cells of F1 embryo and its underlying mechanisms.

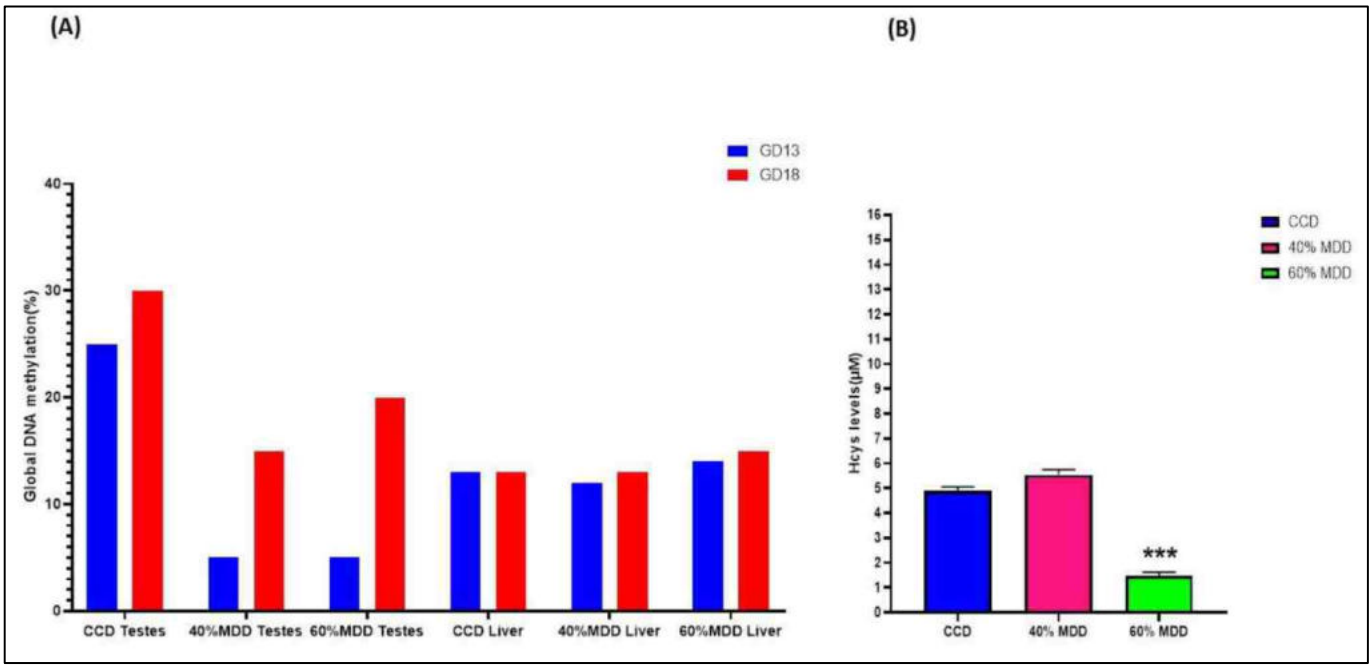


Figure 1: Effect of gestational methyl donor deficiency on (A) Global DNA methylation levels of F1 fetal gonads; (B) Serum homocysteine levels of dams

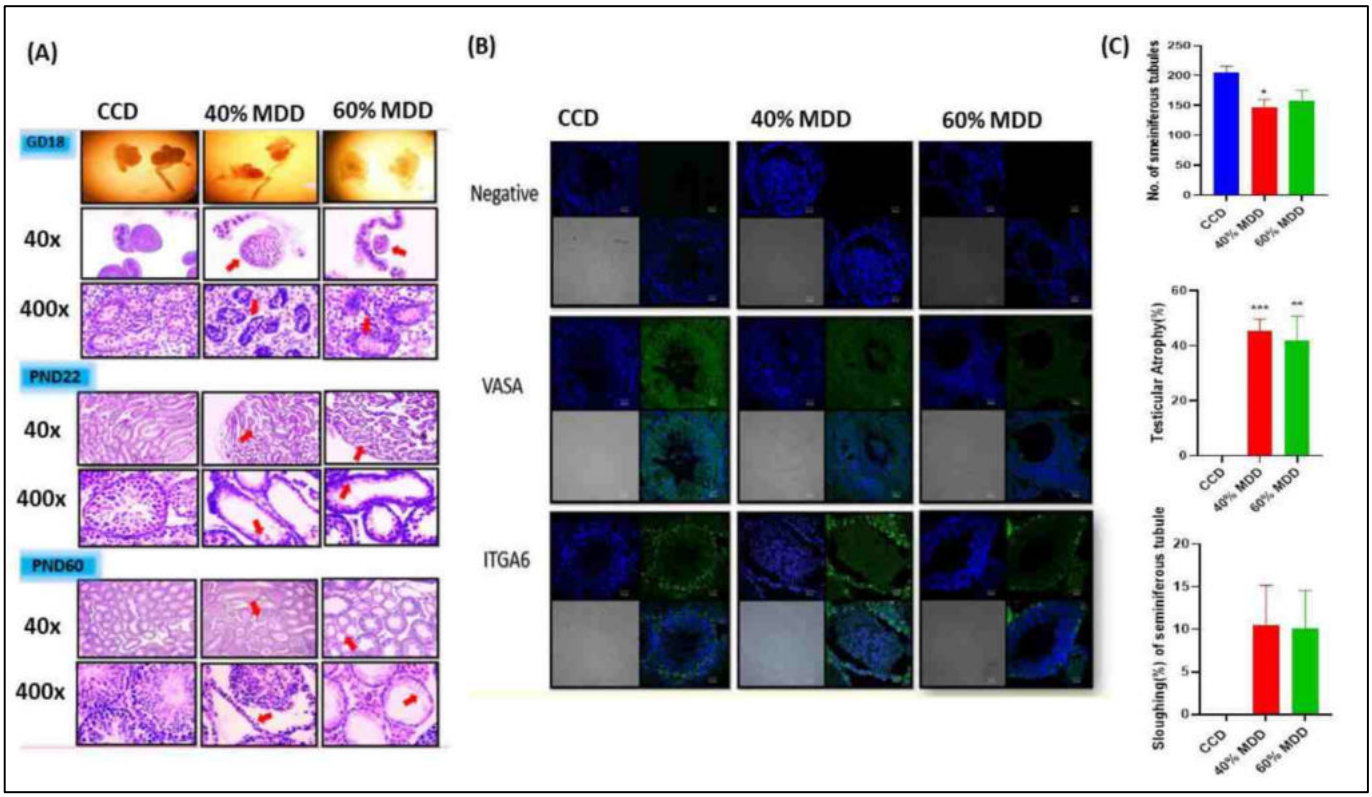


Figure 2: Effect of gestational methyl donor deficiency on (A) Histopathology of F1 offspring at GD18, PND22 and 60; (B) Spermatogonia and spermatocyte markers at adulthood; (C) testicular atrophy at adulthood

STEM CELL BIOLOGY

7. STEM CELL BIOLOGY

Since its inception, the department has derived and characterized two human embryonic stem cell lines, KIND1 and KIND2. These cell lines have been successfully used for differentiation into various lineages such as pancreatic progenitor and cardiac progenitor cells. These two cell lines have been shared with many researchers across India for research.

The department previously expanded its horizon to the area of adult tissue-resident stem cells such as VSELs (Very Small Embryonic-Like stem cells). Their presence has been demonstrated in mouse bone marrow, testes, ovary, prostate and uterus. It was also shown that the neonatal endocrine disruption could alter the number of VSELs, which can lead to various pathologies in reproductive organs. Additionally, projects have been initiated to study the role of various endometrial stem cells (epithelial progenitor, mesenchymal stem cells and side population) in development of endometriotic ectopic lesion in a mouse model.

Recently, the department has embarked into the area of "induced Pluripotent Stem Cells (iPSCs)". Studies have been initiated to generate iPSCs from patients with neuro-developmental and neuro-psychiatric disorders. iPSCs have been generated and these cells have been differentiated to neuronal lineage to study neuronal function in these disorders.

7.1 Evaluating the Role of Hypoxia in hESCs Differentiation towards Trophoblast Lineage

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

Placentation is a key process in the development of the foetus. Improper placentation could lead to pregnancy-related complications like intra-uterine growth restriction, pre-eclampsia, miscarriage etc. In addition, varying oxygen concentrations are considered to play a major role in development of a placenta. Models to study placental development are highly warranted considering the limitations in sample availability and ethical constraints. The present study focuses on determining the effects of hypoxia on trophoblast lineage specification using human embryonic stem cells (hESCs) as a model. In previous years, we have shown lineage specific differentiation of hESCs to trophoblasts. Using media supplemented with BSA, ITS, BMP4, A83-01 and PD173074, trophoblast cell specific transcripts like TFAP2A, TFAP2C, GATA3, CDX2 were observed. In media comprising of N2, B27, Glutamine, A83-01, CHIR99021, EGF, Rspo-1, Y27632, N-Ac-L-Cys, PgE-2, HGF and bFGF (Trophoblast Organoid Media, TOM media), differentiation into syncytiotrophoblast (STB) lineage was observed. In this reporting year, we evaluated the effect of hypoxia on the differentiation process. Briefly, hESCs were primed towards trophoblast cells using BMP4 containing media for 6 days. Following this, the differentiation using TOM media was monitored in 4% oxygen (hypoxic) and 21% (normoxic) oxygen concentrations. Following differentiation, the cells expressed HCG (Fig. 1A&B) under both normoxic and hypoxic conditions. Quantitation was done to calculate the percentage of cells expressing HCG in both normoxic and hypoxic conditions. Results have shown that cells cultured in normoxic conditions exhibited higher number of cells expressing HCG (41.1%) compared to hypoxic conditions (13.5%).

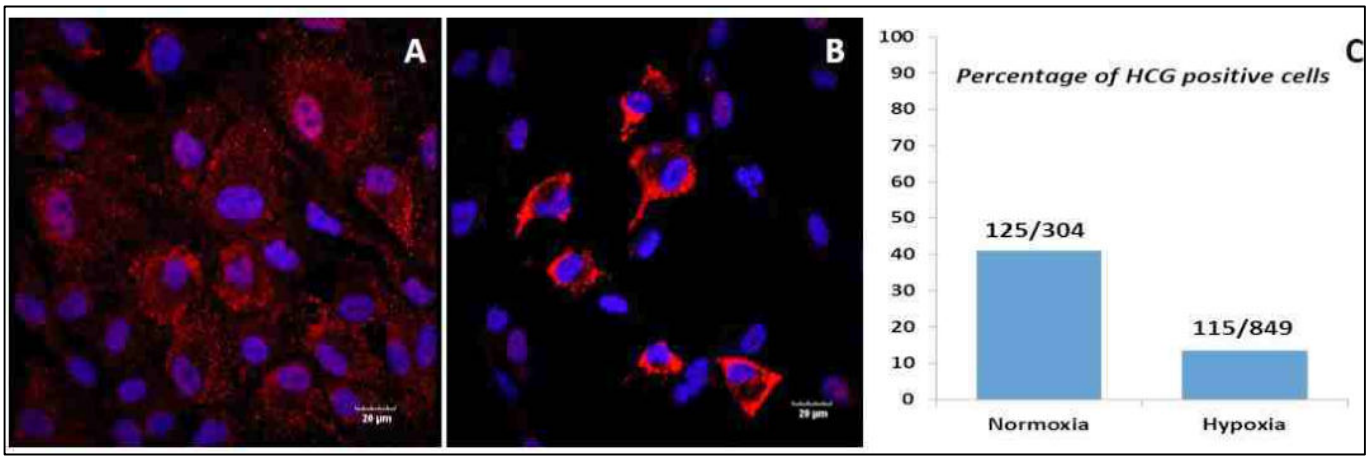


Figure 1: Expression of HCG (red) was observed following differentiation of KIND1 hESCs into STBs under normoxic conditions (A) compared with the hypoxic conditions (B). Cells were counterstained with DAPI (blue). Bar=20µm. (C) Percentage of HCG positive cells following differentiation into STBs under normoxic and hypoxic conditions.

7.2 Investigating the Role of Microdeletion Syndrome in Neuronal Functions using Induced Pluripotent Stem Cells

Principal Investigator	:	**DK Das**
Co-Principal Investigator	:	S Pande
Project Associate	:	B R Shekhar
Duration	:	2023-2028

Microdeletions across the human genome in various loci lead to numerous neuro-developmental genetic disorders. These regions harbour many genes or critical areas of genome which contribute to the pathology of the disorders. That recurrent deletion or copy number variations (CNV) affects complex brain architecture is supported by various evidences in literature. However, the exact role of these CNVs in brain disorder is still elusive. Amongst the microdeletion syndromes, 22q11.2del and 15q11.2-13del are most common. The objective of this study is to generate iPSCs from patients with microdeletion syndrome and differentiate them into cortical neurons. Structural and functional defects in neurons derived from the patient will be assessed compared to the controls. During this reporting year, one patient with 22q11.2del was recruited. This patient was clinically diagnosed at Genetic Research Centre and the deletion was confirmed using FISH. The PBMCs from the whole blood were isolated and subjected to iPSCs generation using Sendai virus kit. Colonies of the iPSCs were generated and mature colonies were derived (Fig. 1). Further, they were characterized for ther expression of pluripotency markers OCT4, SOX2 and NANOG (Fig. 2). This project is ongoing and the iPSCs will be differentiated to cortical neurons for structural and functional analysis.

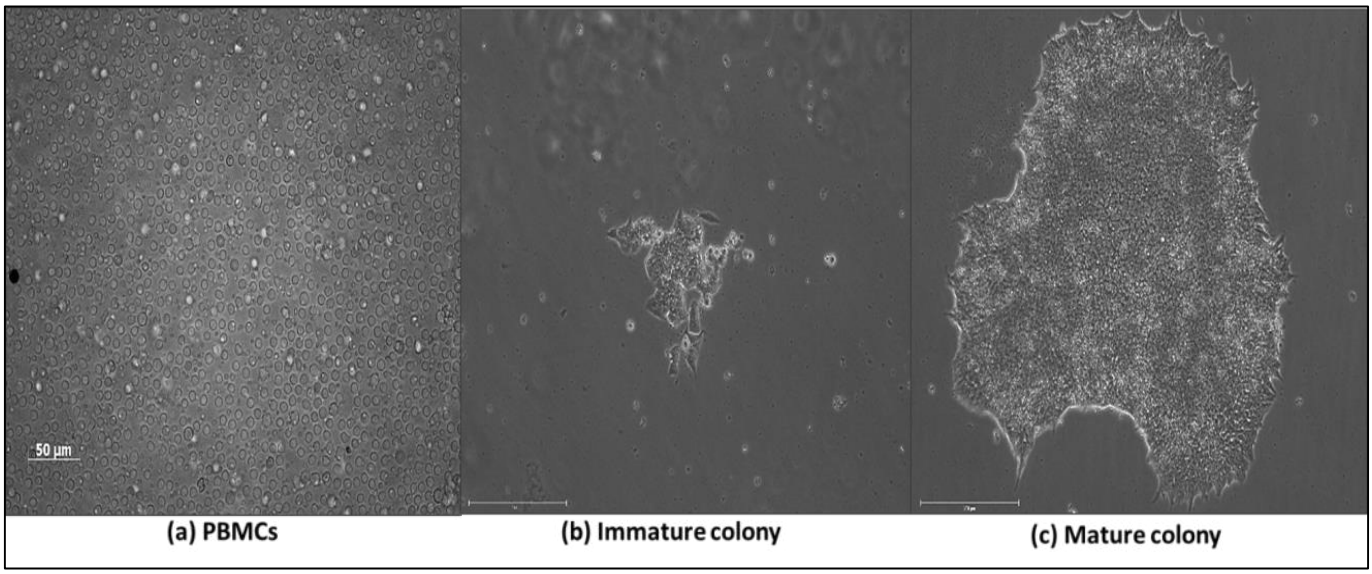


Figure 1: Generation of iPSCs from PBMCs. (a) Isolation of PBMCs from whole blood; (b) PBMCs attached to the matrigel coated plate post-transduction of Sendai virus; (c) matured iPSCs colony on matrigel coated plate.

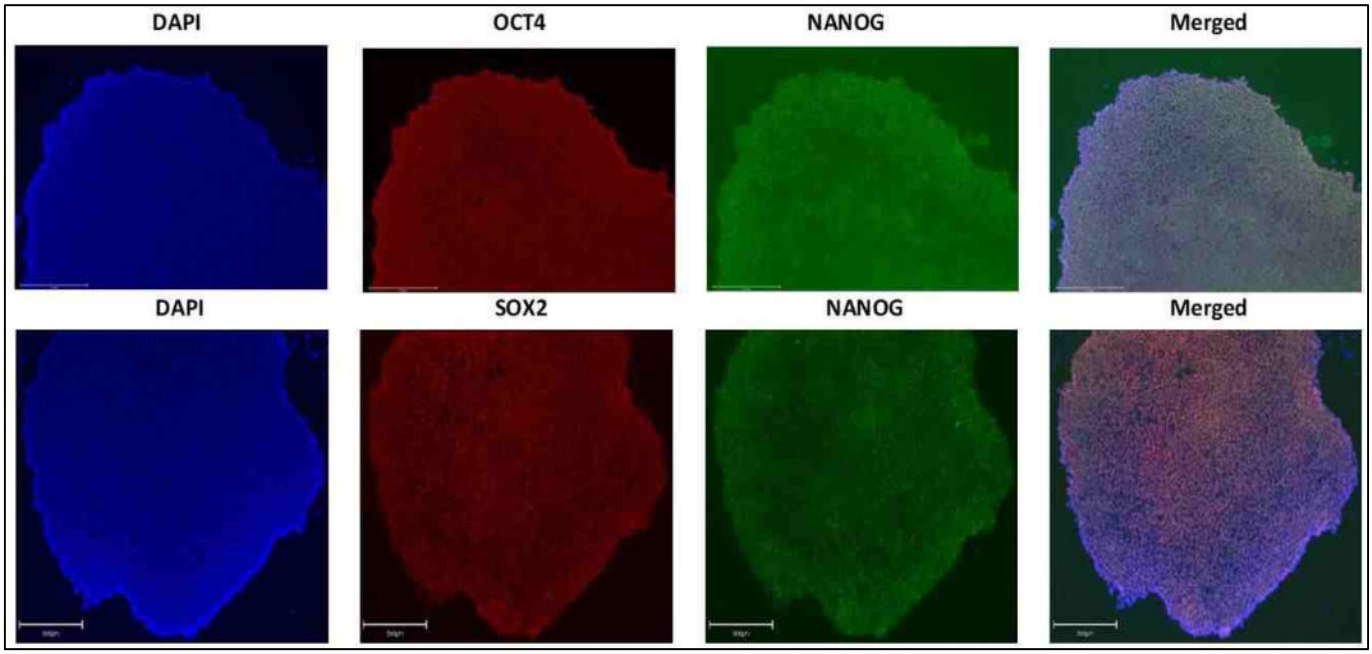


Figure 2: Characterization of iPSCs using pluripotency markers. Each panel showing the iPSCs colony stained with DAPI (column1), OCT4/SOX2 (column 2), NANOG (column 3) and merged image (column 4).

REPRODUCTIVE CANCERS

8. REPRODUCTIVE CANCERS

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

Principal Investigator	:	**Taruna Madan**
Project Associates	:	Kasturi Ganguly
Collaborators	:	U Kishore, UAE University, UAE SM Metkari
Duration	:	2018-2023

Cancer immunotherapies with the blockade of checkpoint inhibitors are attaining impressive clinical success but does not provide substantial benefits to prostate cancer (PCa) patients due to immune-suppressive "cold" tumor microenvironment (TME). Retinoic acid-inducible gene- I (RIG-I)-like receptors (RLRs), cytosolic pattern recognition receptors (PRRs), can detect double-stranded and single-stranded exogenous RNAs, and induce type I interferons and other pro-inflammatory cytokine secretion. Recently, there is an increasing interest in harnessing the potential of RLR activation for antitumor immunity. However, the expression pattern and the role of two major RLRs: RIG-I and Melanoma Differentiation Associated (MDA)-5, in PCa still remain largely unexplored. In the present study, the transcripts and protein expression of RIG-I, MDA-5 and their common adaptor, mitochondrial antiviral signalling protein (MAVS), were analysed in early and late stage of PCa using a spontaneous murine model of PCa, Transgenic Adenocarcinoma Mouse Prostate (TRAMP). RIG-I, MDA-5 and MAVS expression were found to be significantly increased during early stage whereas their expressions were found to be downregulated during the late stage of PCa in TRAMP (Fig. 1).

Further, the potential of RIG-I activation to induce immunogenic cell death in murine PCa cells, TRAMP-C2 and in syngeneic subcutaneous tumor implantation model were analysed by using RIG-I agonist: short 5′-triphosphate-modified RNA (5′ppp-RNA). Cytoplasmic delivery of 5′ppp-RNA (1.25μg/ml and 2.5 μg/ml) for 48h induced apoptosis in TRAMP-C2 cells (Fig. 2A). Additionally, intra-tumoral treatment of 5′ppp-RNA (10 μg/dose for 3 times on every alternative day) induce tumor apoptosis and ecto-calreticulin expression (Fig. 2B).

Further, RIG-I activation leads to immunomodulation via significantly increase infiltration of CD45+ leukocytes, CD4+ and CD8+ cells into tumor thus, turning up the heat of the TME. This study highlights yet another novel dysregulated innate immune surveillance mechanism of RLRs. Targeting RIG-I signalling represents an attractive target for PCa therapy.

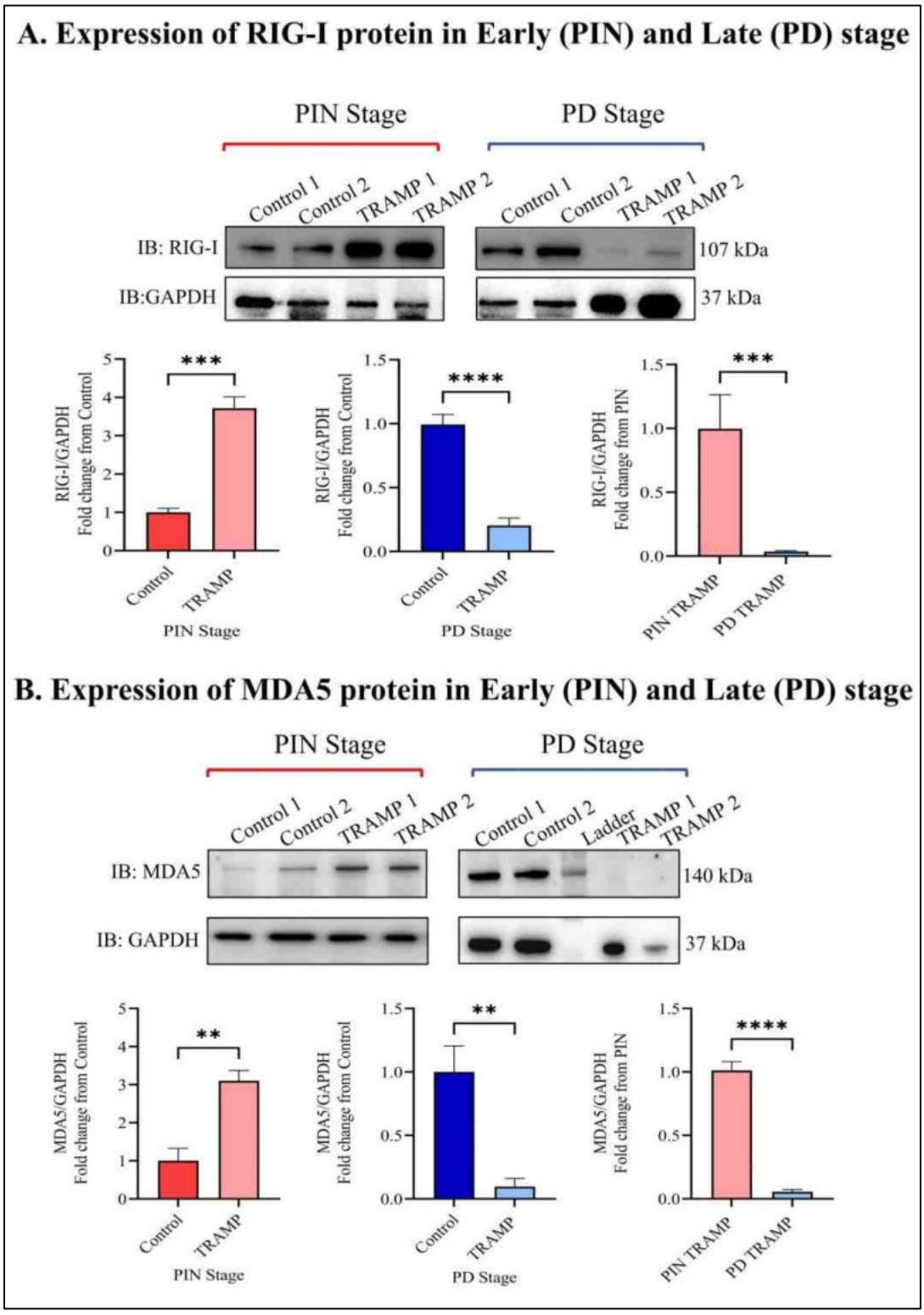


Figure 1: Dysregulated expression of RIG-I and MDA5 proteins in different stages of prostate tumor progression using TRAMP mice. Western blot analysis revealed that RIG-I protein (A) and MDA5 protein (B) expression is significantly increased during pre-cancerous prostatic prostatic intraepithelial neoplasia (PIN) stage whereas its expression significantly goes down during late poorly differentiated adenocarcinoma (PD) stage of TRAMP.

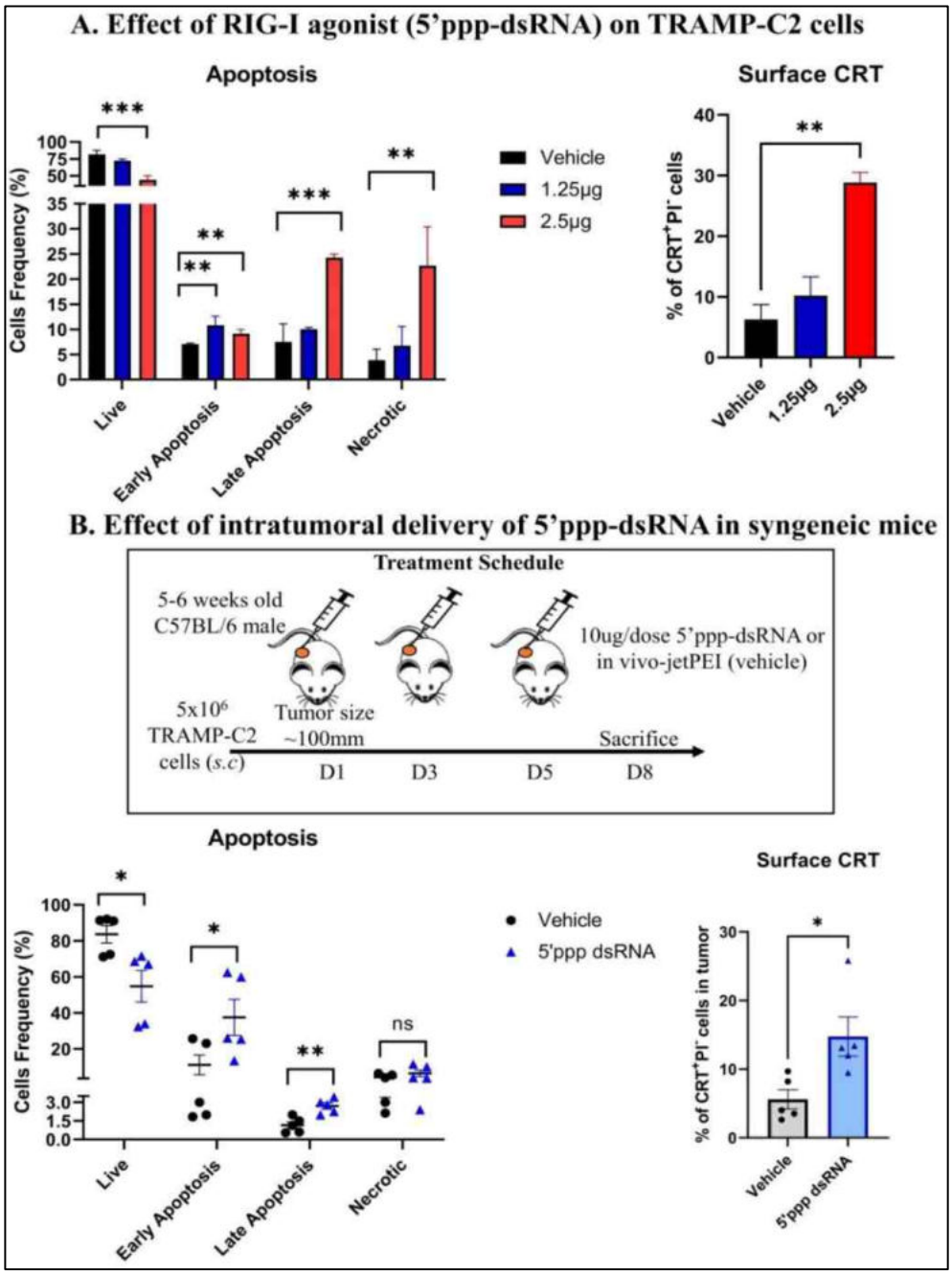


Figure 2: Anti-tumorigenic potential of RIG-I agonist (5′ppp-dsRNA). (A) Treatment on TRAMP-C2 cells with 5′ppp-dsRNA for 48 h showed increased cell death and surface calreticulin (CRT) expression. Late apoptotic and necrotic cells frequency altered in a dose-dependent manner. (B) 5-6 week old C57BL/6 male mice were injected with 5x10^6 TRAMP-C2 cells in their right flank. After the tumor volume reaches ~100 mm3, 10µg 5′ppp-dsRNA were injected intratumorally with in-vivo JetPEI at Nucleotide to Protein (N/P) ratio 6. After three doses of 5'ppp-dsRNA, mice were euthanized on day 10 for further analysis. Mice treated with only *in-vivo* JetPEI were used vehicle control. Annexin -V and PI staining showed increased cell death in the treated group whereas, surface CRT expression found to be significantly enhanced in the tumors followed by RIG-I agonist treatment. n=3-5/group. Statistical significance was tested using two-tailed t test (*p < 0.05, **P < 0.01,). Data represented as mean ± SEM.

8.2 Investigating the *In Vitro* and *In Vivo* Potential of Rfhsp-D to Inhibit Prostate Cancer Metastasis and Mechanistic Involvement of GRP78 *(Partly Funded by Indian Council of Medical Research)*

Principal Investigator	:	**Rambhadur Subedi**
Co-Principal Investigator	:	Taruna Madan
Collaborators	:	U Kishore, Brunel University, London SM Metkari
Duration	:	2021-2024

Surfactant Protein D (SP-D) is a type of Pattern Recognition Receptor (PRR). It is among the most studied soluble PRRs that have been shown to be involved in host defense against pathogens and allergens, and modulates the inflammatory responses. Previous studies from our group reported induced apoptosis in the tissue explants from patients of metastatic prostate cancer (Annual report 2017-18, p. 95). Furthermore, our research (Annual report 2021-22, p. 103) has shown that treatment with rfhSP-D results in a concentration-dependent reduction in the migration of PC3 and LNCaP cells. Additionally, we observed that rfhSP-D treatment leads to an increase in the expression of E-Cadherin and a decrease in the expression of β-catenin at the protein level. These findings suggest that rfhSP-D has the potential to inhibit the metastatic properties of prostate cancer cells. We further tested the effectiveness of rfhSP-D in hindering prostate cancer cell invasion using the Transwell cell invasion assay.

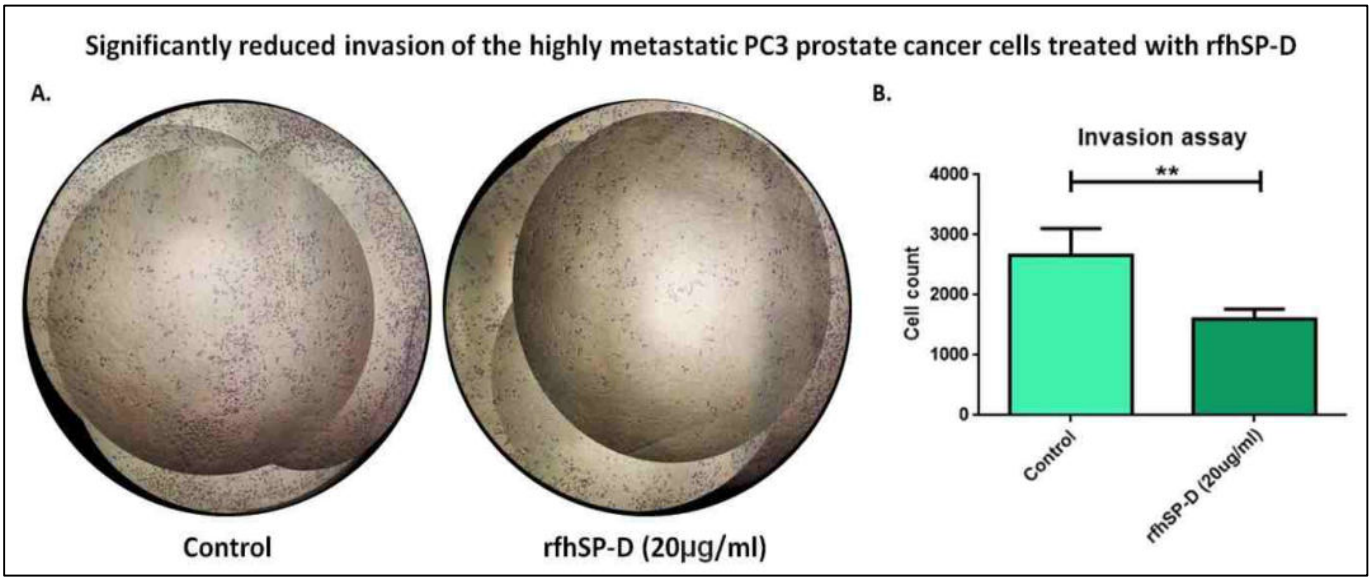


Figure 1: rfhSP-D inhibits invasion of highly metastatic PC3 cells. A. The full-view image of the Transwell cell insert was created by collating five images from different locations of the same insert. Each circle represents cells viewed under the 4X magnification. B. The quantification of migrated cells through the Transwell assay showed a significant decrease in the number of cells invading through the cell insert coated with matrigel. ** $p<0.01$

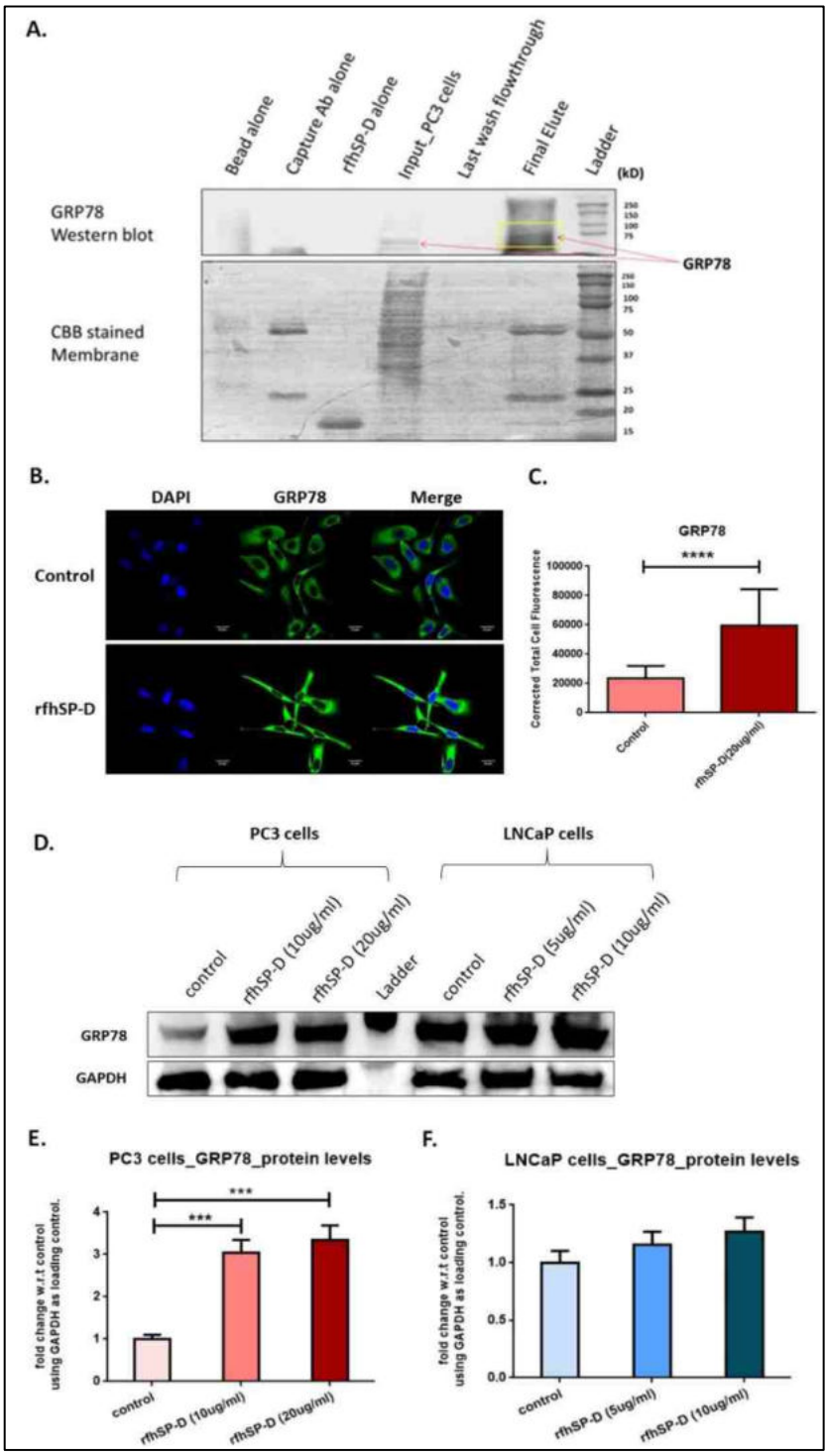


Figure 2: Immunoprecipitation studies provided evidence of the interaction between rfhSP-D and GRP78 (A). The yellow box in the image represents the GRP78 band that was successfully isolated through a pull-down assay using rfhSP-D with PC3 cell lysate. Treatment of PC3 cells with rfhSP-D results in a notable increase in GRP78 protein expression (B, C, D, E). Similarly, LNCaP cells also show an increase in the GRP78 expression upon rfhSP-D treatment, although this increase did not reach statistical significance (D, F). *** $p<0.001$, **** $p<0.0001$

As shown in Fig. 1, treatment with 20µg/ml of rfhSP-D resulted in a notable decrease in the invasion of PC3 cells. Previous studies from our lab had identified GRP78 as one of the top ranked proteins of the rfhSP-D-PC3 membrane interactome. ELISA study also validated the interaction between GRP78 and rfhSP-D in PC3 cell lysates (Fig. 2A). The impact of rfhSP-D on the protein expression of GRP78 in prostate cancer cells was further investigated. PC3 cells were treated with 10µg/ml and 20µg/ml rfhSP-D for treatment whereas, LNCaP cells were treated with 5µg/ml and 10µg/ml rfhSP-D. Notably, both prostate cell lines exhibited an increase in GRP78 protein expression upon treatment with rfhSP-D, as illustrated in Fig. 2B-F. However, statistical significance was observed only in PC3 cells treated with rfhSP-D. Studies are being conducted to further understand the mechanisms underlying the involvement of GRP78 in rfhSP-D-mediated inhibition of prostate cancer cell metastasis.

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

Principal Investigator	:	**Geetanjali Sachdeva**
Project Associates	:	Junita Desouza, 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

Estrogens play a key role in the regulation of different physiological and pathological conditions. Estrogens mediate their actions through their receptors that act like transcription factors and this mode is described as the genomic mode of action. Estrogens are also known to act via extranuclear mode of action i.e. through binding proteins/receptors present in extra-nuclear locations - plasma membrane and cytosol. At the plasma membrane, estrogens can trigger rapid signaling responses that are mediated through activation of intracellular signaling cascades independent of nuclear translocation. In addition to conventional estrogen receptors, GPR30, a G protein coupled receptor, localized to the membrane, is reported to bind estradiol. There exist evidence to suggest prostate cancer (PCa) cell lines, activation of GPER30 via G1 agonist inhibits growth of PCa cells via ERK1/2 and p21 activation while it does not affect quiescent benign prostatic hyperplastic (BPH1) cells. Our previous study demonstrated the relevance of non-genomic estradiol signaling in epithelial to mesenchymal transition (EMT) in prostate cancer cells (Annual report 2018-2019, pp. 94-95). However, it remained uncertain which of the estrogen receptors mediates EMT via

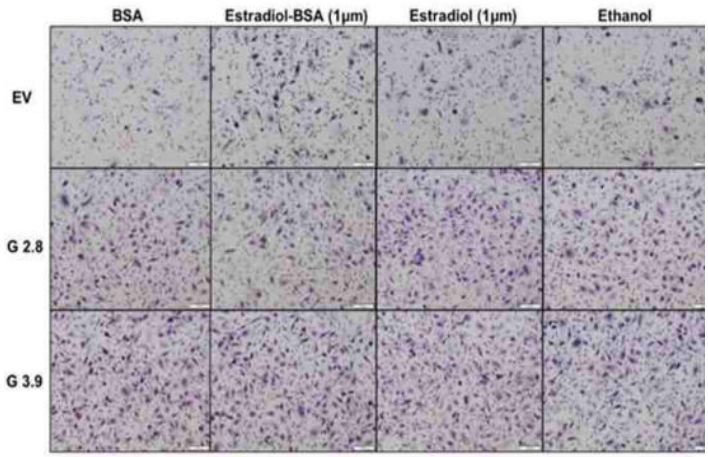


Figure 1: Trans-well migration of GPR30 PC3 knockdown clones (G 2.8 and G 3.9) in response to various stimuli. EV: Empty vector clone

non-genomic estrogen signaling, as our study demonstrated the presence of conventional ERα/β as well as GPR30 on the plasma membrane of prostate cancer cells. The present study was undertaken to determine whether GPR30 mediates EMT via non-genomic estradiol signaling. Towards this, stable GPR30 knockdown clones of PC3 (Annual report 2021-2022, pp. 98) were generated and stimulated with 1µm cell-permeable (estradiol, E2) and impermeable estradiol (estradiol tagged BSA, E2BSA). A significant increase was observed in the invasion of control (empty vector) clones on stimulation with E2-BSA. Further, E2-BSA compared to cell-permeable E2, induced significantly higher invasion. However, GPR30 silenced clones failed to show the similar effect (Fig. 1). To understand the relevance of GPR30 in prostate carcinogenesis the expression of GPR30 in TRAMP (Transgenic Adenocarcinoma of Mice Prostate) mice, a model for prostate cancer, across different stages of PCa progression was assessed. We observed a significant decrease in the number of GPR30 positive cells at the well differentiated (WDC) and PDC (poorly differentiated) stages while a significant increase was found at the high-grade intraepithelial neoplasia (HGPIN) stage compared to age matched control (Fig. 2). At the transcript level, a trend towards increase in GPR30 expression was found at the HGPIN stage. A significant decrease in the GPR30 expression was observed at the WDC stage when compared to age-matched controls. Further studies are underway to treat TRAMP mice with G1, an agonist, at the pre-cancerous stage to investigate if GPR30 activation modulates the disease progression.

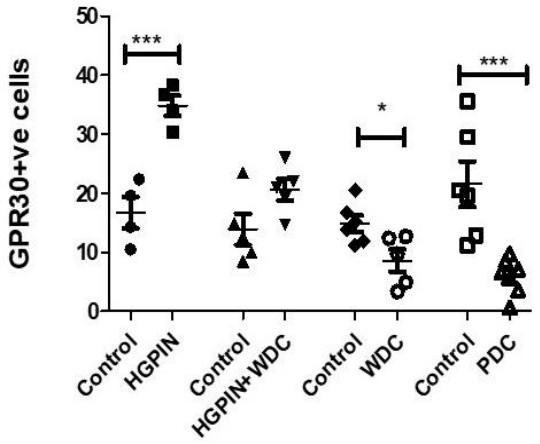


Figure 2: GPR30 positive cells in the prostatic cells at different stages of prostate cancer progression in TRAMP mice, as assessed using flowcytometry. Each group has included at least 4 animals. The data was analyzed using one-way ANOVA and Tukey Post-hoc test was used.
* denotes significance $p<0.05$. HGPIN: High grade intraepithelial neoplasia, WDC: Well differentiated carcinoma, PDC: Poorly differentiated carcinoma.

8.4 Deciphering the Role of PSP94 and CRISP Family Proteins in Ion Channel Modulation *(Partly Funded by Science and Engineering Research Board)*

Principal Investigator	:	**Bhakti R Pathak**
Project Associates	:	Vaidehi Miya, Antara Banerjee, Ananya Breed
Duration	:	2019-2024

CRISPs (Cysteine RIch Secretory Proteins) are evolutionary conserved proteins that show presence of 16 conserved cysteines. Out of these 16 cysteines, 10 reside in the C-terminal domain known as cysteine-rich domain (CRD). In several snake venom CRISPs, a C-terminal subdomain of the CRD is shown to possess ion-channel regulatory activity and this subdomain is also known as ion-channel regulatory (ICR) domain. Though there are many reports on ion-channels regulated by CRISPs present in the venom, information about channels regulated by mammalian CRISPs is limited. Mammalian CRISPs show expression bias towards reproductive tract tissue. Humans express three members of the CRISP family while rodents express an additional CRISP in the epididymis i.e. CRISP4. TRPM8 (Transient

receptor potential cation channel melastatin 8) present on the sperm was reported to be regulated by mouse Crisp4. However, this channel is non-functional on ejaculated sperm. Apart from TRPM8, rodent CRISP1 purified from epididymis is shown to inhibit >50% of CatSper1 (Cation ion channel on Sperm 1) currents by patch-clamping, and by yeast-two hybrid system mouse Crisp2 has been reported to interact with CatSper1 channel. In order to gain insights about ion-channels regulated by mammalian CRISPs as well as to identify novel binding partners, we employed proteomics-based approach. CRISP4 interactome from rat caudal spermatozoa was deciphered by immunoprecipitation followed by mass spectrometry (MS). In the reporting year, the MS data was manually curated and proteins unique to test with at least ≥2 unique peptides were filtered yielding a list of 197 putative rat CRISP4 (rCRISP4) binding partners. Targets were subjected to Gene Ontology (GO) analysis using Metascape server. Fig. 1 highlights the top 5 cellular components, biological processes, and molecular functions that were significantly enriched in our analysis. Being a secreted protein, its interactome showed enrichment of proteins involved in ER-Golgi network and protein folding pathways. Molecular processes also showed enrichment for cholesterol binding which hints towards association of CRISP4 in cholesterol enriched microdomains.

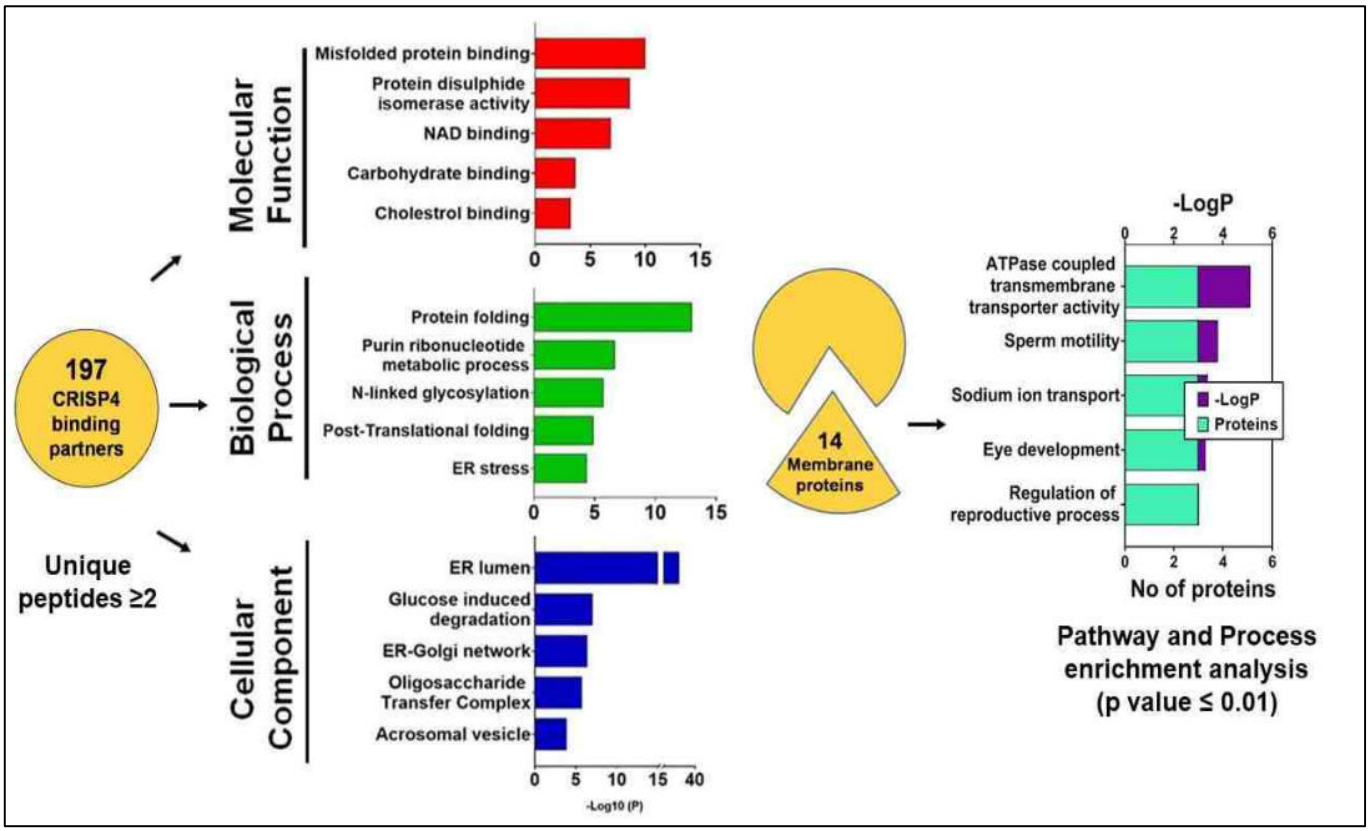


Figure 1: Protein-protein interaction and pathway enrichment analysis for rCRISP4 interactome. Top 5 significantly enriched GO (−log10 (p-value)) annotated terms illustrated proteins to be involved in cholesterol binding, trafficking and protein folding. 14 membrane localizing proteins were shortlisted where ATPase coupled transmembrane transporter activity and sperm motility associated proteins were most enriched. PMCA4b was shortlisted for further validation.

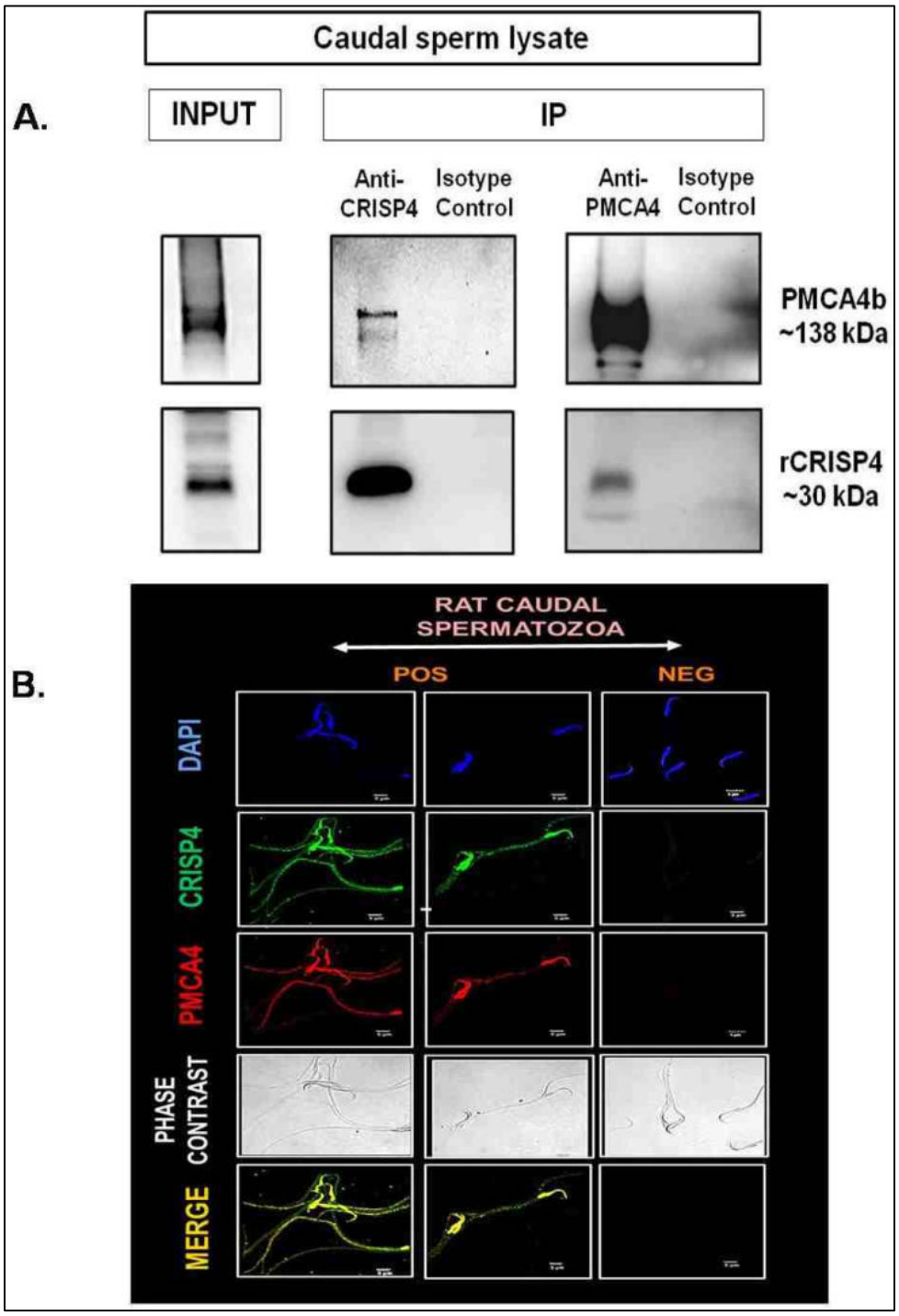


Figure 2: CRISP4 and PMCA4b interact with each other by co-immunoprecipitation a) Lysates from rat caudal spermatozoa were subjected to IP with antibodies against CRISP4 as well as PMCA4. The left panel indicates protein expression in 10% of the total lysate while the right panel shows enrichment of the bait proteins where PMCA4 and CRISP4, respectively got co-IPed.
b) Immunofluorescence of rat caudal spermatozoa for CRISP4 (green) as well as PMCA4 (red) indicates that both the proteins co-localize on sperm head and faintly on the tail. Cell nucleus is stained with DAPI (blue) and sites of co-localization are depicted as yellow foci. Scale bar = 5 µm

The list was further scrutinized for membrane spanning proteins which revealed significant enrichment of proteins involved in ATPase coupled with transmembrane transporter activity and sperm motility from which Plasma Membrane Ca2+ ATPase (PMCA4) isoform b, was pursued for further validation. PMCA4 is the principle channel on sperm that ensures calcium homeostasis. Male mice lacking PMCA4 are reported to be sterile highlighting importance of this channel in sperm function. Interaction of CRISP4 with PMCA4b was validated via co-immunoprecipitation on caudal sperm lysates and co-localization experiments (Fig. 2). Functional implications of this interaction are being investigated further.

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

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

Triple negative breast cancer (TNBC) is a subtype of breast cancer (BC), which lacks hormone receptors (ER/PR/Her2). This malignancy, representing 15–20% of breast cancers, with slightly higher prevalence in Indian population (up to 25%), is a clinical challenge due to the lack of targeted treatments, higher intrinsic aggressiveness, and worse outcomes than other breast cancer subtypes. An extensive molecular heterogeneity within tumors is frequently observed across different TNBC patients. Emerging evidence indicates the essential role of epigenetic mechanisms such as DNA methylation, histone modifications, and the modulation of gene regulatory elements in regulating gene expression and transcriptional regulation of pro- and anti-cancer immune response genes in tumor-microenvironment. Many studies have highlighted the importance of epigenetic deregulations in promoting immune escape mechanisms in different cancers including TNBC. These alterations are particularly interesting since they can be reverted through the inhibition of epigenetic regulators. Members of toll-like receptors (TLR), a family of pattern recognition receptors (PRR) have been previously associated with immunoregulatory roles and chemotherapy response in TNBC and other cancers. Epigenetic alterations like DNA methylation has been shown to regulate the TLR expression in different diseases like type 1 diabetes. However, regulatory role of epigenetic alterations in TLR genes in TNBC is poorly understood. Objective of this study was to quantify the methylation level of TLR4 gene promoter in a panel of TNBC cell lines mimicking different molecular subtypes of TNBC tumors (MDAMB 231: Mesenchymal stem cell subtype, MDA MB 468, HCC70: Basal-like subtype, HCC1187: immunomodulatory subtype) and normal breast epithelial cells (MCF10A). Four CpG sites in TLR4 promoter were analyzed by methylation-sensitive pyrosequencing of bisulfite-modified DNA, extracted from these cell lines. Mean methylation level in 3 of the breast cancer cell lines tested was 3-6% while it was 95% in HCC1187 TNBC cells and 31% in normal like breast epithelial cells respectively (Fig. 1). This suggests a variation in TLR4 promoter methylation levels exists in TNBC and warrants further studies to understand its effect on TLR4-driven mechanisms of pathogenesis of disease. Currently, we are extending analysis to TNBC tumor samples collected from TNBC patients.

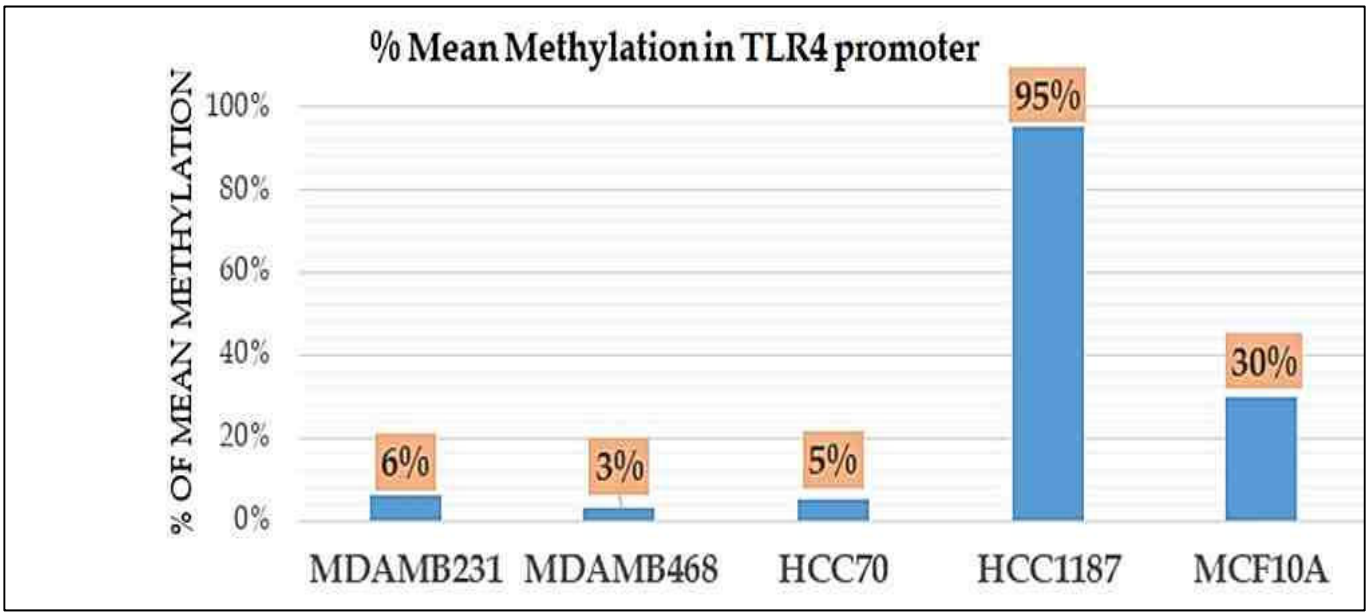


Figure 1: TLR 4 promoter methylation level in breast cancer cell lines: Histogram showing the mean DNA methylation levels at four CpG sites in TLR4 promoter region in TNBC cell lines: MDA MB 231, MDA MB 468, HCC1187, HCC70, TNBC cells, and MCF10 A cells (normal mammary epithelial cells). Four CpG sites in TLR4 promoter were analyzed by pyrosequencing of bisulfite-modified DNA extracted from the cell lines. The boxes in orange contain the percentage of average DNA methylation.

8.6 Identification of Circulating microRNA Signatures as Diagnostic Markers for Early Stage and Metastatic Breast Cancer *(Funded by Indian Council of Medical Research)*

Principal Investigator	:	**Sadhana M Gupta**
Project Associate	:	Rinki Doloi
Collaborators	:	S Mehta, Saifee Hospital, J Anam, Surgical Speciality Oncology Hospital, V Maniar, Mumbai Oncocare Hospital
Duration	:	2021-2024

The aim of the study is to identify circulating microRNA profiles in breast cancer patients as blood-based markers for detection of early stage and metastatic breast cancer. A total of 120 women with breast cancer and 30 normal healthy controls have to be enrolled for this study. We have divided our study participants into 4 groups (30 cases each), according to their molecular subtypes i.e. Luminal A (ER+, PR+ and HER2), Luminal B (ER+, PR- and HER2-), HER2 positive and Triple Negative (ER-, PR- and HER2). So far, 73 women with breast cancer and 24 healthy controls have been recruited. Profiling of microRNA has been carried out in 11 serum samples (7 breast cancer patient samples and 4 normal healthy control samples) using Next Generation Sequencing (NGS). The NGS analysis has revealed that out of 1554 differentially expressed miRNAs, 23 miRNAs were significantly expressed (Fig. 1 - top panel). Of these, 12 miRNAs were significantly downregulated and 11 were significantly upregulated. Validation by Real-time PCR of 8 miRNAs (miR-4306, miR-30b-5p, miR-125b-5p, miR-141-3p, miR-490-5p, miR-200a-3p, miR-4508 and miR-548aq-5p) is under process. miR-200a-3p and miR-141-3p are

downregulated in serum samples of breast cancer patients as compared to the controls, in agreement with the NGS data (Fig. 1 - bottom panel).

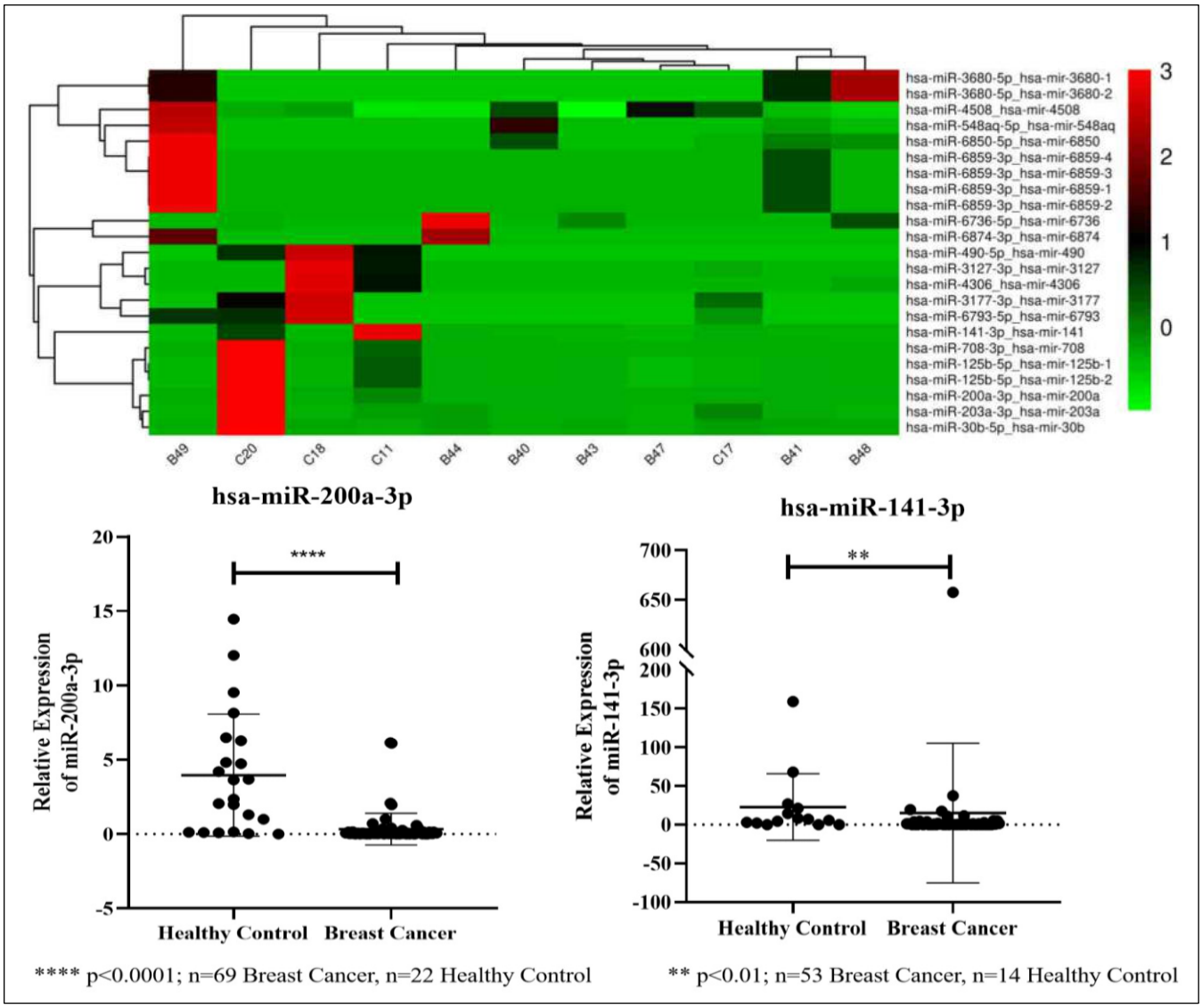


Figure 1: Top panel - Heatmap showing the differentially expressed miRNAs in breast cancer patients. Bottom panel - Downregulation of expression of miR-200a-3p and miR-141-3p in breast cancer patients compared to healthy controls

Further, GO enrichment analysis for the 23 differentially expressed miRNAs in biological process (BP), cellular component (CC), and molecular function (MF) associated genes was carried out. The significantly enriched GO terms related to BP were peptidyl-serine phosphorylation, histone modification, negative regulation of gene expression, transforming growth factor beta receptor signaling pathway, cellular senescence, cellular response to glucose starvation and G1/S transition of mitotic cell cycle. KEGG pathway analysis showed that signaling pathways regulating pluripotency of stem cells, EGFR tyrosine kinase inhibitor resistance, multiple pathways in cancer were significantly altered (Fig. 2).

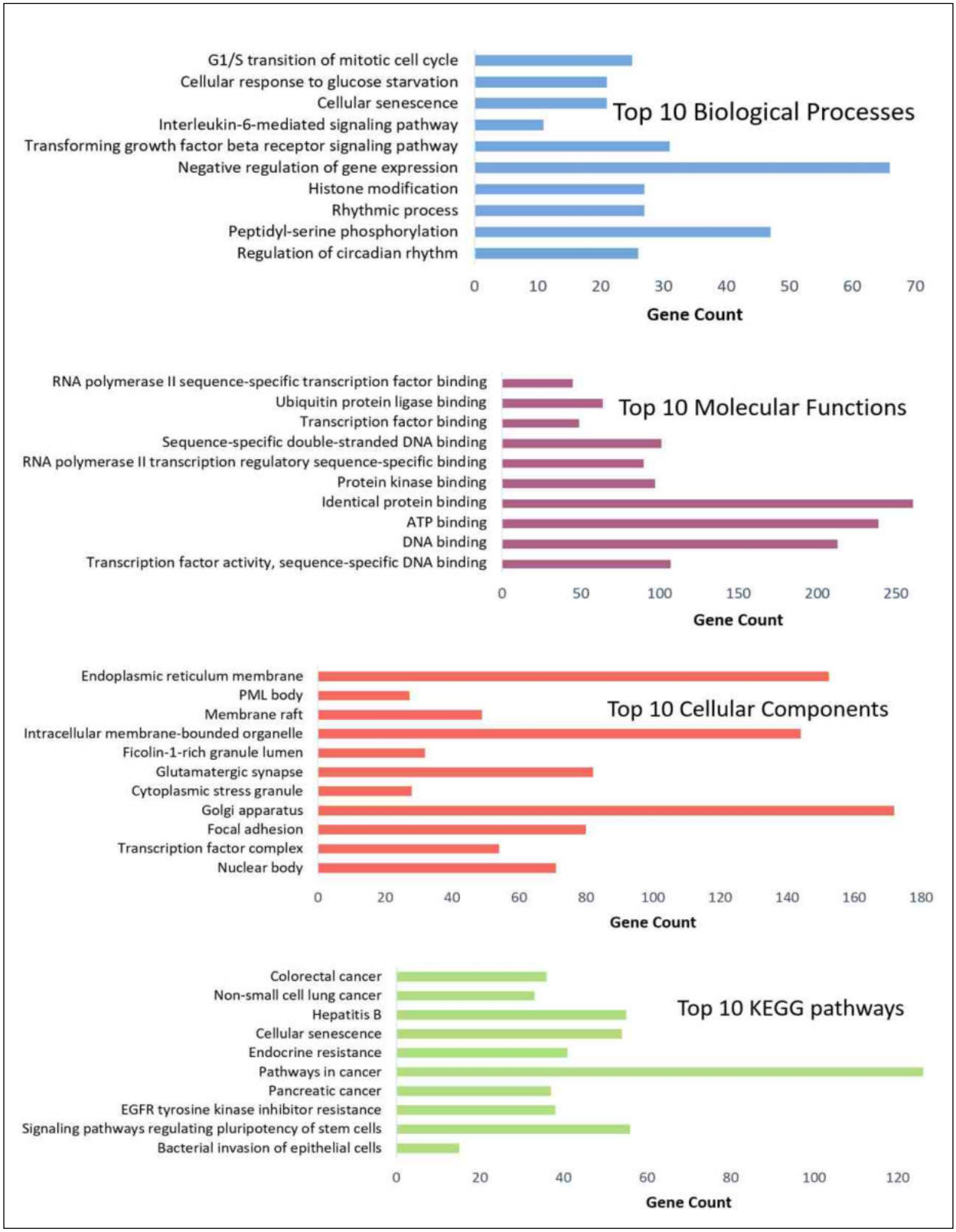


Figure 2: GO Enrichment Analysis of the differentially expressed miRNAs for biological processes, molecular function, cellular components and KEGG pathway analysis

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

Principal Investigator	:	**Nupur Mukherjee**
Co-Principal Investigators	:	Geetanjali Sachdeva, D Modi, Susan Thomas
Collaborators	:	Vandana Bansal, Norwojee Wadia Maternity, Hospital, Mumbai Jyoti Bajpai, Tata Memorial Hospital, Mumbai
Duration	:	2022-2025

Pregnancy Associated Breast Cancer (PABC) is a subtype of breast cancer (BC) diagnosed during pregnancy or within two years postpartum. Interestingly, a number of studies have reported presence of inflammatory changes in breast tumors of PABC patients. Although rare, PABC incidence is rising in urban settings due to women delaying the age of first pregnancy to age ≥30 years. Pregnancy-associated changes in the maternal system have been previously shown to potentially play a role in BC pathogenesis. One of these contributing factors is the placental tissue secretions, which affect physiological functions in the mother such as breast tissue remodeling to prepare the breast tissue for lactation. Previous studies have shown that placental tissue secretome can significantly alter BC cell phenotype and function (cell proliferation, invasion, and migration). We also previously reported that placental cell secretome could alter BC cell proliferation and clonogenic abilities. However, molecular mechanisms involved in the process are not clearly understood. The aim of the current study was to identify the molecular pathways regulating the pathogenesis of PABC and evaluate potential effect of placental tissues/cells on pathogenesis of PABC. Firstly, analysis of publicly available microarray datasets on PABC tumors (GSE31192) was done using various bioinformatics tools such as GEO2R and ENRICHR to identify enriched gene networks and pathways significantly associated with PABC tumors. These analyses indicate an enrichment of TLR4 signaling pathway in PABC tumors. The expression of TLR4 was further measured by qRT-PCR in breast cancer cells (MDA MB 231) exposed to placental cell (HTR8) supernatants. As observed in the microarray datasets, the qRT-PCR analysis showed an approximate 2-fold increase in TLR4 expression in BC cells (MDA-MB-231) exposed to spent culture medium of HTR8 cells, mimicking first-trimester placental cells, compared to untreated BC cells (controls) (Fig. 1) thereby suggesting a potential role of TLR4 signaling pathway in the pathogenesis of breast cancers diagnosed during pregnancy. This preliminary analysis reflects the emerging roles of immune regulated pathways in PABC pathogenesis. In-depth analysis using both cell culture-based assays and patient samples is required to better understand the molecular mechanisms associated with breast carcinogenesis associated with pregnancy.

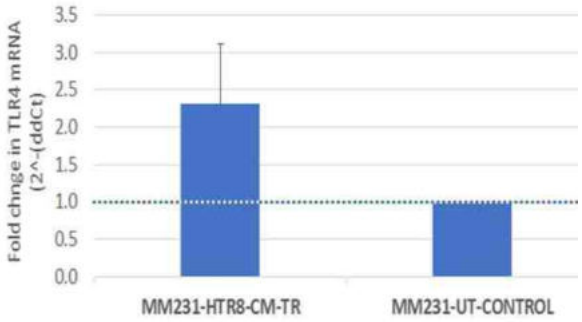


Figure 1: Fold Change in TLR4 gene expression in MDA-MB-231 cells treated with HTR8 conditioned medium, determined by q-RT PCR analysis. Fold change in expression was calculated using 2-ΔΔCt method with 18S rRNA as reference. Experiments were repeated in triplicates. Dotted line represents basal level of TLR4 expression.

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 membrane-bound structures classified into exosomes, micro-vesicles, and apoptotic vesicles based on their origin. Molecular cargo of EVs which consists of nucleic acid, proteins and lipids, determines their effects on recipient cells. EVs, in particular exosomes, play important role in cancer progression, chemoresistance and metastasis. Ovarian cancer is the most lethal gynaecological malignancy due to late detection and chemoresistance. Present study aims at characterization of proteomic and transcriptomic profile of exosomes derived from ovarian cancer cell lines and evaluation of their paracrine effects. Cells grown under 3D culture condition mimics the tumor in vivo. Therefore, EVs liberated by ovarian cancer cells grown under 2D and 3D condition were isolated and analysed. EVs were isolated from ovarian cancer cell line OVCAR4 (2D and 3D) by differential centrifugation method and its morphological characterization was done by TEM analysis at ACTREC. Particles in the size range of 50-200nm were seen in TEM images (Fig. 1A). Exosomes were isolated from both 2D and 3D cultures and were probed with known exosomal markers like CD9 and CD81 as well as with markers of intracellular components like GAPDH and calnexin to confirm isolation of a pure exosome population devoid of any cellular contaminants (Fig. 1B).

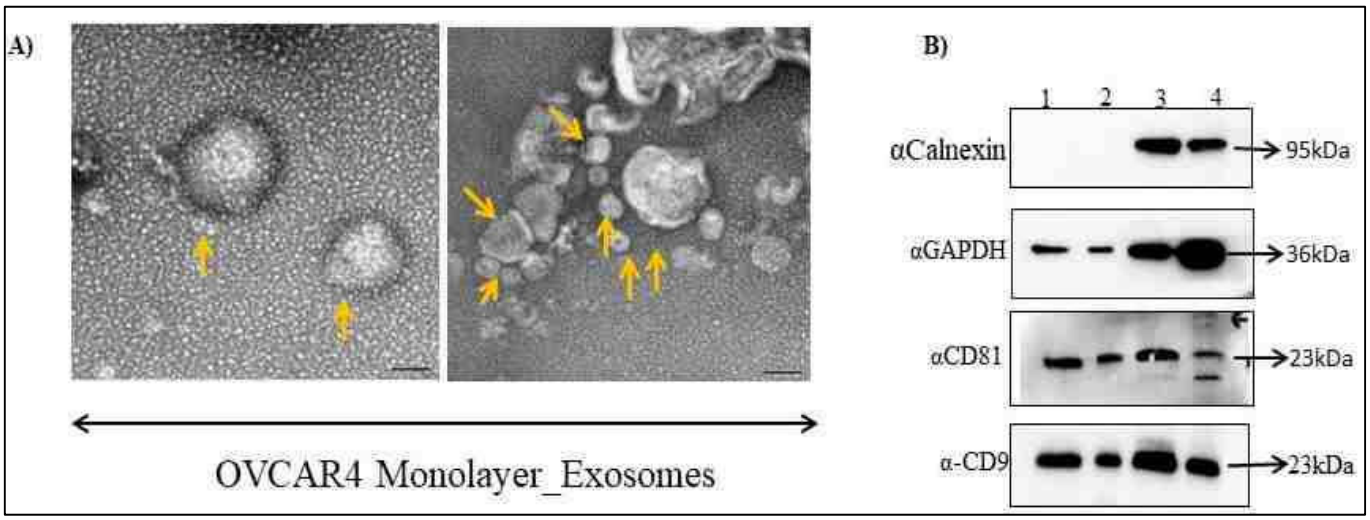


Figure 1: Characterization of EVs A) TEM (Transmission Electron Microscopy) images of EVs isolated from OVCAR4 cell line grown in 2D. Arrows indicate different size EVs. Average size of the population in one field lies in the range of ~50-150nm (scale bar=100nm) B) Detection of CD81 and CD9 by western blotting in EVs isolated from monolayer (lane1) and spheroid (lane2) of OVCAR4. Lysates from monolayer and spheroids are in lanes 3 & 4. GAPDH is shown as loading control for total protein in lysates. Calnexin was detected exclusively in lysates.

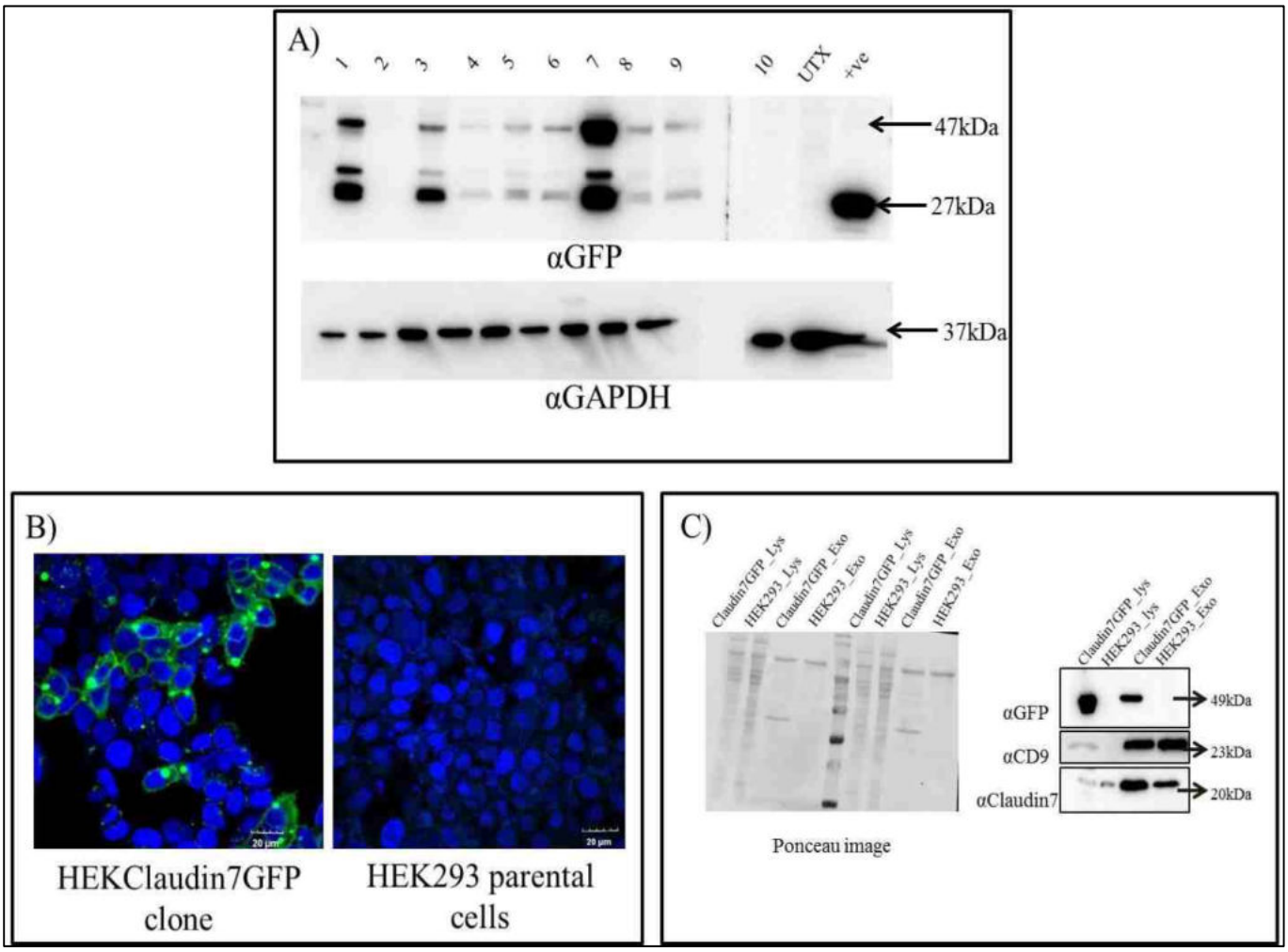


Figure 2: A) Western blotting for screening claudin 7-GFP clones. Fusion protein of ~47kDa was detected using anti-GFP antibody in lane 1 and 3-9. GAPDH was shown for loading control. B) Confocal images of Claudin7 GFP stable clone and HEK293 (parental cells). GFP positive cells were observed as green fluorescent cells in Claudin7GFP stable clone under 60X magnification (scale bar=20uM). C) Western blotting showing GFP-tagged claudin7, CD9 and claudin 7 in EVs isolated Claudin7GFP clone and parental untransfected cells.

Approximately 30ug of total proteins from EVs was subjected to label-free quantitative proteomics. A list of differentially and uniquely expressed protein cargo in 2D vs 3D culture derived exosomes was obtained which is being analysed. Earlier investigations from our laboratory have reported presence of Trop2 and claudin-7 in the EVs liberated from ovarian cancer cells. Since the current study also aims at evaluating EV uptake, EVs expressing GFP-tagged marker proteins would be needed. Hence, GFP-tagged claudin-7 construct was procured. Stable clones expressing claudin7-GFP were generated in HEK-293 cells. Briefly, 48hrs post-transfection with claudin-7GFP construct, cells were allowed to grow in selective media containing G418 (500µg/ml) for about 2weeks. G418-resistant clones were picked up and expanded. They were observed for green florescence under fluorescent microscope and also screened by western blotting using anti-GFP antibody. Total 12 clones were picked up and screened. Many of the clones were found to be expressing claudin 7-GFP, however, one of them was strong expressor of claudin7-GFP as compared to other clones (lane 8, Fig. 2A&2B). It was propagated further

along with the parental cells to isolate EVs. Both these batches of EVs were subjected to immunoblotting and probed with anti-Claudin 7, anti-GFP and standard EV marker anti-CD9. Exclusive presence of signal with anti-GFP antibody in claudin7-GFP clone derived EVs along with CD9 confirms that stable clone liberates EVs with claudin 7-GFP as their cargo (Fig. 2C). These GFP-tagged EVs can be used to track the pathway of uptake by recipient cells.

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, Dhanashree Jagtap
Collaborators	:	Amita Maheshwari, B Rekhi, Tata Memorial Hospital, Mumbai
Duration	:	2017-2024

Trop2 is a transmembrane glycoprotein, which is overexpressed in multiple cancers including ovarian cancers. Ovarian cancer is usually diagnosed at an advanced stage where patients often show presence of ascites. This study investigated expression of Trop2 and its binding partner claudin-7 in the ascitic cells from ovarian cancer patients. Trop2 is reported to undergo proteolytic cleavage and its ECD is released in circulation. We have also reported it to be present in the extracellular vesicles. Therefore, its presence in the ascitic cells as well as in the cell-free ascitic fluid was investigated and reported (n=36) (Annual report 2021-22, p. 112).

Autoantibodies generated against certain tumor antigens have a potential to act as prognostic biomarkers. The serum samples from the same patients and healthy control women were investigated for the presence of autoantibodies against Trop2 using Surface Plasmon Resonance (SPR). Presence or absence of detectable anti-Trop2 autoantibodies was further correlated with the level of Trop2 expression in the ascitic cells in those patients. Recombinant Trop2 protein at 2.5µg/ml was coupled to carboxmethylated dextran matrix sensor chip (CM5). Sera of ovarian cancer patients were passed over it to evaluate binding of Trop2 protein with ovarian cancer patient's serum protein or immunoglobulins. Sera of ovarian cancer patients showed binding to Trop2 at varying levels (Fig. 1A). Further, anti-human IgG was passed over the bound sera to confirm the presence of anti-Trop2 antibodies in the sera. It was observed that human Trop2 IgG autoantibodies are present in the sera of ovarian cancer patients (Fig. 1B). After applying a cut-off (>25 RU) we found that out of 29 ovarian cancer cases, five showed autoantibodies against Trop2 (~17%) and out of 40 healthy controls, three showed autoantibodies against Trop2 (~7%). Trop2 levels in ascitic cells were compared with the anti-Trop2 autoantibodies in the sera of ovarian cancer patients. Those showing Trop2 levels above the 75th percentile were designated as high expressors of Trop2 (n=7) and below 75th percentile were considered as low Trop2 expressors (n=21). Out of 7 high Trop2 expressors, 3 patients (42.8%) showed anti-Trop2 autoantibodies whereas 2 out of 21 (9.5%) low Trop2 expressing patients showed anti-Trop2 autoantibodies.

Our data indicates that there is a correlation between high Trop2 expression by ovarian cancer cells and presence of anti-Trop2 autoantibodies. Literature indicates that high Trop2 expression is linked to worst disease prognosis in cancer patients. Therefore, anti-Trop2 autoantibodies may act as surrogate marker for stratifying ovarian cancer patients with high Trop2 expression and they have the potential to act as prognostic biomarker for ovarian cancer.

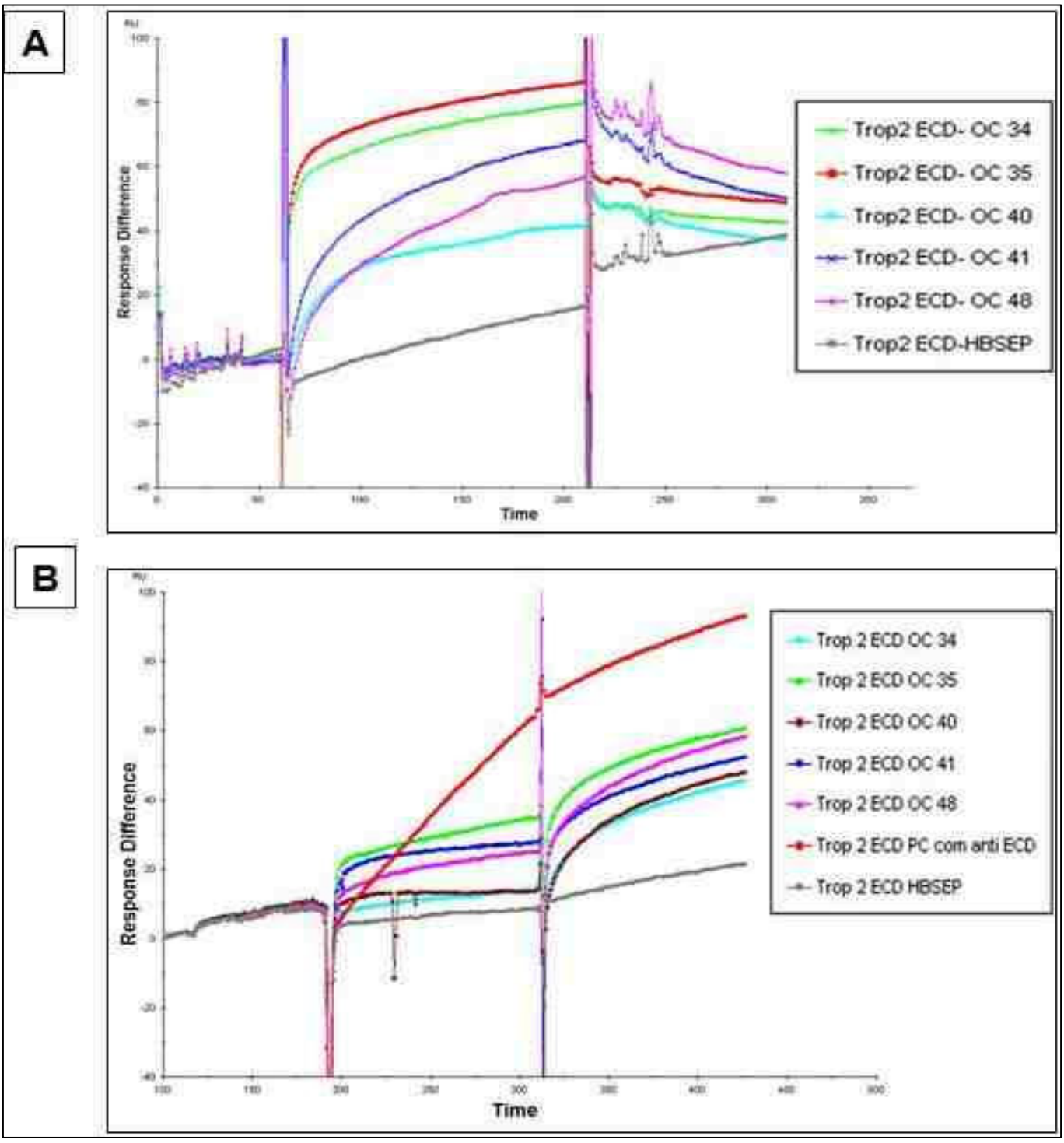


Figure 1: A) Binding analysis of immobilized Trop 2 protein with sera of ovarian cancer patients. Representative sensogram of five ovarian cancer patients (OC 34, 35, 40, 41 & 48) is shown. HBSEP served as a buffer control in the experiment. B) Sera of ovarian cancer patients were passed over immobilized Trop2 followed by human IgG antibody. Representative sensogram of five ovarian cancer patients is shown. Commercial anti-Trop2 antibody was used as positive control (PC) followed by anti-goat IgG antibody. HBSEP served as a buffer control in the experiment.

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 Investigators	:	Anushree Patil, Sharmila Pimple, S Biswas
Project Associates	:	S Chauhan, Suchitra Surve, Shahina Begum, Deepti Tandon
Collaborators	:	Sharmila Patil, Sanjay Biswas, Kedar Deodhar, Tata Memorial Hospital (TMH), Mumbai
Duration	:	2019-2023

Cervical cancer and premalignant lesions constitute a major problem in women´s health. Although screening for cervical cancer by visual inspection with acetic acid (VIA) and Lugol's Iodine (VILI), the Papanicolaou test (Pap smear) and HPV DNA testing is known to reduce mortality by early detection and treatment, however; in low resource settings, its successful implementation is challenging due to a dearth of financial, technical, and logistic inputs. In recent times, hybrid capture II (HCII, Qiagen, Germantown, MD, USA) test system has emerged as a gold standard for detection for HPV DNA detection. A recent modification of the HC2 system is the careHPV test system (Qiagen, Germantown, MD, USA) is rapid, less expensive, requires minimal training, lesser reagents and has greater sensitivity than cytology. These advantages make it conducive for training in Low and Middle Income Countries (LMICs). However, the performance of this assay has not been evaluated extensively in LMICs. In this community based study, eligible women were screened for cervical precancers and cancers by low cost molecular careHPV test, HC2 test, Pap smear and VIA test. Awareness activities and camps were conducted at both urban and rural study sites to motivate the eligible women for cervical cancer screening. Total 1542 study participants, 1024 from Mumbai and 518 from Dahanu, Palghar were recruited after conducting formative study on knowledge, attitude and practices (KAP) on initial 1000 women. A total 939 women with median age 38 years ± 9.24 years met the inclusion criteria. Out of them, about 44% women, 55% from urban and 31% from tribal community had heard about cervical cancer. Majority of KAP participants were unaware about symptoms 250 (60%) and risk factors 296 (71.15%) for cervical cancer. Lack of information and fear of pain were the major perceived barriers besides shyness and fear of getting cancer after undergoing screening. Among 939 KAP participants, 71.72% underwent cervical cancer screening. We did comprehensive screening of total 1542 women from urban and tribal communities. Socio-demographic characteristics of study population in urban (n=1024) and rural/tribal (n=518) population have been analyzed. Majority of 1356 (87.93%) women were married and belonged to upper lower class at Mumbai as well as Dahanu site. At Mumbai site, about 775 (75.68%) women were educated with at least high school level but at Dahanu, majority 262 (50.57%) recruited women were illiterate. High-risk HPV positivity for women was correlated with various socio-demographic factors. It was found to be significantly correlated with use of smokeless tobacco, alcohol, number of sexual partners≥2 and high grade lesions on PAP smear. Pap test reports revealed Atypical Squamous cells of Undetermined Significance (ASCUS) in 14 cases, Low grade squamous intra epithelial lesion (LSIL) in 7 women and High grade squamous intra epithelial lesion (HSIL) in 6 women. All screen-positive women were referred to TMH for follow up. Two women

underwent cryotherapy for Cervical Intraepithelial Neoplasia (CIN 2 lesion) and 2 participants underwent Loop Electrosurgical Excision Procedure (LEEP) for CIN2/3 lesion on biopsy. Considering HC2 test as gold standard, the sensitivity of careHPV was 94.52%, specificity 98.43%, positive predictive value was 75% and negative predictive value as 99.72%. The accuracy was found to be 98.25%. Genotyping was done for the HPV positive samples by genexpert HPV test. 12 samples showed only HPV 16 genotype. More than one out of HPV subtype 16, 18/45 and other high risk HPV types were seen in 14 samples. Out of 74 samples tested by both genexpert and NIRRCH HPV PCR concordance was seen in 67 (90.54%).

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 Indian Council of Medical Research*)

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

This project is a collaboration between ICMR-NIRRCH 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 ANMs and facility survey were done to assess readiness of health system. Findings of formative research highlight that there is limited knowledge on non-communicable diseases (NCDs) and common cancers among tribals as literacy is low. Women accessed the services of traditional healers like Bhagats. Barriers were transportation, time spent at the public health care facility and behaviour of the staff. ASHAs are key connecting link between women and health system. ASHAs need incentives for transport and some financial remuneration. Hence, awareness and health seeking behaviour need to be improved through health education activities involving the ASHA workers. Health care facilities need to be upgraded in terms of infrastructure, equipment and trained manpower to implement screening services. These research findings were used during the implementation phase for creating awareness in tribal area through different activities, for training medical officers for screening NCDs, for motivating ASHAs and ANMs in encouraging tribal women to undergo screening for NCDs and providing incentives and travel compensation to ASHAs. A total of 503 participants were screened during the study period. We found that 78.3% (n=394) of the recruited population belonged to the Warli tribe. About 49.9% (n=251) were illiterate and 41.4% (n=208) women were working as labourer in Chickoowadi. 51.3% (n=258) had less than Rs 10,000 monthly income and 69.6% (n=350) women had dal and rice as staple food. We found that 5% (n=25) women were unmarried but sexually active and 8.7% (n=44) were consuming tobacco (tapkiri) and 7.6% (n=38) women consumed alcohol (tadi). We screened the participants for NCDs as well gynecological examination, clinical breast examination and oral cavity examination. Out of 503 participants, 23.1% (n=116) women were underweight, 31.2% (n=258) women had low haemoglobin, 21.3% (n=107)

women were hypertensive, and 7.2% (n=36) women had high random blood sugar. In gynecological examination, we found that 5.4% (n=27) women were positive by VIA and 6.7% (n=34) women were tested positive for HPV. Gram staining reported 5.9% (n= 30) positive for Candida and 42.1% (n= 212) were positive for bacterial vaginosis. In oral screening, melanoplakia was observed in 2.6% (n= 13) women and 9 of them were smokeless tobacco users, 0.39% (n=2) had cyst under gum line. We also found one patient had leukoplakia and one patient had suspicious growth. In breast examination, we found that 4.6% (n=23) women had nipple discharge other than milk, 4.8% (n=24) had fibro-adenoma of breast. Screen-positive women were not ready to travel to Mumbai for the follow up at Tata Memorial Hospital, so a follow up was arranged at MRHRU.

Figure 1: Awareness activity at Anganwadi at Sarawali Sub Center on September 3, 2022

Figure 2: Counseling and educating ASHA workers at SDH, Dahanu

HTA AND DRUG DISCOVERY

9. HTA AND DRUG DISCOVERY

9.1 To Determine Cost-effectiveness of Rapid Diagnostic Tests (Hemo-Type-SC, Sickle Scan and Gazelle) in Comparison to Solubility Test Followed by HPLC for Sickle Cell Disease/Trait Diagnosis among High-risk Population in India *(Partly Funded by Department of Health Research)*

Principal Investigator	:	**Beena Joshi**
Co-Principal Investigator	:	Kavitha Rajshekhar
Project Associates	:	Gyani Gaurav, Kirti Tyagi, Rehana Mohammed, H Chaurasia, Revathy Panicker
Collaborators	:	PGIMER, HTA Resource Hub HTAIn DHR
Duration	:	2022-2023

In response to the request from the Ministry of Health and Family Welfare, health technology assessment was undertaken to inform which point of care rapid tests available in Indian market for sickle cell screening (Hemotype SC, Sickle Scan) are cost-effective to be used for population based screening of Sickle Cell Disease in Indian context, compared to the standard of care (solubility test along with HPLC). Clinical effectiveness of both tests were comparable and superior over current standard of care solubility test. The disease burden and evidences on the cost-effectiveness of point of care (POC) tests along with their sensitivity and specificity were collated through literature review. The health system costs for sickle cell screening were estimated from the standard treatment guidelines, Ayushman Bharat, Costing of Health Services in India (CHSI) database and expert opinions. Age group considered for the screening was 0-30 years and population considered for the model was high-risk population in selected states of the country with high disease prevalence. Literature review and expert consultation done as a part of landscape analysis of this study suggested that solubility test is not suitable for testing Sickle Cell Disease and Trait in newborns, as number of false negatives will be high. Therefore, in this part of the model, solubility as a comparator was not considered.

Using Markov model, cost per individual screened and cost per test) using the POC tests Hemotype SC and Sickle Scan were estimated. Cost per test for Hemotype SC and Sickle Scan was INR 250.17 and for Solubility + HPLC was INR 53.32. Incremental Cost-effectiveness Ratio (ICER) per case detected for Hemotype SC was 3,46,437 and 3,47,466 for 2-30 years and 0-30 years, respectively. ICER per case detected for Sickle Scan is 3,04,090 and 3,04,284 for 2-30 years and 0-30 years, respectively. ICER per case detected suggests that at current price these POC tests are not cost-effective. However, if Hemotype SC Kit and Sickle SCAN Kit can be procured below INR 100, these tests may become cost-effective. The POC tests as compared to HPLC is cost effective in 0-2 years, as solubility test can not be done for newborns due to interference of foetal hemoglobin. POC tests may be considered for adoption in Sickle Cell Screening programme for neonates (0-2 years).The results from the study were used by the Ministry to inform the states to procure the POC kits at the suggested rates (below INR 100) to ensure that the testing is cost-effective.

9.2 Identification of Enriched Biochemical Networks and Polypharmacological Targets for Metabolic Syndrome *(Partly Funded by Science and Engineering Research Board, Department of Science and Technology - STAR)*

Principal Investigator	:	**Susan Thomas**
Project Associate	:	Indra Kundu
Duration	:	2021-2024

GeDiPNet, an integrated knowledgebase for genes, pathways and networks associated with diseases, was used for identification of polypharmacological targets for Metabolic Syndrome (MetS). The polypharmacological target prediction tool of GeDiPNet is based on the principle that genes commonly associated with a group of diseases will be multifunctional, present in multiple pathways and have important network features. Hence, the first step in the algorithm was to identify the genes associated with more than one disease. In the second step, overlapping genes were subjected to pathway enrichment analysis using KEGG pathways. In the third step, network analysis was performed on the genes associated with significantly enriched pathways along with their interactome. The protein-protein interaction data, from curated databases or experimentally determined, available in STRING database was used for network creation. The critical genes (nodes) of network were identified based on network topological properties such as degree, weighted degree, closeness centrality and betweenness centrality (Fig. 1). Genes associated with the four MetS components namely obesity, hyperglycemia, hypertension and dyslipidemia were used as input for the prediction algorithm. 187 genes were commonly associated with all four MetS components. Utilising the common genes the algorithm identified 32 genes that included INSR, JAK2, REN, GSK3, DPP4, MAPK, ESR1 and MC4R as potential targets (Fig. 1).

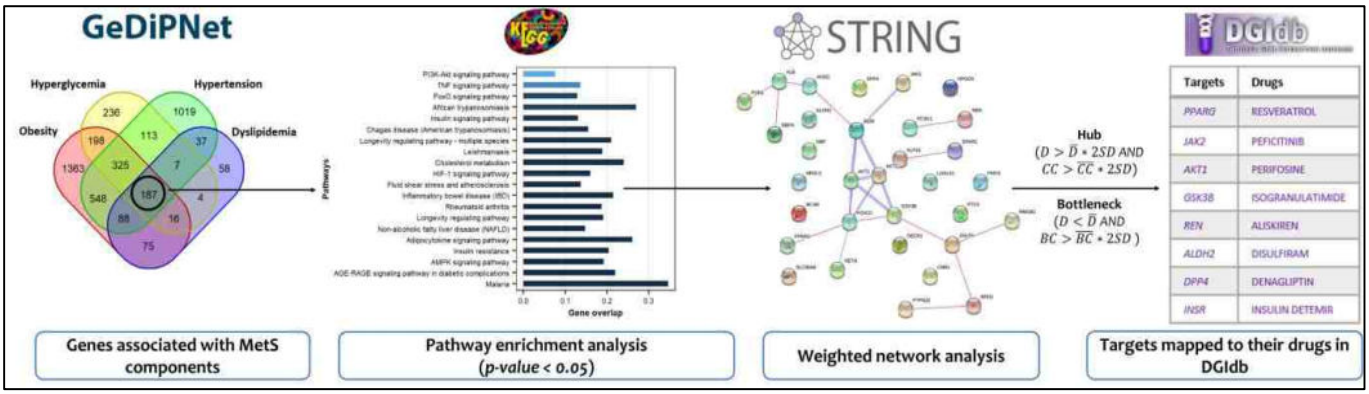


Figure 1: Algorithm employed for identification of MetS polypharmacological targets

Many of these targets are currently being used in the management of isolated components of MetS, for example: i) hyperglycemia is managed using inhibitors of JAK2 and GS3K; as well as agonists of PPARG and INSR, ii) hypertension is managed using inhibitors of REN and JAK2, and iii) hypertriglyceride is managed by PPARG agonist. In addition to these known targets, few targets identified by the algorithm such as DECR1, involved in fatty acid metabolism and inflammation, and HPGDS, involved in renal programming, are worthy of further validation as therapeutic targets for

MetS. Subsequent to target identification, the algorithm was used to map the identified targets to their known drugs from DGIdb. The predicted targets DECR1 and HPGDS that are involved in fatty acid metabolism and inflammation will be validated as therapeutic targets for MetS in future studies.

9.3 Evaluation of Drug-Cytochrome P450 Enzyme Interaction through Fluorometric High Throughput Screening Assays *(Partly Funded by Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Govt. of India)*

Principal Investigator	:	**V Dighe**
Co-Principal Investigator	:	Susan Thomas
Project Associate	:	Amruta V Gadade
Collaborator	:	Pallavi Jamadagni, RARI, Pune
Duration	:	2021-2024

Cytochrome P450 superfamily has been implicated as one of the most important drug-metabolizing enzyme families because its members are reported to be involved in metabolizing a wide range of pharmaceutical drugs. In the proposed study, known ayurvedic formulations are being studied for their Herbal Drug interactions against the key markers: CYP1A2, CYP3A4, CYP2D6, and CYP2C19. It is important to include preclinical (*in-vitro*) and clinical (*in-vivo*) interaction studies the drug development process. In the *in-silico* study, structures of 45 active principles of 30 traditional medicinal formulations were downloaded from the PubChem database. Molecular Docking of 45 active principles with CYP450 was performed using the CDOCKER docking optimization tool. Ten 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 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 P450 isozymes. Herbal Drug interaction study is undertaken with the commercially available liver microsomes. Herbal formulations, Laksha Guggulu, Ayush GG, Ayush AG, and Ayush D show higher IC50 than the positive control. The higher IC50 than the positive inhibitor indicates that test formulations and their constituents have moderate interaction in drug metabolism and suggests that traditional use of this drug is safe. So Laksha Guggulu, Ayush GG, Ayush AG, and Ayush D are proven safe as ayurvedic formulation for traditional use.

9.4 Exploring the Therapeutic Potential of Peptides Targeting Lysophosphatidic Acid Receptors in Ovarian Cancer *(Partly Funded by Department of Biotechnology)*

Principal Investigator	:	**VD Dighe**
Co-Principal Investigator	:	Taruna Madan
Project Associates	:	Bhavana Bhat, Yugandhara Jirwankar, Akanksha Nair, S Jadhav, P Salunke
Duration	:	2021-2024

Ovarian cancer is one of the leading gynecological cancers affecting women globally. Lysophosphatidic acid (LPA) is one of the key molecules elevated in ovarian cancer and its receptor LPAR2 and LPAR3 are overexpressed in ovarian cancer. LPA –LPAR interaction activates the signaling pathways leading to cell proliferation, invasion and metastasis in ovarian cancer. Hence, the present study aims to explore the therapeutic potential of the peptides targeting LPAR3 in ovarian cancer.

Full-length recombinant LPAR3 (rLPAR3) was used as bait for in vitro panning using phage display library. Three rounds of in vitro panning were carried out for the identification of LPAR3 binding peptides. The phage recovery titer after each round of in vitro panning was quantified by qPCR approach by standard curve method where M1 ssDNA was used as standard. There was an increase in the phage titer in each round of *in-vitro* panning. This suggests the enrichment of phages in the subsequent rounds of in vitro panning.

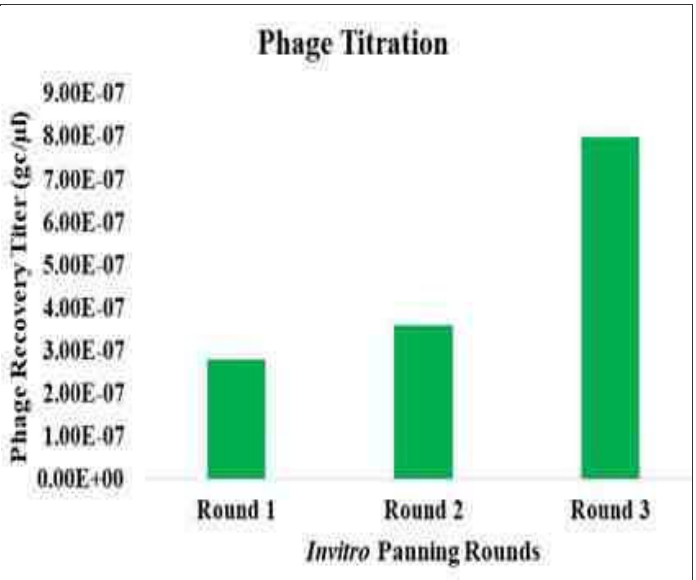


Figure 1: Phage titration of three rounds of in vitro panning by standard curve method

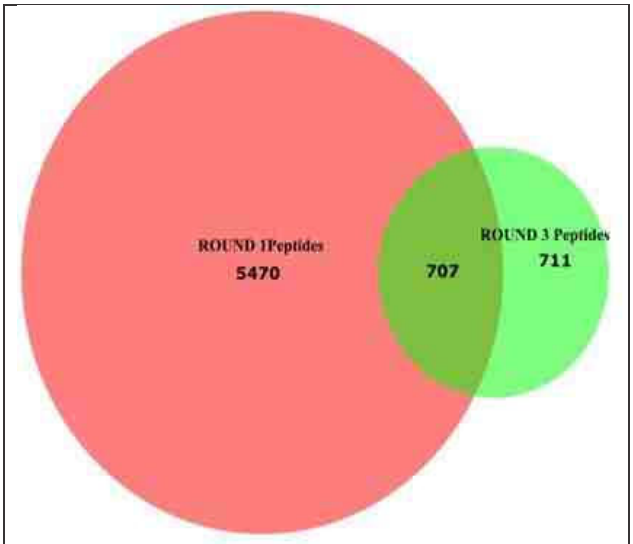


Figure 2: Venn diagram showing the number of peptides in the in vitro rounds of panning (DeepVenn, 2020-2023, Tim Hulsen)

Table 1: List of top 10 peptides with their frequencies in Round 1 and Round 3

Peptide Name	Peptide sequences	Peptide Frequency in Round1	Peptide Frequency in Round 3
LP 1	XQMXXXYXQSXX	132472	250369
LP 2	TXXRXXVXSXXA	18727	19170
LP 3	XHXAXXLGXVXX	10416	89348
LP 4	FXXDXWXXVXIX	10132	10353
LP 5	XXPVXKXAXDXX	9351	9555
LP 6	SAXXXMXLXDXX	7833	8038
LP 7	WXXHXVEXXPXX	7712	7845
LP 8	SXEXKXXDXQXX	7526	7681
LP 9	XXVKXXPXFXXG	6870	6992
LP10	XXDWXXSPXXAX	6410	80027

To identify the rLPAR3 binding peptides, the phage DNA pool of Round 1 and Round 3 was subjected to NGS sequencing. After analysis, in Round 1 of in vitro panning, the total peptide count obtained was 365241, and the unique peptide sequences were 6177. While for Round 3 in vitro panning, the total peptide count obtained was 314384, and unique peptide sequences were 1418. This suggests the enrichment of the peptide sequences in Round 3 compared to Round 1. Total 707 peptide sequences were common to both rounds of in vitro panning. The peptide diversity decreased from Round 1 to Round 3. XQMXXXYXQSXX and TXXRXXVXSXXA are the top two peptides whose frequency was higher than other peptides. These peptides will be synthesized commercially and studied for their specificity to rLPAR3 in vitro and in vivo.

9.5 Sertoli and Leydig Cell Homing Peptides as Molecular Steering for Testicular Targeted Drug Delivery *(Partly Funded by Department of Health Research)*

Principal Investigator : **VD Dighe**
Project Associates : Yugandhara Jirwankar, Akanksha Nair, A Tiwari, Bhavana Bhat, P Salunke
Duration : 2021-2024

Targeted drug delivery to testis can solve the issues faced by conventional drug delivery methods. Homing peptide provides an excellent platform for the development of active ligand-targeted drug delivery. In this study, we have made efforts to identify Sertoli cell homing peptides (SCHPs) and Leydig cell homing peptides (LCHPs) using a phage display peptide library. To identify SCHPs and LCHPs, TM4 (mouse Sertoli cell line) and TM3 (mouse Leydig cell line) were used as a bait for *in-vitro* bio-panning experiment. *In-vivo* bio-panning was performed on Balb/C mice to select SCHPs and LCHPs able to target testis after *in-vitro* bio-panning. After the phage display bio-panning, the phage pools were subjected to next generation sequencing to identify the SCHPs and LCHPs. Based on the frequency of the peptides two SCHPs and two LCHPs were selected for validation of their homing potential to Sertoli and Leydig cells. *In-vitro* homing potential was validated via treating TM4 and TM3 cells with the FITC labeled peptides and analyzed with confocal microscopy and flow cytometry.
During the reported period, *in-vivo* bio-distribution of the selected SCHPs and LCHPs was assessed using *in-vivo* imaging. 10µg of Cy5.5 labeled peptides and free Cy5.5 dye were injected to the male Balb/C mice intravenously and imaging of the testis and other vital organs was performed at 1 hr, 6 hrs and 24 hrs. CTP, a 12-mer peptide, was used as a control peptide. The *in-vivo* bio-distribution at 1 hr, 6 hr and 24 hrs time point suggested higher uptake of SCHP1, SCHP2, LCHP1 and LCHP2 in testis at selected different time points compared with other organs whereas, free Cy5.5 dye and CTP did not show significant uptake in testis (Fig. 1). This differential pattern of bio-distribution of SCHPs and LCHPs compared with Cy5.5 dye and CTP suggests testicular targeting potential of the SCHPs and LCHPs.

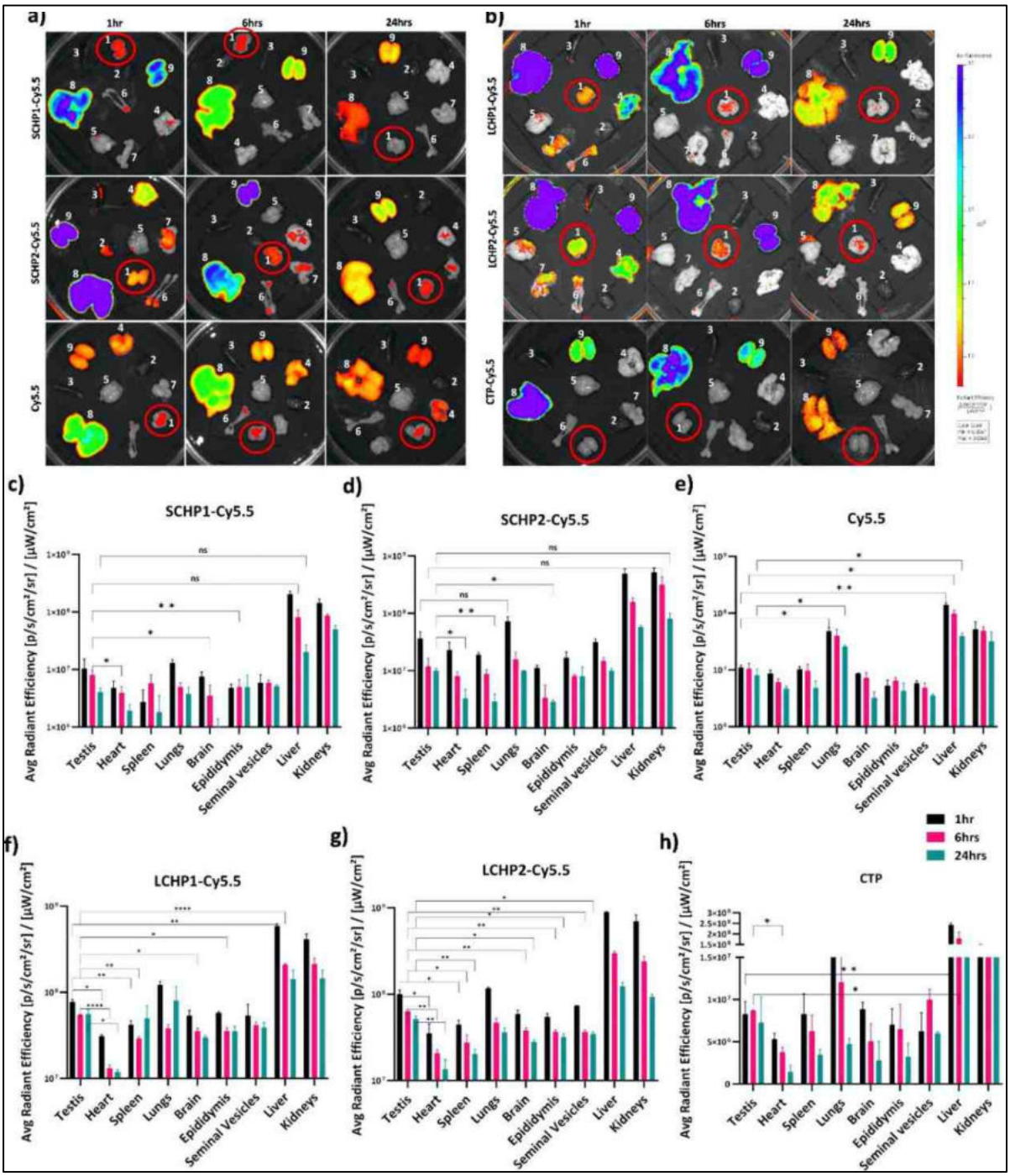


Figure 1: *In-vivo* bio-distribution of SCHP1-Cy5.5 (a and c), SCHP2-Cy5.5 (a and d), Cy5.5 dye (a and e), LCHP1-Cy5.5 (b and f), LCHP2-Cy5.5 (a and g), and CTP-Cy5.5 (a and h) at 1 h, 6 h, and 24 h time points. Cy5.5 conjugated peptides were injected intravenously, and mice were sacrificed after 1 hr, 6 hrs, and 24 hrs, and organ imaging was performed with Perkin Elmer's IVIS Lumina III *in-vivo* imaging system and analyzed with Living Image software. In a and b the numbers show 1. Testis, 2. Heart, 3. Spleen, 4. Lungs, 5. Brain, 6. Epididymis, 7. Seminal vesicles, 8. Liver, 9. Kidneys and testis is encircled with red circle. All data represents the mean ± SD; n = 3; * represent $p < 0.05$, ** represents $p < 0.001$, and **** represents $p < 0.0001$ for two-way ANOVA, followed by Dunnett's multiple comparisons test.

RESEARCH SUPPORT FACILITIES

10. RESEARCH SUPPORT FACILITIES

10.1 Family Welfare Clinics

The Family Welfare Clinics at the institute provide family planning services and serve as a model for integration of family planning services with other aspects of reproductive health care. Services offered routinely include IEC on the various contraceptives and motivation to accept a reliable method, creating awareness, screening and treatment of reproductive tract infections (RTIs), screening for cervical cancers and pelvic ultrasonography for women. The women attending these clinics are then enrolled for clinical and 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 into high rate of follow-ups (more than 95%) for the project participants. Details of the services provided during year 2022-2023 is given in Table 1 below:

Table 1: Services provided by the Family Welfare Clinic (Wadia Clinic)

Family Welfare Services	Number
Total number of women who attended the clinic	1186
Women counselled for contraception	455
Women who accepted CuT-380	17
Women who accepted Oral Contraceptive Pills (OCPs)	14
Condoms distributed	4650
Women who accepted Injectable Contraceptive	14
Women who accepted Centchroman Contraceptive Pills (Chaya)	8
No of women tested for urine infections	87
Women who were tested by Papanicolaou test	57

10.2 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 disease 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. Details of the services provided during year 2022-2023 are as below:

Table 1: Child health services provided by the clinic

Child Health Services	Number
Total number of children who attended the clinic	1170
Total number of new children	589
Children treated for deworming	355
Children treated for anemia	134
Children who received dietary counseling	603
Children treated with Calcium and Vitamin D supplementation	469
Puberty counseling	31
Children recruited and followed up for research	193
Paediatric Endocrinology	32
Children referred from schools (growth+ puberty)	338

Parents are advised mainly on new-born care and breastfeeding, growth and development of children, nutrition, issues related to menarche and puberty. In order to address issues related to precocious puberty, obesity, short stature, the pediatric endocrinology OPD is held once a month by Dr Sudha Rao, paediatric endocrinologist from Bai Jerbai Wadia Hospital for Children. The linkages with schools have been established and children are referred for evaluation of growth and puberty.

10.3 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. Telephonic consultation was offered to infertile couples. Pre Conception and Pre-natal Diagnostic Techniques (PCPNDT) report are compiled and sent to Municipal Corporation of Greater Mumbai on monthly basis. During the year, Premature Ovarian Insufficiency (POI) clinic was initiated. The details about the clinical services provided are given in the following table.

Table 1: Clinical services provided by the clinic

Clinical Services Provided	Numbers
Total infertile couples registered and consultation provided	224
Total number of telephonic consultations	921
Infertile couples - new, old and follow up	New-301 Old-1745
Follow up patient visits for diagnosis, counseling and management	1745
Ultrasound for serial follicular monitoring	293
Number of pregnancies reported	10
Number of POI cases detected	11
POI clinic conducted	2
Total clients who attended the POI clinic	9
Number of couples referred for IUI and IVF at Wadia Hospital	76/62

10.4 Multidisciplinary PCOS Clinic

Our Institute has a multidisciplinary team conducting research in clinical, epidemiological, genetic and bio-informatics aspects of PCOS. Metropolitan cities have increasing number of women of reproductive age being diagnosed with PCOS. Sixty four percent of women referred at our infertility clinic had PCOS. 55% were obese (BMI above 25) and 70% had waist hip ratio more than 0.8. This observation reiterated that PCOS is a cause of infertility and is increasing among adolescents. Recognizing that apart from medical management, PCOS needs a holistic care under one roof for weight reduction, diet, cosmetic and psychological issues, a model of Multidisciplinary PCOS Clinic was developed. This is one of its kinds in India in a government health research institute multidisciplinary PCOS clinic. Women with PCOS are managed on a regular basis and once in a month a joint multidisciplinary clinic is conducted. A multidisciplinary team of eminent doctors like infertility specialist, dermatologist, psychiatrist, nutritionist and Yoga expert provide holistic management to women with PCOS. Cohorts of adolescent and infertile women with PCOS are enrolled and followed at the clinic. Metabolic parameters are monitored. An electronic database is created of physical, hormonal, biochemical, ultrasound and emotional health parameters of the women with PCOS.

Outreach education programmes for adolescent school girls are also conducted in schools and colleges to increase awareness about the condition and to improve health seeking behavior and early diagnosis.

Table 1: Clinical services provided by the PCOS clinic

Clinical Services Provided	Numbers
Total PCOS diagnosed and consultation provided	473
Number of new PCOS registrations	66
Registration for married women	40
Registration for unmarried girls	26
Ultrasonography for PCOS	124
Total no. of multidisciplinary PCOS clinic conducts	6

10.5 Multidisciplinary Clinic for Premature Ovarian Insufficiency

A new Multidisciplinary Clinic for Premature Ovarian Insufficiency has been initiated from June 2022 constituting of a gynaecologist, an IVF expert, a clinical immunologist, an endocrinologist and a genetic expert. The aim of the clinic is to evaluate the clinical spectrum, common autoimmune disorder, genetic factors and quality of life of women diagnosed with Spontaneous Premature Ovarian Insufficiency (POI). POI though affects only 1-2% of women less than 40 years of age, has significant medical, psychological, and reproductive implications. These women are prone to osteoporosis and cardiovascular morbidities. After the diagnosis of POI, there is significant impact on the psychosocial health of women. Research studies from India on POI are majorly in the form of case reports or small case-control studies and there is a lack of comprehensive database for women diagnosed with spontaneous POI with focus on their clinical features, etiologies, common genetic factors, associated autoimmune causes and psychosocial health

10.6 Andrology Clinic

Services including clinical examination, scrotal doppler, semen analysis, diagnosis, treatment, and counseling of the infertile men were provided during the reporting period through the andrology clinic and laboratory. Study participants were recruited for the collaborative research projects of Dr Dhanashree Jagtap, Dr Dipty Singh, Dr Periyasamy Kuppusamy and Dr DVS Sudhakar.

Table 1: Clinical services provided by the Clinic

Clinical services provided	Numbers
Total number of andrology consultations	26
Total male infertility registrations new)	49
Total male infertility cases (new + old)	266

10.7 Bone Health Clinic

The community based Bone Health Clinic is a unique model to address osteoporosis while attending to knee/ spine problem 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 18 new registered and 48 old registered clienteles repeat scans done (total 66 scans). The routine services with the bone health were treated via teleconsultation (>2000 teleconsultations) and clinic consultations. Total clinic attendees for

the current year was 891 attendees and were given various referral as required for cardiac opinion, urology, physiotherapy, diabetologist and follow up for hypertensive treatment with physician. All clienteles were given six months stock of routine calcium supplements, multivitamins, vitamin C and vitamin D due to COVID pandemic. Regular follow up was taken for all the registered clients every 2 monthly via telephonic calls regarding intake of medicines. Spine OPD (clinic consultation) with 10 OPD's for the year with attendance of 63 clienteles and two endocrine OPD with 11 attendees were conducted.

10.8 Women's Health Clinic

Women's Health Clinic, a community-based service cum research clinic located at BDD Chawl 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 current year, gynaecology consultation at clinic was done for 77 women. Services included Copper-T 380A insertions, condom distribution and 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. A specialized gynaecology OPD was held with 3 OPD and 13 attendees.

10.9 Genetic Research Centre

Genetic testing and counselling are provided to the patients visiting the Genetic Research Centre. In the reporting year, 475 patients visited the Genetic Research Centre OPD for consultation. This include 180 new patients with bad obstetric history (BOH), infertility, antenatally detected malformed foetuses, still birth/ IUFD foetuses , 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, advanced maternal age and other genetic disorder etc. and rest were follow-up cases. The new patients underwent genetic counselling and wherever necessary, karyotyping was done.

Table 1: Clinical Services provided by the Center

Clinical Services Provided	Number
Karyotyping	198
FISH	70
Molecular genetic tests	130
Genetic Counselling	475

PCR for 11 STS markers for Y chromosome microdeletion has been standardized by the Genetic Research Center for genetic testing of patients with male infertility. The center also provided training to medical doctors in a 4-week DHR workshop on paediatric medical genetics organized for 19 pediatricians and fetal medicine experts. The center also conducted a one-day CME on Genetic Testing and Counselling in Medical Practice for 128 participants.

10.10 National Center for Preclinical Reproductive and Genetic Toxicology

The National Center for Pre-clinical Reproductive and Genetic Toxicity has a mandate of research, services and capacity building. Services to various academic bodies and industries are being undertaken following Organization for Economic Co-operation and Development (OECD) guidelines and Good Laboratory Practices (GLP). Following research projects were undertaken in collaboration with academic institutions during 2022-2023:

1. Acute, subchronic and genotoxicty of WSSV DNA vaccine *[Funded by ICAR - Central Institute of Fisheries Education (CIFE), Mumbai]*
2. 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 of Technology, Mumbai)*
3. Study the intra-articular retention time of dye-loaded formulations *(Funded by Indian Institute Technology, Mumbai)*
4. Pharmacokinetics, synovial clearance and efficacy studies of nanoparticles loaded in-situ gel by intra-articular route in rats *(Funded by Indian Institute of Technology, Mumbai)*
5. Acute oral toxicity of organic ITK *(Funded by Regional Agricultural Research Station, Karjat, Maharashtra)*
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., Pune)*
7. Genotoxicty studies for Cap.PCOSNIL in rats *(Funded by Acuere Biosciences, Pvt. Ltd., Pune)*

10.11 COVID-19 Diagnostics Testing and Reporting

The facility carried out testing of over 32,000 samples by RT-PCR in the reporting period.

10.12 HTA Resource Hub

The HTA Resource Hub supported by HTAIn DHR has trained manpower to extend support for conduct of costing or costeffectiveness studies to generate evidence for policy decision making for local, state and national health departments

10.13 Institutional Animal Ethics Committee

The Institute is registered for breeding and experimentation on laboratory rodents including non-human-primates with Committee for the Control and Supervision of Experiments on Animals

(CCSEA), Department of Animal Husbandry and Dairying, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India (vide Registration No. 78/GO/ReBi/SL/99/CPCSEA dated March 11, 1999) and this registration has been renewed periodically.

The Institutional Animal Ethics Committee (IAEC) members including CPCSEA nominees critically review, approve and monitor research protocols on the laboratory animals. The IAEC also conducts inspection of animal house facility periodically to ensure animal welfare activities 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 2022-23, three IAEC meetings were convened on April 7, 2022 and October 7, 2022 and March 14, 2023 respectively. Total 21 animal study protocols were reviewed and approved by the IAEC.

10.14 Experimental Animal Facility

The Institute has well maintained animal facility distributed over three floors and houses different species of laboratory animals viz mice, rats, rabbits, bonnet monkeys and marmosets. The animals are maintained and well taken care of by qualified and trained staff everyday including weekends and holidays. The details of animals bred and supplied during the year is given in Table 1.

Table 1: Animals bred and supplied after due approval of IAEC during April 2022 - March 2023.

Species	Animal Bred	Animals Supplied
Swiss Mice	1123	118
Balb/c Mice	1199	421
C57BL/6	1064	439
DBA2/J	532	06
FVB-NJ	557	61
GFP- BL/6	547	NIL
GFP- FVB	570	05
Transgenic Mice WB/Rej/Kit	642	NIL
Transgenic Mice C57BL/6/ Kit/J	555	NIL
Transgenic Mice Mgat1 -/-	72	14
Transgenic C57BL/6-Tg (TRAMP)	297	200
Transgenic Mice B6:CBA Tg- Oct4	922	216
Wistar rats	1976	1260
Rabbits	NIL	NIL
Bonnet Monkeys 12 (In house)	NIL	NIL
Marmosets	19	07

10.15 NIRRCH Ethics Committee for Clinical Studies

Prof Shubhada Chiplunkar continued as the Chairperson with support from Dr Vikrant Bhor as Member Secretary and Dr Suchitra Surve as Joint Member Secretary for the ICMR-National Institute for Research in Reproductive & Child Health Ethics committee for Human Studies. In the reporting year, the following new members were inducted to the IEC: Dr Rakhi Tripathi as clinical pharmacologist (May 2022); Dr Ashwini Karve as alternate clinical pharmacologist (June 2022); Dr Smitha Nair as alternate social scientist (June 2022); Dr Bipin Kulkarni as basic scientist (September 2022); Dr Shailesh Pande as affiliated clinician (September 2022). Mrs Vaishali Bhogate, Mrs. Zakia Ansari, Mr. Ananda Hankare continued as the ethics secretariat staff. A total of 7 full board meetings were held in the reporting period. Overall, 56 new projects including clinical trials were reviewed along with a number of amendments, revisions, continuing review reports and completion reports. A follow-up visit of the SIDCER-FERCAP Survey team comprising of the local coordinator, Dr Nandini Kumar, was held on March 9, 2023.

10.16 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 45 online journals including a video journal package. These journals focus on reproductive and child health and can be accessed via the institutional intranet. 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 also collates information for the monthly/quarterly/annual reports on the significant achievements and publications of the Institute. In addition to the library activities, the library staff is actively involved in updating the institutional website and maintaining the website and social media handles of the institute including Twitter, Facebook, LinkedIn, Instagram and YouTube. The library also coordinated visits by students/staff from other institutions who are keen to learn about the research and facilities at the institute.

10.17 Core Facilities

10.17.1 Confocal Imaging

The facility provided assistance to staff and students of the Institute and other academic / research Institutes for co-localisation, FRET, live cell imaging, 3D imaging, on LASER Confocal System. From April 2022 to March 2023 47 researchers used the facility both from the Institute and from other institutes.

10.17.2 Droplet Digital PCR (ddPCR)

Droplet Digital PCR (ddPCR) is used for quantification of nucleic acids by either the dye chemistry or primer/probe assays. ddPCR involves the partitioning of the PCR reaction into individual reactions. The droplet-based quantifications allow direct and independent estimation of nucleic acid without standard curves. This brings in higher sensitivity and accuracy. Thus, ddPCR is used for extremely low-target quantitation with consistent and acceptable reaction efficiency. The facility trains the users and allows them to carry out the assay themselves. Support is provided in designing and optimizing the assays. The equipment usage and applications were demonstrated to the participants of various workshops and student visitors. 15 participants of the DHR workshop and 5 institute research scholars have been trained in the reporting year.

10.17.3 Flow Cytometer and DNA Sequencing

This facility provides services toward institutional projects and inter-institutional projects. During reporting year, 1200 samples were processed for flow cytometry and DNA sequencing were performed for 6500 samples.

10.17.4 Histology Work Station

The histology workstation in the National center for Preclinical Reproductive and Genetic Toxicology is a state of art facility equipped with automatic tissue processor, automatic slide stainer, tissue embedder, microtome and automatic cover slipper. This facility is a central facility and utilized by researchers in the institute as well as other academic and private institutions. In the reporting year, total 1742 tissue samples were processed and embedded and paraffin blocked was prepared. 4420 tissue blocks were sectioned was done and 1831 slides were stained using Hematoxylin and eosin stating.

10.17.5 Live Cell Imaging System

Live Cell Imaging system is a core facility for fluorescent and DIC image capture and also live time-lapse imaging for cells. In the reporting year, this facility was used extensively by the students and staff of various departments to carry out imaging in fluorescence and live mode. The participants of the DHR training workshop were also trained.

MODEL RURAL HEALTH RESEARCH UNIT (MRHRU)

11. MODEL RURAL HEALTH RESEARCH UNIT (MRHRU)

Name of Facility: Model Rural Health Research Unit (MRHRU) Dahanu, Maharashtra
Nodal officer: Dr. Ragini Kulkarni, Scientist 'F' ICMR-NIRRCH, Mumbai
Member secretary: Dr. Kiran Munne, Scientist 'C' ICMR-NIRRCH, Mumbai

MRHRU is collaboration between NIRRCH as a research and mentoring body; Directorate of Health Services, Government of Maharashtra as facilitator and Grant Medical College and Sir JJ group of hospitals, Mumbai as a linked medical college.

11.1 COVID-19 Diagnostic Facility

From April 2022 to March 2023, a total of 5228 samples were processed for the presence of SARS-CoV-2, of which 215 samples were detected positive (4.11%). Positivity was high in Sep 2022 (9.93%) followed by July and August 2022 with 7.59% and 6.48%, respectively. A gradually decreasing trend in the positive cases was observed after October 2022. Another rise in the number of positives was recorded in March 2023 with a positivity rate of 4.25%.

11.2 Sentinel Surveillance for Dengue and Chikungunya Fever

Dengue Fever: from April 2022 to March 2023, a total of 1741 serum samples were received and tested for the presence of anti-dengue IgM antibodies by IgM capture ELISA (NIV, Pune Kit). Of them, 257 (14.76%) were detected positive.
Chikungunya Fever: During this reporting period, 82 suspected blood samples were received for chikungunya, among which 16 (19.5%) were positive.

11.3 Leptospirosis Diagnostic Facility

Leptospirosis diagnostic service (April 2022 – March 2023) Diagnosis for Leptospirosis was initiated in the month of October 2021. From April 2022 to March 2023, a total of 13 samples were tested for Leptospira IgM antibodies using Leptospira IgM ELISA kit (Panbio, USA), of which 3 (23.07%) were positive.

11.4 Influenza Diagnostic Facility

The real time RT-PCR based diagnosis facility was initiated for qualitative detection and characterization of Influenza A(H1N1) pdm09, Influenza A(H3N2) and Influenza B Yamagata and Victoria lineage in November 2022. A total of 23 samples were tested using ICMR-NIV Multiplex Combo Kit, of which 01 (4.34%) was tested positive for H3N2 and 4 (17.39%) were detected positive for SARS-CoV-2.

11.5 Ongoing Projects:

1. Assessment of neonatal screening approaches for sickle cell disease and the effect of early Intervention in management of the disease in tribal population *(Funded by Indian Council of Medical Research)*

2. 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)*
3. ICMR Multicentre study: 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 (participating centre) *(Funded by Indian Council of Medical Research)*
4. Implementation research to explore operational feasibility, acceptability and cost-effectiveness of using IV Ferric Carboxy Maltose (FCM) in management of Iron Deficiency Anaemia (IDA) among pregnant women through sub district health system of Palghar district in Maharashtra *(Funded by Department of Health Research)*
5. Molecular analysis of HLA-G in pregnant tribal women and its role infectious etiologies modulating intrauterine inflammation- A prospective cohort study *(Funded by Indian Council of Medical Research)*
6. Integrated palliative, elderly and mental health care (I-PEM) under for establishment of Model Rural Health Research Unit (MRHRU) under the Umbrella Scheme of "Development of Infrastructure" *(Funded by Indian Council of Medical Research)*
7. Primary screening of High risk HPV DNA by molecular test in urban and rural community of Maharashtra (study site) *(Funded by Department of Health Research)*
8. Sentinel Surveillance Hospital (SSH) for the diagnosis of Dengue and Chikungunya Fever *(On-going since September 2017, MRHRU recognized as the 38th Surveillance centre in Maharashtra-NVBDCP, Government of Maharashtra)*

PUBLICATIONS

12. PUBLICATIONS

12.1 Peer Reviewed Publications during 2022

1. Aathi MS, Kumar C, Prabhudesai KS, Shanmugarajan D, Idicula-Thomas S. Mapping of FSHR agonists and antagonists binding sites to identify potential peptidomimetic modulators.
   Biochim Biophys Acta Biomembr 1864: 183842, 2022 **[IF: 3.4]**

2. Agrawal A, Raval A, Velhal S, Patel V, Patravale V. Nanoparticles eluting stents for coronary intervention: Formulation, characterization and in vitro evaluation.
   Can J Physiol Pharmacol 100:220-233, 2022 **[IF: 2.1]**

3. Aranha C, Goriwale M, Begum S, Gawade S, Bhor V, Patil AD, Munne K, Bansal V, Tandon D. Evaluation of cytokine profile in cervicovaginal lavage specimens of women having asymptomatic reproductive tract infections.
   J Obstet Gynaecol 42: 3106-3111, 2022 **[IF: 1.3]**

4. Ashary N, Singh A, Chhabria K, Modi D. Meta-analysis on prevalence of vaginal group B streptococcus colonization and preterm births in India.
   J Matern Fetal Neonatal Med 35: 2923-2931, 2022 **[IF: 1.8]**

5. Arya D, Balasinor N, Singh D. Varicocele associated male infertility: Cellular and molecular perspectives of pathophysiology.
   Andrology 10: 1463-1483, 2022 **[IF: 4.5]**

6. Bane K, Desouza J, Rojewale A, Katkam RR, Fernandes G, Sawant R, Dudhedia U, Warty N, Chauhan A, Chaudhari U, Gajbhiye R, Sachdeva G. Dysregulation of X-ray repair cross-complementing 4 expression in the eutopic endometrium of women with endometriosis.
   Reproduction 163: 95-105, 2022 **[IF: 3.8]**

7. Beirag N, Kumar C, Madan T, Shamji MH, Bulla R, Mitchell D, Murugaiah V, Neto MM, Temperton N, Idicula-Thomas S, Varghese PM, Kishore U. Human surfactant protein D facilitates SARS-CoV-2 pseudotype binding and entry in DC-SIGN expressing cells, and downregulates spike protein induced inflammation.
   Front Immunol 13: 960733, 2022 **[IF: 7.3]**

8. Bhartiya D, Kaushik A, Singh P, Sharma D. Cancer initiates due to excessive self-renewal and blocked differentiation of tissue-resident, OCT-4 positive VSELs.
   Stem Cell Rev Rep 18: 3112-3114, 2022 **[IF: 4.8]**

9. Bhartiya D, Mohammad SA, Singh P, Sharma D, Kaushik A. GFP tagged VSELs help delineate novel stem cells biology in multiple adult tissues.
   Stem Cell Rev Rep 18:1603-1613, 2022 **[IF: 4.8]**

10. Bhurke AV, DasMahapatra P, Balakrishnan S, Khan S, Mortlock S, Das V, Nirmala C, Sowmini CV, Srivastava A, Majumdar A, Pasi A, Sachdeva G, Montgomery GW, Gajbhiye RK. Clinical characteristics and surgical management of endometriosis-associated infertility: A multicenter prospective cohort study.
Int J Gynaecol Obstet 159: 86-96, 2022 **[IF: 3.8]**

11. Birje S, Patil AD, Munne KR, Chavan V, Joshi BN, Akula A, Salvi N, Nair S, Valawalkar SP, Tandon D, Chauhan S, Patil D, Babu BV. Enablers & challenges of tribal women & health system for implementation of screening of non-communicable diseases & common cancers: A mixed-methods study in Palghar district of Maharashtra, India.
Indian J Med Res 156: 319-329, 2022 **[IF: 4.2]**

12. Chaaithanya IK, Mujumdar Y, Anwesh M, Aranha C. Novel genotypes of vaginal Candida glabrata isolates from premenopausal asymptomatic women with vulvovaginitis.
Eur J Obstet Gynecol Reprod Biol 276: 249-250, 2022 **[IF: 2.6]**

13. Chaudhari UK, Newcomb JD, Jen KC, Hansen BC. Iron deficiency, but not anemia, is identified in naturally occurring obesity and insulin resistance in male nonhuman primates.
J Med Primatol 51:165-171, 2022 **[IF: 0.7]**

14. Chauhan S, Kulkarni R, Bharat S, Joshi B, Unisa S, Singh A, Subramanian T, Chaudhuri RN, Baishya AC, Patil A and Pasi A. Community perceptions and treatment seeking behaviour for infertility in India: A qualitative research study.
Indian J Social Work 83: 439-454, 2022 **[IF: NA]**

15. Chavan VR, Ahir S, Kerkar S, Ansari Z, Samant-Mawani P, Nanavati R, Mehta P, Mania-Pramanik J. Th1 cytokine gene polymorphism and the corresponding plasma cytokine levels: A comparative study in HIV-1 positive and exposed uninfected infants.
J Med Virol 94(2): 625-633, 2022 **[IF: 12.7]**

16. Colaco S, Achrekar S, Patil A, Sawant U, Desai S, Mangoli V, Jirge PR, Modi D, Mahale SD. Association of AMH and AMHR2 gene polymorphisms with ovarian response and pregnancy outcomes in Indian women.
J Assist Reprod Genet 39(7): 1633-1642, 2022 **[IF: 3.1]**

17. Dama T, Chheda P, Limaye S, Pande S, Vinarkar S. Evaluation of single exon deletions in DMD/BMD: Technical and analytical concerns.
Neurol India 70: 1615-1617, 2022 **[IF: 2.7]**

18. Das DK, Udani V. Coexistence of Rett & Angelman syndrome: A rare clinical presentation.
Indian J Med Res 155(1): 79-80, 2022 **[IF: 4.2]**

19. Dixit A, Ghule M, Rao N, Battala M, Begum S, Johns NE, Averbach S, Raj A. Qualitative examination of the role and influence of mothers-in-law on young married couples' family planning in rural Maharashtra, India.
Glob Health Sci Pract 10(5): e2200050, 2022 **[IF: 4.0]**

20. Gadkar S, Thakur M, Desouza J, Bhowmick S, Patel V, Chaudhari U, Acharya KK, Sachdeva G. Estrogen receptor expression is modulated in human and mouse prostate epithelial cells during cancer progression.
Steroids 184: 109036, 2022 **[IF: 2.7]**

21. Gajbhiye RK, Mahajan NN, Sachdeva G. Preparedness and strategies for addressing monkeypox infection in pregnant women in India.
Lancet Reg Health Southeast Asia 5: 100066, 2022 **[IF: NA]**

22. Gajbhiye RK, Mahajan NN, Waghmare R, Surve SV, Howal P, Bhurke A, Pious M, Modi DN, Mahale SD. Protocol for a prospective, hospital-based registry of pregnant women with SARS-CoV-2 infection in India: PregCovid Registry study.
BMJ Open 12(3): e050039, 2022 **[IF: 2.9]**

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Front Immunol 13: 930449, 2022 **[IF: 7.3]**

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BMC Pediatr 22(1): 602, 2022 **[IF: 2.4]**

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Schizophr Res 243: 214-216, 2022 **[IF: 4.5]**

26. Gupta K, Desai R, Jawade K, Jagtap D, Modi D, Jain R, Dandekar P. Determination of functional similarity of biosimilar H9P2S from an investigational CHO clone with Adalimumab.
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28. Irani D, Borle S, Balasinor N, Singh D. Maternal cypermethrin exposure during perinatal period dysregulates gonadal steroidogenesis, gametogenesis and sperm epigenome in F1 rat offspring.

Reprod Toxicol 111: 106-119, 2022 **[IF: 3.3]**

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Arch Gynecol Obstet 306: 639-662, 2022 **[IF: 2.6]**

31. Kaushik A, Bhartiya D. Testicular cancer in mice: interplay between stem cells and endocrine insults.
Stem Cell Res Ther 13: 243, 2022 **[IF: 7.5]**

32. Kulkarni RN, Chauhan S, Maternal Near Miss Working Group. Experiences and challenges during implementation of operational guidelines of Maternal Near Miss Review of the Government of India at tertiary hospitals in Maharashtra.
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33. Kulkarni R, Chauhan S, Joshi B, Balsaraf K, Chaudhari R, Donde G, Juneja K. Assessing the potential of self-help group women for improving reproductive health of women in a tribal block of Maharashtra, India.
*Indian J Med Re*s 156: 191-197, 2022 **[IF: 4.2]**

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12.2 Books and Book Chapters

1. Bhartiya D, Singh P, Kaushik A, Sharma D. The role of very small embryonic-like stem cells (VSELs) in reproductive tissues. *In:* Virant-Klun I (ed.). Stem cells in reproductive tissues and organs-from fertility to cancer. Series: Stem Cell Biology and Regenerative Medicine. Springer International Publishing, pp. 263-288, 2022

2. Bhide A, Aboo A, Sawant M, Majumder A, Paul D, Modi D. Placenta on chip: A modern approach to probe feto-maternal interface. *In:* Mohanan PV (ed) Microfluidics and Multi Organs on Chip. Springer, Singapore, 2022

3. Gunasekaran VP, Naidu S, Nishi K. Y-Box-Binding Protein-1: A promising therapeutic target for cancer. *In*: Chakraborti S (ed.) Handbook of Oxidative Stress in Cancer: Therapeutic Aspects, Springer, Singapore pp: 3497-3512, 2022

4. Poojari P, Paramanya A, Singh D, Ali A. Polycystic Ovarian Syndrome: Causes and therapies by herbal medicine. *In*: Sarwat M, Siddique H (eds.) Herbal Medicines: A boon for healthy human life, Academic Press, Elsevier, pp. 435-451, 2022

12.3 Papers / Posters presented at Conference / Symposia

International

1. **United Kingdom-International Coronavirus Network-sponsored conference, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Srinagar, J&K, May 11, 2022**
   - **Gajbhiye RK.** Maternal and neonatal outcomes amongst pregnant women with Sars-CoV-2 infection: Evidence from Pregcovid registry

2. **15th World Congress on Inflammation, Ergife Palace Hotel & Conference Centre, Rome, Italy, June 5-8, 2022**
   - **Bhowmick S.** sMAdCAM:IL-6 ratio influences disease progression and anti-viral responses in SARS-CoV-2 infection

3. **International Conference on Reproductive Biology, Comparative Endocrinology and Development and 39th Annual Meeting of the Society for Reproductive Biology and Comparative Endocrinology, Hyderabad, September 14-16, 2022**
   - **Panchal D.** N-Formyl-L-Aspartate mediates chemotaxis in sperm via the beta-2-adrenergic receptor.

- **Singh I** Unravelling the epigenetic landscape of testis specific histone variants TH2A and TH2B in murine sperm.
- **Modi D**. Dissecting the inflammatory networks that drive embryo implantation
- **Bhide A.** Placental infection of SARS-CoV-2 and its mother-to-child transmission
- **Salvi R.** Chinese hamster ovary cells and testis deficient in N-linked glycosylation may be critical for Insulin growth factor-1 receptor signalling.

4. **International Conference on Epigenetics and Epigeneomics in Health and Disease, Brussels, November 17-19,2022**
   - **Irani D.** Sperm DNA methylation landscape of idiopathic recurrent pregnancy loss cases

5. **International Conference on Bioinformatics (InCoB2022), King Abdullah University of Science and Technology, Thuwal, Saudi Arabia,** virtual **mode, CBRC in partnership with APBioNET, November 21-23, 2022**
   - **Kundu I**. GeDiPNet: Online resource of curated gene-disease associations for polypharmacological targets discovery

National

6. **Student Indian Peptide Symposium sIPS 2022: Therapeutics, Biomaterials, and Beyond, Virtual by Indian Institute of Technology Bombay, March 31-April 1, 2022**
   - **Kamble P.** Identification and characterization of the immunodominant epitopes of Trop2: an important tumor antigen

7. **Life Conference, organised by the International Institute for Training and Research in Reproductive Health (ITRRH), The Lalit Ashok, Kumara Krupa Road, High Grounds, Bangalore- 560001, May 14-15, 2022**
   - **Modi D.** Recalcitrant endometrium - The toughest valley

8. **Virtual Conference on Snakebite, Amrita Institute of Medical Sciences and Research Centre, Kochi (online), May 28, 2022**
   - **Gajbhiye RK.** Community empowerment and Public Health System Capacity building for addressing the burden of snakebite envenomation: an experience from Maharashtra, India

9. **TPACON 2022, The Pathologist Association (TPA) State Level Conference, Thane, June 11-12, 2022**
   - **Pande S.** Genetics and genetic counselling in medical practice

10. **Nutrition and Health Research Conference (NHRC 2022): Translational Research in Human Health and Nutrition, Hirabai Cowasji Jehangir Medical Research Institute, Pune, July 29-30, 2022**
    - **Surve S.** Experience of working in research studies in children under 5 years

11. **LE STUDIUM Conference - Gonadotropins in the Physiopathology: Current Advances in the Mechanisms of Action, (virtual mode), September 14 -15, 2022**
    - **Banerjee A.** Functional Characterization of two naturally occurring mutations V221G and T449N in the follicle stimulating hormone receptor

12. **7th Annual PCOS Conference 2022., Leela Hotel, Mumbai, September 16-18, 2022**
    - **Shukla P.** Analysis of Mitochondrial DNA copy number and Variants in Mitochondrial DNA Encoded Genes Using Next Generation Sequencing in Women with PCOS

13. **Symposium on Male Infertility, Lokmanya Tilak Municipal General Hospital and Lokmanya Tilak Municipal Medical College, Sion, Mumbai, October 2, 2022**
    - **Pande S.** Genetic evaluation in male infertility

14. **The Cytometry Society 2022 Annual Meeting, Central University, Hyderabad, October 15-16, 2022**
    - **Patel V.** Delineating immune correlates of HCMV congenital transmission
    - **Kasarpalkar N**. Opposing roles for sMAdCAM and IL-15 in COVID-19 associated cellular immune pathology

15. **Microphysiological Systems: Advances and Applications in Human-relevant Research (EMBO), CSIR–Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, October 31-November 4, 2022**
    - **Bhide A**. Placenta-On-Chip devices to assess migration and barrier function
    - **Borkotoky M**. Comparative analysis of spheroids derived from ovarian cancer cell lines

16. **SYMRESEARCH 2022, National Conference on Research in Health & Biomedical Sciences , Symbiosis, Pune, November 3-5, 2022**
    - **Joshi B**. Health Technology Assessment for Universal Health Coverage

17. **7 World Cancer Congress - 2022, Bangalore, November 19-20, 2022**
    - **Ganguly K.** Surfactant protein-D mediated innate immune surveillance in prostate cancer.

18. **49th Annual Conference & General Body Meeting of Indian Immunology Society, Immunocon 2022, PGIMER, Chandigarh, November 22-26, 2022**
    - **Devadiga P**. Longitudinal analysis of integrin α4ß7 expressing T Lymphocytes in people living with HIV.
    - **Jondhale M.** Antitumor potential of Toll like receptors agonists in Triple negative breast cancer.

19. **9th World Ayurveda Congress and Ayurveda Expo, World Ayurveda Foundation (Initiative by Vijnana Bharti) with Focal Theme: Ayurveda for One Health, Panjim, Goa, December 8-11, 2022**
    - **Savardekar L.** Implementing a Public Health Initiatives Study for Screening of Osteoporosis among Women in Community by using Ayurveda – Allopathy Collaborative Approach: Experiences shared.

- **Gadade A**. Ashotone +Zpter reverse the polycystic ovary syndrome induced by letrozole in animal models

20. **18th Annual Conference of the Indian Fertility Society, FERTIVISION 2022, Hyderabad International Convention Centre, Hyderabad, December 9-11, 2022.**
    - **Modi D.** The endometrium in recurrent implantation failure

21. **Five-Days Workshop on Sampling Methods in Population and Health Research, International Institute for Population Sciences Alumni Association (IIPS AA), IIPS, Mumbai, December 12-16, 2022**
- **Begum S.** Estimation of sample size using epi info and other software

22. **International Conference on Virus Evolution, Infection and Disease Control (ICVEIDC)-2022, Department of Biotechnology & Bioinformatics, School of Life Sciences, University of Hyderabad, Hyderabad, December 15-17,2022**
    - **Bhowmick S.** CD4+ T Regulatory cells dysfunction associated with Latent TB infection in PLHIV

23. **13th Annual Conference South Asian College of Clinical Pharmacology (SAC-ACCP) "Clinical Pharmacology: Goal Setting for Next Decade" at Vivanta Ahmedabad SG Highway, Ahmedabad, January 6-8, 2023**
    - **Gadade A**. Ashotone +Zpter reverse the polycystic ovary syndrome induced by letrozole in animal models
    - **Desai S**. The effect of Triclosan, an endocrine disruptor exposure on prostate pathobiology
    - **Dighe V**. Pre-clinical drug development for women's diseases: Experience of NIRRCH

24. **45th All-India Cell Biology Conference, Banaras Hindu University, Banaras, January 20-22, 2023**
    - **Colaco S.** Diagnosis of Y chromosome microdeletions and development of an algorithm for prediction of ART outcomes in men with Y chromosome microdeletions
    - **Miya V.** Analysing CRISP4 interactome from rat caudal sperm reveals plasma membrane associated ion channel, PMCA4, as a novel binding partner
    - **Negi B**. Identification of NLRP3 regulated transcriptome in mouse uterus during embryo implantation

25. **International Conference on Kaleidoscopic Insights into Reproductive & Child Health, Mumbai, January 23-25, 2023**
    - **Anand S, Das D.** Human embryonic stem cell model for studying early trophoblast development
    - **Arya D.** Status of sperm quality and DNA methylation in infertile men with clinical varicocele
    - **Bhanarkar S.** Circulating levels of Kisspeptin and Neurokinin B among prepubertal Indian girls
    - **Bhingardeve S.** Epigenetic crosstalk between miRNA and DNA methylation in pathophysiology of PCOS

- **Bhandari P**. Association between oral contraceptive use and the prevalence of hypertension in Indian women: recent evidence from 2019-21 National Family Health Survey
- **Bhonde G.** Non-invasive evaluation for detection of congenital cytomegalovirus infection in newborns
- **Breed A**. Urinary levels of Placental Growth factor (PLGF) in normal pregnancies and their association with SGA infants and preterm deliveries
- **Chakraborty S**. Novel peptidomimetics as promising SAP inhibitors for combinatorial drug therapy against *Candida albicans*
- **Chaaithanya KI**. Molecular characterization of *Candida glabrata* isolates from premenopausal asymptomatic women with vulvovaginitis
- **Colaco S.** Improved methods for diagnosis and prediction of ART outcomes in men with Y chromosome microdeletions
- **Dadachanji R.** Evaluating coagulation and fibrinolytic profiles contributing to PCOS pathophysiology
- **Desouza J.** GPR30 and its role in estrogen mediated non-genomic signaling in prostate cancer
- **Devadiga P.** Altered gut homing potential of B Lymphocyte subsets in people living with HIV
- **Ganguly K.** Intra-tumoral delivery of RIG-I agonist regressed tumor in a syngeneic murine model of prostate cancer
- **Imran M.** Therapeutic metformin concentrations regulate endometrial epithelial cell proliferation by activating mTOR and upregulating mitochondrial strength
- **Irani D.** Global DNA methylation and sperm DNA fragmentation in male partners of fertile and idiopathic recurrent pregnancy loss cases
- **Gawai P**. Profile of birth defects in newborns in tribal district of Maharashtra
- **Jagtap D**. Diagnostic utility of serum PSP94/PSA ratio (PP Index) in minimizing prostate biopsies in lower urinary tract patients with serum PSA levels between 4-20 ng/ml
- **Joshi N.** Association of ApoE allele with risk of PCOS and related traits
- **Joshi K**. Analyzing the methylation status of the H2BC1 gene in human spermatozoa
- **Karandikar K.** Bifidobacterium species predominantly colonize the healthy infant gut microbiome
- **Khandvilkar A.** HMGB1-RAGE axis is essential for efficient breakdown and repair of endometrium in rat model
- **Khavale S.** Influence of YAP1 polymorphism on PCOS risk and its related traits in Indian women
- **Khan S**. Health system's response to family planning services during COVID-19 : A rapid assessment survey in a district of Maharashtra
- **Khambata K.** Estrogen receptors, ERα and ERβ, differentially regulate the sperm DNA methylome in male rats
- **Kokate P.** Impact of providing comprehensive Multidisciplinary model of care for PCOS affected women
- **Kore Y**. Iron status and hematological profile of Sickle cell disease babies at MRHRU, Dahanu, Maharashtra
- **Kundu I**. Identification of polypharmacological targets for metabolic syndrome using GeDiPNet

- **Kuppusamy P.** Prevalence and contributing risk factors of high-risk pregnancy among Indian women during 2019-21
- **Mehta N**. 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
- **Mukherjee N.** Role of Wnt/β-catenin signaling pathway in placenta mediated breast carcinogenesis during pregnancy
- **Mukherjee S**. Glimpses of PCOS Research at NIRRCH
- **Munne K**. Knowledge, attitude, practice and barriers of cervical cancer screening among women living in urban and tribal communities of Maharashtra: A cross sectional study
- **Naigaonkar A.** Communication between oocyte and granulosa cells for metabolic cooperation - which wire has gone haywire in PCOS
- **Negi B**. Analysis of NLRP3 Regulated Transcriptome in Mouse Uterus during Embryo Implantation.
- **Panchal D.** The role of N-Formyl-L-aspartate in sperm chemotaxis
- **Pande S**. Genetics of infertility and RPL
- **Patil K.** *In-silico* analysis for the identification of mRNA-miRNAs network regulating angiogenesis in women with PCOS
- **Patankar A.** Analyzing the methylation status of the H2BC1 gene in spermatozoa
- **Patel A**. Anti-microbial and anti-cervical cancer activities of metabolites produced by *Lactobacillus salivarius* and *Lactobacillus reuteri* isolated from the vaginal microbiota
- **Periyappa V**. Insights gained from use of fourth generation QuantiFERON-TB Gold Plus IGRA for Latent TB diagnosis in paediatric population.
- **Prusty RK**. Does intimate partner violence against women increase with marital duration? Insights from India's 2019-21 National Family Health Survey
- **Rao A**. Biophysical and functional characterization of circulating placental exosomes in preeclamptic and normotensive pregnancies
- **Raut S**. Role of prolactin in male fertility and molecular mechanisms involved
- **Salvi R**. Unravelling the proteome dynamics in spermatids of murine testis deficient in Mgat1 required for spermatogenesis in mammals
- **Samant M.** Understanding the genetic profile in polycystic ovary syndrome by whole exome sequencing
- **Samant S.** Role of Wnt/β-catenin signaling pathway in placenta mediated breast carcinogenesis during pregnancy
- **Shekhar BR**. Association of CNTNAP2 as a causative gene in familial schizophrenia
- **Shinde G.** Neonatally estrogenized, cystic ovarian mouse model reveals DNA methylation associated transcriptional changes in estrogen-responsive tissues during adulthood
- **Shukla P.** Evidences of mitochondrial dysfunction in the pathophysiology of PCOS
- **Singh A.** Developmental and molecular effects of gestational methyl donor deficiency in mice offspring
- **Singh I.** The murine sperm epigenome of testis specific histone variants TH2A and TH2B
- **Sonawane S.** Effect of epigallocatechin-3-gallate (EGCG) supplementation on spermatogenesis of cypermethrin exposed rats

- **Tandon D.** Evaluate the effect of various contraceptives on Nugent score, vaginal cytokine profile and vaginal microbiome: Prospective longitudinal cohort study
- **Verma P.** Profilin-2, a novel interacting partner of HDAC6 identified on testis
- **Wakle A.** Spatiotemporal expression of endocannabinoid system in mouse placenta across gestation
- **Yevate S**. Profilin-2, a novel interacting partner of HDAC6 identified in testis

26. **EMBO (India) Lecture Course on `Modeling Development and Disease with Human Tissue Organoids, Institute for Stem Cell Science and Regenerative Medicine, Bengaluru, February 6-9, 2023**
    - **Anand S, Das DK.** Development of an in vitro model system reflecting early trophoblast development using human embryonic stem cells

27. **International Conference on Reproductive Health with Emphasis on "Innovation in Reproductive Sciences and Technologies: Hope, Risk and Responsibilities" & 33rd Annual Meeting of the ISSRF, Ravenshaw University, Cuttack, February 24-26, 2023**
    - **Aranha C.** Biological and physicochemical properties of biosurfactants produced by vaginal Lactobacillus salivarius and Lactobacillus reuteri
    - **Bhingardeve S.** Epigenetic alteration of miRNA genes may contribute to dysregulation of key pathways involved in PCOS pathophysiology
    - **Chakraborty S.** Identification and validation of novel peptidomimetics as SAP inhibitors in Candida
    - **Desouza J.** GPR30: Does it contribute to increased invasiveness via non-genomic estrogen signaling in prostate cancer
    - **Gajbhiye RK.** Endometriosis Clinical and Genetic Research in India (ECGRI): An update on improving awareness, knowledge, and care of women with endometriosis
    - **Gaonkar R.** Toxicity assessment of L-NAME in male wistar rats
    - **Imran M**. Therapeutic effects of metformin on endometrial thickness in a rat model
    - **Mukherjee S.** Delineating putative mechanisms of compromised oocyte quality in women with PCOS
    - **Panchal D.** The role of N-formyl-L-aspartate in Sperm Chemotaxis.
    - **Patil K.** Altered angiogenic milieu in follicles contributes to the luteal defects in women with PCOS
    - **Pawar A.** TACSTD2 family protein expression in the rat placenta is influenced by the gestational age, oxidative stress, and inflammatory milieu
    - **Rajendran R.** Innovations in reproductive sciences and technologies: Hope, risk and responsibilities
    - **Rao A.** Proteomic profile of circulating placental exosomes from early-onset preeclamptic patients at parturition
    - **Salvi R.** Proteomic profiling of spermatids with Impaired N-glycosylation revealed dysregulated spermatogenesis
    - **Singh I** The epigenomics of murine testis specific histone variants TH2A and TH2B

28. **BIOFACET 2023, Impact of Bioinformatics Tools in Biological Sciences, Webinar, Department of Biotechnology & Microbiology, February 25, 2023**.
    - **Thomas S.** Role of machine learning in biological research, online.

29. **AI for Public Health: Potential and Challenges in Low Resource Settings, Virtual Lecture Series by The School of Business Management (SBM) in Association with Shastri Indo-Canadian Institute (SICI), February 27-28, 2023**
    - **Thomas S.** Operational constraints in deploying machine learning in public health

30. **Sickle Cell Disease-Diagnosis and Management, ICMR-Centre for Research, Management and Control of Hemoglobinopathies (ICMR-CRMCH), Chandrapur, February 28, 2023**
    - **Surve S.** Management of new-borns with SCD

31. **International Conference on "Contextualising Health in Social Sciences: Global and National Perspectives", Sharda School of Humanities and Social Sciences, NOIDA, UP, March 15, 2023**
    - **Prusty RK.** What explains the rapid increase in hypertension among Indian women of reproductive age? Evidences from recent two rounds of National Family Health Surveys

32. **EmbryoConnect, Manipal Reproductive Science Summit, Kasturba Medical College, Manipal, March 18–20, 2023**
    - **Parte P.** The role of n-formyl-l-aspartate in sperm chemotaxis

CAPACITY BUILDING

13. CAPACITY BUILDING

13.1 Workshop / Training Program Organized

- **Dighe V.** 13th Annual Conference of South Asian College of Clinical Pharmacology (SAC-ACCP) on "Clinical pharmacology: Goal setting for next decade" at Taj Vivanta, Ahmedabad, January 6-8, 2023.
- **Gajbhiye R.** Workshop on "Prevention, diagnosis, and management of snakebite envenomation" at Eklavya Residential School, Aheri, District Gadchiroli, June 13-14, 2022.
- **Madan T.** Mini-symposium on "Challenges in clinical management of reproductive infectious diseases: Research opportunities" at ICMR-NIRRCH, Mumbai, April 27, 2022.
- **Modi D, Mukherjee S.** DHR sponsored workshop on "Omics in biomedical research and clinical practice" at ICMR-NIRRCH, Mumbai, May 9 - June 3, 2022.
- **Mukherjee S.** Lecture and demonstration of advanced techniques for biological research for students of KET's VG Vaze College at ICMR-NIRRCH, Mumbai, February 3, 2023.
- **Mukherjee S, Kulkarni R.** "International Conference on Kaleidoscopic Insights into Reproductive & Child Health (ICKIR)", Swatantrya Veer Savarkar Auditorium, January 23-25, 2023
- **Pande S.** DHR sponsored training course on "Pediatric medical genetics" at ICMR-NIRRCH, Mumbai, June 20 - July 15, 2022.
- **Pande S.** CME on "Genetic testing and counseling in medical practice", at Swatantrya Veer Savarkar Auditorium, Mumbai, January 23, 2022.
- **Patil A.** Classes for M. Sc. students – SVT College of Home Science, SNDT Women's University, Mumbai at ICMR-NIRRCH & community visit, August 1 - September 30, 2022.
- **Surve S.** CME on "Precocious puberty among girls: An update on evaluation, therapeutics and knowledge gaps" at ICMR-NIRRCH on November 6, 2022.
- **Surve S.** E-Symposium on "Child health research: Achievements, challenges and way forward" at ICMR-NIRRCH on April 1, 2022.
- **Thomas S.** Workshop on "Genomics in health and diseases" at ICMR-NIRRCH on October 1, 2022.

13.2 Community Outreach Activities

- **Joshi B**: "All about growing up" for girls of class 8-10 at Mumbai Public School, Abhyudaya Nagar on September 30, 2022.
- **Munne K.** Awareness program for cervical cancer screening for Teli Galli community members on January 5, 2023.
- **Munne K.** Awareness program for cervical cancer screening for Veermata Jijabai Bhaosale Udyan and Zoo staff on September 28, 2022.
- **Savardekar L.** Traditional diet foods recipe contests as a part of National Nutrition Month activities in collaboration with Maharashtra Labour Welfare Board Mumbai, Naigaon, Maternity Home, Brihanmumbai Mahanagar Palika and DB Kulkarni High School in the months of September and October.

- **Savardekar L.** Community-based camp in collaboration with Sarvajanik Navratrotsav Mandal Naigaon and Rehab team of India As part of International Day for Elders, and National Nutrition Month during October 2022.
- **Savardekar L.** Community based program for body fat analysis of the camp attendees (women) in collaboration with the Health Department of Municipal Corporation of Greater Mumbai as part of Mata Surakshit tar Kutumbh Surakshit on October 3, 2022.
- **Savardekar L.** World Osteoporosis Day as a joint collaborative activity with the Padmashali Yuvak Sangh, Naigaon on October 20, 2022.
- **Savardekar L.** Ahar and Arogya Program as part of Sampark din celebration of Lok Seva Sangha for students of Dr DB Kulkarni High School and Dr Prabhavati Kulkarni High School (5th -10th standard) to sensitize children towards nutritious food habits through IEC program on the theme "Healthy vs Junk foods, My Plate Concept and general nutrition".
- **Savardekar L.** "Healthy Foods for a Healthy Family" was organized in collaboration with Maharashtra Labour Welfare Board, Mumbai at Lalit Kala Kendra, Kamgar Kalyan Mandal, Naigaon on March 9, 2023.
- **Surve S**. "Nutrition and Health" at Mahalaxmi Mumbai Public School for 120 parents of Junior to Grade 9th children on August 10, 2022.
- **Surve S.** Community activity orientation of 33 M.Sc Nutrition Students from SVT College, SNDT University on August 10, 2022.
- **Surve S**. Activities like painting, games & story telling for children and educating mothers on nutrition & child health on the occasion of Children's Day on November 14, 2022.
- **Surve S**. Activity session on the occasion of World Health Day on the theme "Health for all", at Child Health Clinic on April 6, 2022.
- **Surve S.** Talk on "Know your body and Touch" by a clinical psychologist for parents and kids on the occasion of Christmas on December 29, 2022.
- **Surve S.** Invited session on "Immunization for children" and "Puberty and menstrual hygiene" for social workers from different schools of Akanksha foundation on June 4, 2022.
- **Surve S**. Health and menstrual hygiene, parents meeting, Abhyudaya Nagar School, September 7, 2022.
- **Surve S.** Parents meeting on "Nutrition and Health" at **Wadibunder Mumbai Public S**chool on October 20, 2022.
- **Surve S**. "Immunization for children" and "Puberty and menstrual hygiene" sessions for social workers from different schools of Akanksha foundation on July 04, 2022.
- **Surve S**. Talk on Nutrition at Natawar Nagar Mumbai Public School, Andheri on January 30, 2023.
- **Surve S**. Awareness talk on "Pubertal changes among girls" at Sai Baba Path Municipal School, February 04, 2023.
- **Surve S.** Talk on "Pubertal changes" at Mumbai Public School, Abhyudaya Nagar on April 21, 2022.
- **Surve S**. Invited session on "Nutrition and child health" as part of Poshan Maah activities at Mumbai Public School, Shindewadi on September 27, 2022
- **Surve S.** Invited session on "Nutrition and health" at The Akanksha Foundation for social workers from schools on July 8, 2022.

13.3 Meetings / Conferences / Seminars / Workshops Attended

- **Aranha C.** "Science Based Evidence on the Benefits of Probiotics for Human Health", 13th India Probiotic symposium, **The Gut Microbiota and Probiotic Science Foundation (India),** Delhi (hybrid), March 25-26, 2023.
- **Aranha C, Banerjee A, Jagtap D**. "Marvelous molecular motors, TNQ distinguished lecture in life sciences by Dr Ronald Vale", Tata Institute of Fundamental Research, Mumbai, January 09, 2023.
- **Banerjee A.** Board of Studies- PG Department of Biotechnology, (virtual), St Xavier`s College, Mumbai, March 17, 2023.
- **Bhanothu V**. Brainstorming session to examine the issue of still births by DG ICMR, online, Delhi, August 22, 2022.
- **Bhanothu V**. Webinar on "Therapeutics for inherited rare diseases", ICMR, New Delhi, December 20, 2022.
- **Bhanothu V**. The application of nanomaterials in newborn screening, (virtual), Refresher course in nanobiochemistry and bioinformatics, UGC-HRDC, Osmania University, Hyderabad, October 20, 2022.
- **Bhanothu V**. Two-day hands-on workshop on "Chick embryo: An emerging preclinical animal model for cancer research", School of Medical Sciences, University of Hyderabad, Hyderabad, December 8-9, 2022.
- **Bhanothu V**. Online meeting on "Preconception carrier screening for genetic disorders", the Society for Indian Academy of Medical Genetics (SIAMG), October 8, 2022.
- **Bhanothu V**. Round table panel discussion on importance of inborn errors of metabolism (newborn screening) - The Indian context, Synergy Medical Systems LLP and Zentech, September 22, 2022.
- **Bhanothu V**. DST-Proteomics Workshop-2022, Monsoon Advanced Proteomics School (MAPS), Department of Biosciences & Bioengineering, Proteomics Facility IIT Bombay, Mumbai, June 18-29, 2022.
- **Bhanothu V**. Awareness on cyber & data security Cyber Jaagrookta Diwas (CJD), Department of Health Research, India, October 6, 2022.
- **Bhor V**. Course on randomized controlled trials, St. John's Medical College & Research Institute, Bengaluru, October 17-22, 2022.
- **Bhor V.** WHO Workshop on piloting the tool for benchmarking ethics oversight of health-related research with human participants, Bengaluru, December 6-7, 2022.
- **Bhor V**. 108th Indian Science Congress, RTM University, Nagpur, January 3-7, 2023.
- **Bhor V, Chaaithanya KI, Tryambake V, Kokate P, Akula A, Kharat S, Kamat S**. 5th Annual Innovation Festival at Nehru Science Centre, Innovation Festival at Nehru Science Centre, February 01-03, 2023.
- **Bhavya MK**. Health Communication Course, ICMR HQ & MICA Ahmedabad, November 2022 - April 2023.
- **Bhavya MK**. Research Methods and Biostatistics, ICMR NIMS, Delhi, February 06-10, 2023.
- **Bhavya MK**. Qualitative Research and Data Analysis, IIPS Mumbai, March 06-10, 2023.

- **Chaudhari U**. Global epidemic of obesity: lesson learnt from spontaneous obese nonhuman primate models for prevention of human obesity, 10th International Conference LASA India animal models for one health programme: challenges and future perspectives at Hyderabad from June 2 -4, 2022.
- **Dighe V, Mehta N, Desai S.** 9th World Ayurveda Congress and Ayurveda Expo organized by World Ayurveda Foundation (Initiative by Vijnana Bharti) with Focal theme: - Ayurveda for One Health, Panjim, Goa, December 8 – 11, 2022.
- **Gajbhiye RK**. Issues related to Snake Bite and steps for mitigating the associated mortality and morbidity, NITI AAYOG (online), May 19, 2022.
- **Joseph S**. DST sponsored - International Conference on OPEN Autism, CAREADD, St. John's National Academy of Health Sciences, Bengaluru, March 4-5, 2023.
- **Khambata K, Joseph S**. International Bioinformatics Online workshop on Data Science & Machine Learning for Bioinformatics with R (4th edition), September 26 - October 22, 2022.
- **Joseph S**. Training on NGS data analysis pipeline, CDFD, Hyderabad, October 26, 2022.
- **Joshi B**. Expert group meeting on Updating India adapted MEC wheel (2015) for inclusion of Centchroman, organised by MOHFW, New Delhi, May 30, 2022.
- **Joshi B.** Brainstorming Meeting on Implementation Research, ICMR-NIIRNCD, Jodhpur, December 29, 2022.
- **Joshi B.** Technical resource group meeting for reference manual on single rod subdermal implant, New Delhi, December 23, 2022.
- **Joshi B, Tandon D.** Inter country WHO Funded project meeting with Dr Ratanprabha Chavan RCH Officer, Navi Mumbai, September 13, 2022.
- **Khambata K**. International workshop on Advanced Statistical Data Analysis Using SPSS, Online, September 24-30, 2022.
- **Kulkarni RN.** Pre Conception Care - Prepare to Create and Care, Virtual mode, June 10 – 11, 2022.
- **Kulkarni RN.** Technical Advisory Committee meeting on the review of the schedule to the Child and Adolescent Labour (Prohibition and Regulation Act), Central labour Institute, Sion Mumbai, October 31, 2022.
- **Kulkarni RN.** Workshop on Data Quality Guidelines at Mumbai, Novotel, Juhu, Mumbai, October 17-18, 2022.
- **Kulkarni RN.** Implementation Research, ICMR headquarter, January 12 -13, 2023.
- **Kulkarni RN.** Regional Consultation for Prioritizing Diseases for Elimination in India, Co-chair - Panel Discussion - Identifying region specific diseases for elimination and Problems -West Zone, ICMR- NIV Pune, March 9 -10, March 2023
- **Kulkarni RN**. Non-Inferiority trials, Hosted by Clinical trial and Projection Unit, ICMR in virtual mode, March 2, 2023.
- **Kulkarni RN**. Capacity Building Workshop on Operational Research, Operational Research Concepts and Importance in Program, MDACS, Wadala, Mumbai, March 16, 2023.
- **Kumar C**. Training program on structure bioinformatics and molecular docking, online, January 31, 2023.
- **Miya V**. Post-conference workshop on Scientific Communication, BHU, Varanasi, January 23, 2023.
- **Munne K, Akula A**. Awareness among women for common cancers screening, International Women's Day, Xavier's college of Mumbai, March 8, 2023.

- **Munne K.** Hands-on Workshop "SARS-CoV-2 Whole Genome Sequencing", Department of Microbiology, BJ Medical College, Pune, November 16, 2022.
- **Munne K.** Workshop on "Mathematical modelling of infectious diseases focusing on tuberculosis", ICMR- National Institute for Research in Tuberculosis, Chetpet, Chennai, November 14-16, 2022.
- **Munne K.** 1st National Conference "MARSCON 2022" (on Microbiology, Antimicrobial Resistance and Antimicrobial Stewardship), Department of Microbiology, Dr DY Patil Medical College, Hospital and Research Centre, Pimpri, June 3-4, 2022.
- **Munne K.** Elimination of cervical cancer in India, Tata Memorial Hospital, Mumbai, January 13, 2023.
- **Naigaonkar A**. Hands on training for manuscript writing and other lectures, EMBO India Research Partnership programme, IIT Bombay, Powai, February 27, 2023.
- **Patil A.** Towards Elimination of Cervical Cancer in India, Association of Medical Women in India -Mumbai Branch; Cytology Clinic, Cama&Albless Hospital in Association with Department of Preventive Oncology, Tata Memorial Hospital, TMC. Continuing Medical Education (CME), Golden Jubilee Block, Tata Memorial Hospital, Mumbai, January 13, 2023.
- **Pande S.** CME on "Genetics and genetic counseling: What medical professional should know" and Brainstorming meeting to understand research gaps in genetics with clinicians, Bhaktivedanta Hospital and Research Institute, Mira Road, Mumbai, March 16, 2023.
- **Pande S.** CME on "Neonatal endocrinology - bench to bedside" organized by Division of Pediatric Endocrinology and Neonatology B.J. Wadia Hospital for Children, Mumbai, October 14, 2022.
- **Pande S.** ICMR-DHR Biomedical & Research Ethics Updates workshop conducted by ICMR Bioethics Unit, Bengaluru, May 18, 2022.
- **Pathak B, Mukherjee N, Vishwakarma J**, **Borkotoky M**. Cancer genomics & bioinformatics - 5th Edition, decodelife.co.in, online, February 11 – March 7, 2023.
- **Prusty R.** Count EVERYONE, NDQF, ICMR-NIMS Virtual, September 1, 2022.
- **Prusty R.** Availability and Quality of Mortality Statistics in India: Current Appraisal and Future Prospects, NDQF, ICMR-NIMS Virtual, April 7, 2022.
- **Prusty R.** Health Analytics and Disease Modelling, IIT Mumbai, February 23-24, 2023.
- **Surve S**. Data Quality Workshop, Mumbai, Novotel Hotel, Balraj Sahani Marg, Juhu Beach, Juhu, October 17-18, 2022.
- **Surve S**. Protocol improvement workshop on implementation research, ICMR, New Delhi, January 12-13, 2023.
- **Kulkarni RN, Munne K, Surve S**, **Bhanothu V**. Webinar on implementation research by DG ICMR, virtual, ICMR, New Delhi, January 28, 2023.
- **Savardekar L.** "Collaborative projects development workshop: a satellite event", Scientific Committee of the upcoming 9th World Ayurveda Congress, The University of Transdisciplinary Health Sciences and Technology, Bangalore, August 6-7, 2022.
- **Savardekar L.** One-week online course on Randomized Controlled Trials organized by St. John's Medical College & Research Institute in collaboration with faculty from McMaster University, Canada, Duke University, USA, September 20 -24, 2022.
- **Sudhakar DVS**. Hands-on workshop on next generation sequencing: A walk through from sample QC to Data QC, CDFD, Hyderabad, June 20-24, 2022.

- **Sudhakar DVS**. Hands on training on histology and immunohisto/cytochemistry in animal models, School of Life Sciences, University of Hyderabad, March 6-12, 2022.
- **Sudhakar DVS**. Hands-on workshop on Sanger sequencing, Department of Pharmacology and Microbiology, JIPMER, Puducherry, December 5-6, 2022.
- **Sudhakar DVS**. Hands-on workshop on NGS, JIPMER Puducherry, March 9, 2023.
- **Surve S**. Research Perspectives of Idiopathic Precocious Puberty, Clinical meeting, Thane academy of paediatrics, December 12, 2022.
- **Yevate S**. 5th Mouse Cryobiology/IVF workshop, Mouse Genome Engineering Facility (MGEF), Bangalore life Science cluster campus, December 12-17, 2022.

13.4 Invited Lectures

- **Begum S**. "Epidemiological indicators", Workshop on Research Methodology and Biostatistics, Poddar Hospital, October 14, 2022.
- **Bhor, V.** "Gut Microbiome-Immune Axis in Health and Disease", "Swaasthik" Annual Pan-Indian Medical Conference at TN Medical College and BYL Nair Hospital, Mumbai, September 22, 2022.
- **Gajbhiye RK.** "Maternal and neonatal outcomes amongst pregnant women with SARS-CoV-2 infection: Evidence from PregCovid registry" at United Kingdom-International Coronavirus Network-sponsored conference, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Srinagar, J&K, May 10-11, 2022.
- **Gajbhiye RK.** Participated as an expert on snakebite research in the NITI AAYOG meeting (online), May 19, 2022.
- **Gajbhiye RK.** "Community empowerment and Public Health System capacity building for addressing the burden of snakebite envenomation: An experience from Maharashtra, India" at Annual Conference of Indian Society for Toxinology and Snakebite Mitigation (ISTSM) (virtual), May 26-28, 2022.
- **Gajbhiye RK.** "ICMR-NITM Lectures on Snakebite and Development of a proposal for the establishment of the Centre for Advanced Research & Training on Snakebite (CARTS)", ICMR-NITM, Belagavi, June 04-06, 2022.
- **Gajbhiye RK.** "Multi-sectoral model for reduction of mortality and morbidity due to snakebite envenomation in rural India", ICMR-NITM, Belagavi, June 06, 2022.
- **Gajbhiye RK.** "Endometriosis Clinical and Genetic Research in India (ECGRI): An update on improving awareness, knowledge, and care of women with endometriosis" at International Conference on Reproductive Health with Emphasis on "Innovations in Reproductive Sciences and Technologies: Hope, Risk and Responsibilities" & 33rd Annual Meeting of ISSRF and Ravenshaw University, Cuttack, February 25, 2023.
- **Kulkarni RN**. Regional consultation for prioritizing diseases for elimination in India, Co-chair - Panel Discussion - Identifying region specific diseases for elimination and Problems -West Zone, ICMR- NIV, Pune, March 9-10, 2023.
- **Modi D.** "Placenta, sex and the brain", OPEN Symposium, St John's Hospital, Bangalore, June 23-24, 2022.
- **Modi D.** "COVID and male infertility presented", Annual Conference of All India Coordinating Committee RCOG, Mumbai, November 22 2022

- **Modi D.** "Basics to latest advances in genetics for reproductive health", ISAR Gurukul (online), December 12, 2022.
- **Modi D.** "Lhx2 Drives the Decision of the Developing Germ Cells to Enter in Meiosis Recent advances in reproductive health", Central Drug Research Institute, Lucknow, March 30, 2022.
- **Prusty RK**. "Understanding district level household survey data", Research methodology workshop focusing on large scale data analysis, GB Pant Social Sciences Institute, Prayagraj (virtual), June 17, 2022.
- **Prusty RK**. "Family planning programme and interventions, MPS Training", IIPS, Mumbai, December 15, 2022.
- **Pande S**. Case studies on RPL, Indian Society for Assisted Reproduction- ISAR (virtual) on December 13, 2022.
- **Pande S.** Genetics of recurrent spontaneous abortion, ICMR-NIIH, Mumbai, January 4, 2023.
- **Sachdeva G.** Endometrial functions and dysfunction, Kasturba Health Society MRC, Virtual, September 16, 2022.
- **Sachdeva G.** Endometrial permissiveness for SARS-COV2, Association of Clinical Embryologists at Kasturba Medical College, Manipal, October 22, 2022.
- **Savardekar L.** "Roles and responsibilities of Ethics Committee", Basics of Ethics and Functioning of Ethical Committee, Central Council for Research in Homoeopathy (CCRH), virtual, November 23, 2022.
- **Savardekar L.** "Roles and responsibilities of Ethics Committee", Workshop on Bioethics, ICMR-NIIRNCD, virtual, October 7, 2022.
- **Savardekar L.** 1. ICMR ethics Guidelines 2. Roles and responsibilities of the EC, as a FERCAP local surveyor for YEC-1, Yenepoya, Mangalore, September 26-30, 2022.
- **Savardekar L.** "Ethics committee management", Tata Medical Centre, February 18, 2023.
- **Savardekar L.** SIDCER surveyor for the site visit AT Yenepoyya University ethics committee, Manglore, SIDCER survey, February 11, 2023.
- **Savardekar L**. "Role and responsibilities of Ethics Committees", Seminar on Bio-Medical Research Ethics & GCP: NDCT Rules 2019 Perspectivc by ICMR-NI`l`M in association with ICMR-NCDIR, KLE College of Pharmacy, KLF J N Medical College and KLE Academy of Higher Education and Research (KAHER), Belagavi, ICMR-NITM, Belagavi, August 26, 2022.
- **Savardekar L**. Roles and responsibilities of EC members, IEC/GCP update training program for all ICMR-NIRT IEC members, online, ICMR-NIRT, Belagavi, July 9, 2022.
- **Patel V.** "Delineating immune correlates of HCMV congenital transmission", 14th The Cytometry Society Annual Conference & Workshop(s) – 2022, Hyderabad, October 13-16, 2022.
- **Patel V.** "Emerging Viral Infections", BRNS supported PopularScience lectures, Indian Women Scientists' Association, Jai Hind College, Churchgate, Mumbai, November 21, 2022.
- **Patel V.** "Applications of High Dimensional Biology in Advancing Translational Research: Latest Trends and Way Forward", PARADIGM meet, BD Biosciences India, February 20, 2023

13.5 Inter-Institutional Collaborations

13.5.1 National Collaborations

- ACI Cumballa Hill Hospital, Mumbai
- Amity University, Noida
- Bai Jerbai Wadia Hospital for Children, Mumbai
- Breach Candy Hospital, Mumbai
- Cottage Hospital, Jawhar
- Government of Maharashtra
- Grant Government Medical College and Sir JJ Group of Hospitals, Mumbai
- HTAIn Resource hub, PGIMER Chandigarh
- Indian Institute of Science, Banglore
- Indian Institute of Technology, Bombay
- Institute of Life Sciences, Bhubhaneswar
- Jaslok Hospital, Mumbai
- Kasturba Medical College (KMC), Manipal
- Lokmanya Tilak Municipal General Hospital, Mumbai
- Mangeshikar's Clinic, Mumbai
- MGM Hospital, Kamothe, Mumbai
- Mumbai District Aids Control Society, Mumbai
- Mumbai Fertility Clinic and IVF, Mumbai
- Municipal Corporation of Greater Mumbai
- Nalini Speciality Hospital, Mumbai
- National Institute of Animal Biotechnology, Hyderabad
- Nowrosjee Wadia Maternity Hospital, Mumbai
- PD Hinduja Hospital, Mumbai
- Seth GS Medical College & KEM Hospital, Mumbai
- SRL Dr Avinash Phadke Labs, Mumbai
- STI clinic Municipal ART Centre, Nagpada
- **Sub District Hospital, Dahanu**
- **Sub District Hospital,** Kasa
- Tata Memorial Hospital, Mumbai
- TN Medical College and B Y L Nair Hospital, Mumbai

13.5.2 International Collaborations

- Brunel University, London, UK
- Department of Veterinary Medicine, UAE University, UAE
- Institute for Molecular Biosciences, The University of Queensland, Australia
- School of Public Health, The University of Queensland, Australia
- University of California, San Diego, USA

13.6 Trainees

Fifty one summer and winter trainees were inducted in various departments namely Molecular Endocrinology, Gamete Immunobiology, Genetic Research Centre, Cell Physiology And Pathology, Molecular and Cellular Biology, Infectious Diseases Biology, Cellular and Structural Biology, Bioinformatics, Neuroendocrinology, Molecular Immunology and Microbiology, Innate Immunity, National Center For Preclinical Reproductive and Genetic Toxicology, Biochemistry, Infectious Diseases Biology, Clinical Research Laboratory. These trainees were introduced to technological innovations in different fields and were trained in various scientific processes in ongoing projects.

13.7 ICMR National Snakebite Project (INSP) on Capacity Building of Health System on Prevention and Management of Snakebite Envenomation Including its Complications *(Partly Funded by Indian Council of Medical Research)*

Principal Investigator	:	**R Gajbhiye**
Co-Principal Investigator	:	H Bawaskar, Bawaskar Hospital and Research Centre, Mahad, District Raigad, Maharashtra
Project Associates	:	H Munshi M Gavhande, Shahapur, District Thane G Bhad, Aheri, District Gadchiroli JP Dash
Co-Investigators:	:	A Yadav, Directorate of Health Services, Maharashtra State MV Bansode, Sub District Hosipital, Shahapur
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. 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; b) 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; c) empower the health system for management of SBE through the implementation of Standard Treatment Guidelines (STG) of the Government of India; and iv) study the impact of the interventions on reducing SBE mortality and morbidity (Fig. 1). Retrospective data on snakebite cases (n=1415) reported at health facilities in study blocks during the year 2020 and 2021 has been collected. A sub-group analysis is depicted in Table 1. Total 28 focus group discussions (14 males, 14 females) have been conducted in the study blocks on knowledge, perceptions and awareness about snakebite prevention, diagnosis & first aid. About 250 Medical Officers and Community Health Officers have been trained on prevention, diagnosis and management of snakebite envenomation by national experts including Dr Himmatrao Bawaskar, Dr Dayal Bandhu Mazumdar and Dr Sadanand Raut including training on endotracheal intubation and cardio-pulmonary

resuscitation. The project staff has completed the assessment of public health facilities (n=34) in their respective blocks for their preparedness to manage snakebite victims including availability and utilization of anti-venom. Knowledge assessment of frontline healthcare workers including ANMs, MPWs, HAs and LHVs has also been completed (n=350).

Figure 1: The schematic outline of the study

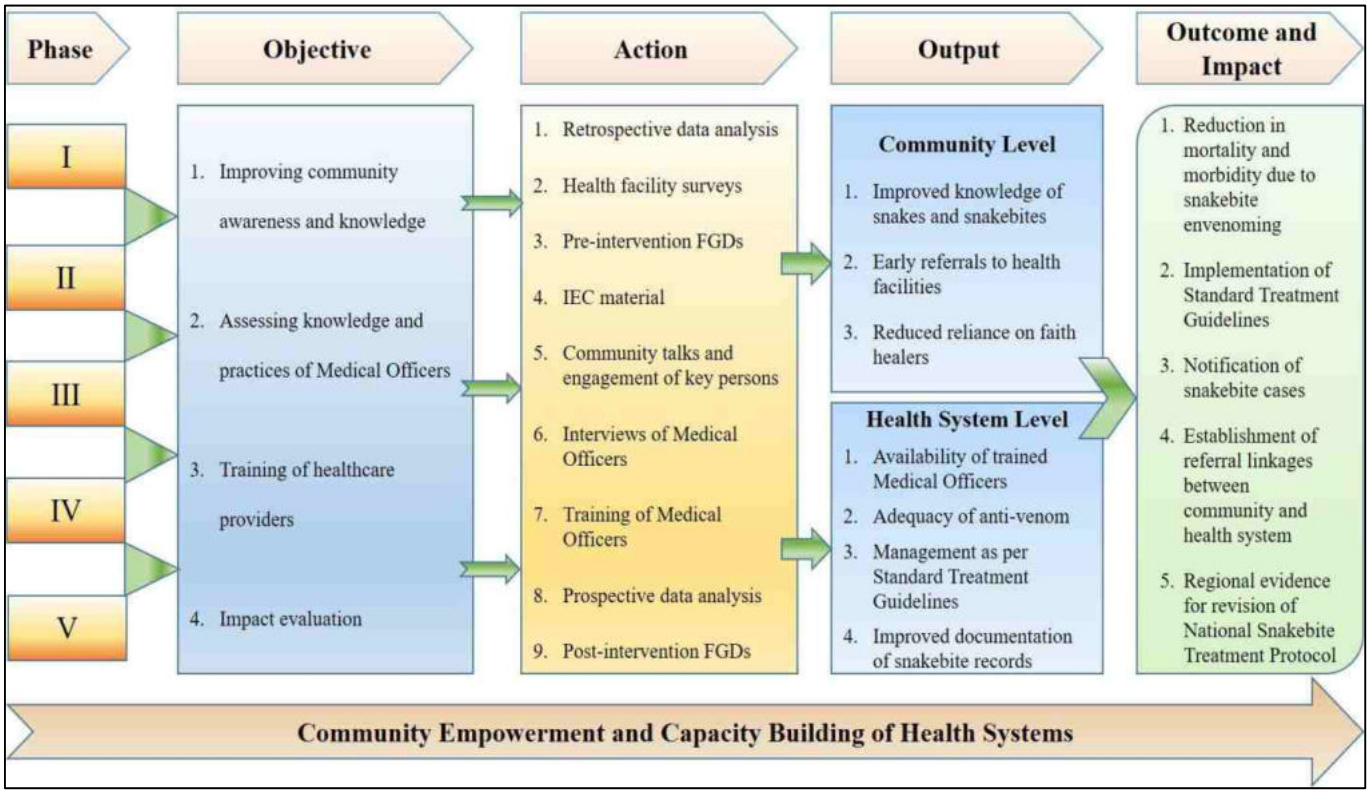

Table 1: Age and gender wise distribution of snakebite cases as per analysis of the retrospective data of snakebite cases from 2020-2021

Age group	Gender		Total (n,%)
	Male (n,%)	Female (n,%)	
0 to 10	20 (2.2)	18 (3.6)	38 (2.7)
10 to 20	89 (9.7)	72 (14.5)	161 (11.4)
20 to 30	245 (26.7)	97 (19.6)	342 (24.2)
30 to 40	235 (25.6)	125 (25.2)	360 (25.4)
40 to 50	191 (20.8)	103 (20.8)	294 (20.8)
50 to 60	84 (9.1)	47 (9.5)	131 (9.3)
60 to 70	40 (4.4)	21 (4.2)	61 (4.3)
70 to 80	15 (1.6)	12 (2.4)	27 (1.9)
> 80	0 (0)	1 (0.2)	1 (0.1)
Total	**919** (100)	**496 (100)**	**1415 (100)**

As per the study protocol, the staff has been collecting prospective data on snakebite victims and data of about 700 cases has been recorded. A comparative analysis of retrospective and prospective data

would aid in evaluating the impact of the interventions. Awareness material including snakebite management flowchart, posters and brochures for communities and snakebite information booklet for healthcare workers has been developed, reviewed and approved by the Technical Advisory Committee. The material has been developed in local languages and will be disseminated on a wide scale for maximizing its utility. A directory of names of snakes as reported by respondents including their local names has been developed to assist the Medical Officers and Healthcare workers in snake identification (Table 2).

Table 2: Local names of the Big Four in study blocks as reported by respondents during focus group discussions

English common name	Scientific name	Shahapur	Aheri	Khordha	Kasipur
Indian Cobra	Naja naja	*Nag; Pandhrya; Nagsap*	*Nag; Naitras; Nagdev; Nagulpam; Kodenagu; Nailnalu; Godmanalu*	*Naga; Kolathia Naga*	*Naga saapa*
Common Krait	Bungarus caeruleus	*Manyar; Chur; Gathya; Satpal*	*Manyar; Chur; Katlapam; Katlataras; Nark-kalle*	*Chiti*	*Kaleta saapa*
Russell's Viper	Daboia russeli	*Ghonas; Kamblya*	*Ghonus; Kamblya; Pinjar Pam; Mudtaras; Urla Pinjar; Budar Pinjar*	*Chandan Boda; Sankha Modia*	*Mandala saapa; Jabada saapa*
Saw Scaled Viper	Echis carinatus	*Fursa; Fodsa; Vatarya*	*Fursa; Fodsa; Fursha; Rakt Pinjar*	*Dhulia Boda; Nadara Boda; Khandi Boda*	*Dhulia Boda; Dhuli Naga*

13.8 Nationwide Study to Estimate Incidence Mortality Morbidity and Economic Burden due to Snakebites in India *(Partly Funded by Indian Council of Medical Research)*

Principal Investigator	:	**R Gajbhiye**
Project Associates	:	A Shaikh, Project Assistant District Nanded, Maharashtra Ashwini Wanzolkar, Project Assistant District Raigad, Maharashtra, K Shelkande Field Worker District
Co-Investigator	:	Smita Mahale Former Director & Emeritus Scientist ICMR-NIRRCH
Duration	:	2021-2023

Snakebite is a major public health problem in India, with majority of victims hailing from a rural agrarian population having poor access to medical facilities. However, the lack of robust record keeping in the most affected regions, combined with many victims not presenting to hospital due to logistical or cultural reasons, lead to underestimations. The present study aims to be the first large scale survey in 13 states located in the 5 different geographical zones covering 1-4 full districts in each state and includes data from outside the hospital, directly from the community and would be first prospective study on snakebite burden in the country. The objectives of the study are – a. to determine the incidence of snakebite cases, b. to determine the pattern of injuries due to snakebites in the community and nature of morbidity and mortality, c. to understand the treatment seeking behavior of the snakebite victims and d. to determine the cost of illness due to snakebites. Three districts – Raigad, Pune and Nanded with a combined population of 17,368,437 are the study sites in Maharashtra. ICMR-NIRRCH, Mumbai has the primary responsibility of coordinating and directing the activities in the three districts. In the preparatory phase of the study, training of ASHAs was carried out in the three districts on filling of case record forms, informed consent and other ethical aspects of the study. Out of the total 6862 ASHAs in the districts, 5414 (78.9%) ASHAs attended the training programs. A pre- and post-training assessment of ASHAs was carried out to evaluate the effectiveness of the one-day training program. The questionnaire assessed ASHAs' knowledge regarding snakes, first aid and preventive measures (Table 1). The project staff and ASHAs have since then started the data collection process in all the three districts. The data is collected from the victims and part of the data regarding the management at healthcare facilities is cross checked with facility records as quality control. The details of data collected till May 21, 2023 is depicted in Table 2. In the coming year, data collection using the case record form by the project staff will be completed. The data entry and analysis of the pre- and post-training ASHA evaluation have been completed and a manuscript is currently in development.

Table 1: District wise details of ASHA trainings and pre- and post- training assessment

District	Total ASHAs	Attended training	% of attendance	Filled pre- and post-questionnaires	% filled both pre- and post-questionnaires out of attending ASHAs
Raigad	1887	1520	80.6	1488	97.9
Pune	3351	2625	78.3	1342	51.1
Nanded	1624	1269	78.1	1229	96.8
Total	**6862**	**5414**	**78.9**	**4059**	**75.0**

Table 2: District wise details of snakebite cases and deaths

District	Cases	Deaths	Case Fatality Rate
Raigad	1300	14	1
Pune	1217	38	3.1
Nanded	445	25	5.6
Total	**2962**	**77**	**2.6**

HONORS AND AWARDS

14. HONORS AND AWARDS

14.1 Awards

1. **Amruta Gadade**. 3rd prize for Pre-clinical studies oral presentation, at SAC-ACCP Taj Vivanta Ahmedabad, Gujrat, January 8, 2023.
2. **Anshul Bhide.** Best Poster Award by Society for Reproductive Biology and Comparative Endocrinology (SRBCE) at the International Conference on Reproductive Biology, Comparative Endocrinology & Development - 39th Annual Meeting of SRBCE, CCMB, Hyderabad, September 14-16, 2022.
3. **Aishwarya Rao.** Best Oral Presentation Award at International Conference on Reproductive Health with Emphasis on "Innovations in Reproductive Sciences and Technologies: Hope, Risk and Responsibilities" & 33rd Annual Meeting of the ISSRF, Ravenshaw University, Cuttack, Odisha, February 24 -26, 2023.
4. **Delna Irani.** Dr Shanta Rao Best Paper Award 2023 at ICMR-NIRRCH, February 23, 2023.
5. **DVS Sudhakar.** Young Investigator Award at the International Conference on Reproductive Biology, Comparative Endocrinology & Development - 39th Annual Meeting of SRBCE, CCMB, Hyderabad, September 14-16, 2022.
6. **DVS Sudhakar**. Young Scientist Award at the International Conference on Reproductive Health with Emphasis on "Innovations in Reproductive Sciences & Technologies: Hope, Risk & Responsibilities" & 33rd Annual Meeting of the ISSRF, Ravenshaw University, Cuttack, Odisha, February 24 -26, 2023.
7. **Kasturi Ganguly.** Best Poster Award at International Conference on Kaleidoscopic Insights into Reproductive and Child Health at ICMR-NIRRCH, January 23-25 2023.
8. **Kasturi Ganguly.** ICMR-NIRRCH Foundation Day Award, 2023 for the Best Research Paper, ICMR-NIRRCH, February 20, 2023.
9. **Kritika Patil.** Prof NR Moudgal Young Scientist Award 2023 at International Conference on Reproductive Health with Emphasis on "Innovations in Reproductive Sciences and Technologies: Hope, Risk and Responsibilities" & 33rd Annual Meeting of the ISSRF, Ravenshaw University, Cuttack, Odisha, February 24 -26, 2023.
10. **Nandini Kasarpalkar, Amit Kumar Singh, Shilpa Bhowmick.** Best Paper Award by Cytometry Society of India at TCS2022 Annual Meeting, Hyderabad, October 15, 2022.
11. **Nandini Kasarpalkar.** Young Investigator Award by Investigator Initiated Research (IIR) 2021 Initiative, ICMR-IAVI Joint Call, April 2022.
12. **Nupur Mukherjee.** Early-career Woman Scientist Award: Oration Award by Indian Immunology Society at the 49th Annual Conference & General Body Meeting of Indian Immunology Society Immunocon-2022 at PGIMER, Chandigarh, November 24-26, 2022.
13. **Pallavi Shukla**. Best Oral Presentation Award at 7th Annual PCOS Conference 2022, Leela Hotel, Mumbai, September 16, 2022.
14. **Shruti Desai.** 2nd Prize for Pre-clinical studies poster presentation, at SAC-ACCP Taj Vivanta Ahmedabad, Gujrat, January 8, 2023.

14.2 PhD Degrees Awarded

1. **Ms Gargi Thakur**

   Thesis title: Elucidating the role of Surfactant Protein D (SP-D) in prostate cancer
   Research Guide: Dr Taruna Gupta

2. **Ms Kaushiki Prabhudesai**
   Thesis title: Rational design and experimental validation of modulators of human follicle stimulating hormone receptor to serve as fertility regulating agents
   Research Guide: Dr Susan Thomas

3. **Ms Krutika Patil**
   Thesis title: Understanding follicular angiogenesis in women with polycystic ovary syndrome
   Research Guide: Dr Srabani Mukherjee

4. **Ms Pratibha Varma**
   Thesis title: Study deciphering the role of HDAC6 in spermatogenesis
   Research Guide: Dr Priyanka Parte

5. **Ms Shaini Joseph**
   Thesis title: Building and analysing gene networks associated with phenotypes leading to infertility.
   Research Guide: Dr Smita Mahale

6. **Ms Varsha Prabhu**
   Thesis title: Characterization of hMR-gp120 interaction & its association with transmission of HIV
   Research Guide: Dr Vainav Patel

ADVISORY COMMITTEES

15. ADVISORY COMMITTEES

15.1 Scientific Advisory Committee

Dr Neerja Bhatla (*Chairperson*)
Professor, Obstetrics and Gynaecology,
All India Institute of Medical Sciences, Ansari Nagar, New Delhi

Dr Subeer S Majumdar
Director General,
Gujarat Biotechnology University, Sector 11, Gandhinagar, Gujarat

Dr BK Thelma
Department of Genetics,
Delhi University, Benito Juarez Marg, New Delhi

Dr Ashutosh Halder
Professor & Head, Reproductive Genetics,
All India Institute of Medical Sciences, Ansari Nagar, New Delhi

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,
A-503, Devdeveshwar CHS,
Telly Galli Cross Lane, Andheri (East), Mumbai

Dr Bharati Kulkarni (*ICMR representative*)
Scientist-G and Head, Division of RBMCH & Nutrition,
Indian Council of Medical Research, V Ramalingaswami Bhawan,
Ansari Nagar, New Delhi

Dr Manisha Madkaikar (*Special Invitee*)
Director, ICMR-NIIH,
Parel, Mumbai

Dr Sudha Rao (*Special Invitee*)
Professor and Head, Department of Pediatrics,
Bai Jerbai Wadia Hospital for Children, Acharya Donde Marg, 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 Sadhana Tayade
Director of Health Services, Govt. of Maharashtra,
Arogya Bhavan, St. Georges Hospital Compound, PD Mello Road, CSTM, Mumbai

Dr Mangala Gomare
Executive 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-NIRRCH, Parel, Mumbai

14.2 Members of ICMR-NIRRCH Ethics Committee for Human Studies

Prof Shubhada Chiplunkar *(Chairperson)*
Former Director ACTREC-TMC,
Principal Investigator, Chiplunkar Lab,
Tata Memorial Centre, Kharghar, Navi 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, Tata Institute of Social Sciences,
Deonar, Mumbai

Dr Rakhi Tripathi
Department of Pharmacology,
Acharya Donde Marg,
Parel, Mumbai

Dr Ketki Kulkarni
Assistant Professor,
Nowrosjee Wadia Maternity Hospital, Parel, Mumbai

Dr Bipin Kulkarni
Scientist E, ICMR-NIIH, Parel, Mumbai

Adv Ajay Shinde
Gulisatan, 3rd floor, CAT Bar Association,
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Flat No. 13, Orchids Complex, Madhumalati CHS, Majiwada, Thane West

Mrs Sudha Sathaye
C/9/8 Sukumar society, Dayaldas Road, Vileparle (East), Mumbai

Dr Bhakti Pathak
Scientist-E,
ICMR-NIRRCH, Parel, Mumbai

Dr Shailesh Pande
Scientist-D,
ICMR-NIRRCH, Parel, Mumbai

Dr Smita Nair
Assistant Professor,
Tata Institute for Social Sciences, VN Purav Marg, Deonar, Mumbai

Adv Mrs. Meghana Shirke *(Alternate Member - Legal Expert (from February 14, 2022)*)
Room No. 81, 3rd floor, Mahavir Sukh Building,
Dr Ambedkar Road, Dadar (East), Mumbai

Dr Ashwini Karve
Associate Professor
Topiwala National Medical College, RTO Colony, Mumbai Central, Mumbai

Dr Suchitra Surve *(Joint Member Secretary)*
Scientist-C, ICMR-NIRRCH, Parel, Mumbai

Dr Vikrant Bhor *(Member Secretary)*
Scientist-D, ICMR-NIRRCH, Parel, Mumbai

15.3 Members of Institutional Animal Ethics Committee

Dr Geetanjali Sachdeva *(Chairperson)*
Director, ICMR-NIRRCH, Parel, Mumbai

Dr K Pani Prasad *(Main Nominee*)
Principal Scientist,
ICAR-Central Institute of Fisheries Education,
Yari Road, Panch Marg, Varsova, Mumbai

Dr Prabhakar Ukale
Veterinarian,
Institute of Chemical Technology,
Nathalal Parekh Marg, Matunga (E), Mumbai

Dr Eshita Kishor Waghela *(Socially Aware Nominee)*
Veterinarian,
Mumbai Veterinary College, Parel Village, Sindhu Nagar, Parel, Mumbai

Dr Sangram Shankarrao Chavan *(Link Nominee)*
Bombay Veterinary College, Parel, Mumbai

Dr Padma Devrajan
Scientist, Dean Research & Innovation,
Institute of Chemical Technology, N. P. Marg, Matunga, Mumbai

Dr Vikas Dighe,
Scientist-E, ICMR-NIRRCH, Parel, Mumbai

Dr Dhanjit Das
Scientist-E, ICMR-NIRRCH, Parel, Mumbai

Dr SM Metkari, *(Member Secretary)*
Principal Technical Officer, ICMR-NIRRCH, Parel, Mumbai

14.4 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 *(Vice-Chairperson*)
Director, ICMR-NIIH, Parel, Mumbai

Dr Abhijit Majumdar *(Stem Cell Expert)*
Associate Professor,
Department of Chemical Engineering, IIT Bombay

Dr Sanjeev Waghmare *(Stem Cell Expert)*
Principal Investigator & Scientific Officer F,
ACTREC, Tata Memorial Centre, Navi Mumbai

Dr Pradip Chaudhari *(Member)*
Scientific Officer G,
ACTREC, Tata Memorial Centre, Navi Mumbai

Dr Bipin Kulkarni *(Member)*
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Dr Nilesh Gawade *(Ethics Expert)*
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Dr Smitha Sasidharan Nair *(Social Scientist)*
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Bandra West, Mumbai

Dr Dhanashree Jagtap *(Member Secretary)*
Scientist-D, ICMR-NIRRCH, Parel, Mumbai

EXTRAMURALLY FUNDED ONGOING AND NEW PROJECTS

16. EXTRAMURALLY FUNDED ONGOING AND NEW PROJECTS

S.No.	Title	PI	Funding agency	Start year	End year
1.	Delineation of the role of isoforms of kisspeptin in mammalian reproduction	Antara Banerjee	SERB	2022	2024
2.	Role of kisspeptin mediated signaling in onset of puberty	Antara Banerjee	SERB	2021	2024
3.	Study of kisspeptin receptor oligomerization and its functional significance	Antara Banerjee	BRNS-DAE	2022	2025
4.	Improving access for screening of common cancers and non-communicable diseases among women in tribal block of Maharashtra: Challenges in implementation	Anushree Patil	ICMR	2019	2022
5.	Evaluating the role and proteolytic processing of Trop1 and Trop2 in normal placentation and placental pathologies	Bhakti Pathak	DBT	2021	2024
6.	Health systems analysis and evaluations of the barriers to availability, utilization, and readiness of family planning and contraceptive services in COVID-19 affected areas of Maharashtra, India	Beena Joshi	WHO	2022	2023
7.	HTA Resource Hub	Beena Joshi	DHR HTAIn	2022	2023
8.	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	2022	2023
9.	Omics of serum exosomes in endometriosis: An attempt to identify a possible biomarker	Dhanjit Kumar Das	ICMR	2022	2025
10.	Deciphering the immunomodulatory roles of Homeobox A 10 in the mouse endometrium during embryo implantation	Deepak Modi	DBT	2021	2024
11.	Evaluating the inflammatory, microbiome profile and co-infections in women diagnosed with treatment failure, relapse or recurrent bacterial vaginosis	Deepti Tandon	ICMR	2022	2025
12.	Longitudinal cohort study to evaluate the effect of various contraception methods on the composition and diversity of the vaginal microbiota	Deepti Tandon	ICMR	2019	2022

13.	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.	Delineating the role of human β-microseminoprotein in male reproduction	Dhanashree Jagtap	ICMR	2023	2025
15.	DNA damage, repair and demethylation in the pathogenesis of Endometriosis	Geetanjali Sachdeva	DBT	2019	2022
16.	Eutopic endometrial cell repertoire in women presenting different subtypes of endometriosis and its association with endometrial receptivity	Geetanjali Sachdeva	ICMR	2023	2026
17.	Uterine alarmins and their relevance in implantation	Geetanjali Sachdeva	SERB	2020	2023
18.	Molecular analysis of HLA-G in pregnant tribal women and its role in infectious etiologies modulating intrauterine inflammation - A prospective cohort study	Krishna Chaaithanya Itta	ICMR	2022	2023
19.	Exploring Exosomal MicroRNA as Potential Biomarker for Endometriosis	Kanchan Sharma	DHR	2022	2025
20.	Improving treatment literacy and adherence among People living with HIV (PLHIV) through innovative strategies	Kiran Munne	NACO	2020	2022
21.	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
22.	Investigating the role of endocannabinoid system in first trimester chorionic villi of women experiencing recurrent spontaneous abortions	Kumari Nishi	SERB	2023	2026
23.	Impact of Mukta Shukti Bhasma and Saubhagya Shunti Churna in a reversal of bone mineral density among lactating women consuming traditional diet foods in Maharashtra: A randomized controlled preliminary clinical study	Lalita Savardekar	CCRAS, Ministry of AYUSH	2023	2026
24.	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	ICMR-IAVI	2022	2023
25.	Immune and microbiome correlates of TB reactivation in PLHIV and a NHP model	Nupur Mukherjee	Indo-US HMSC	2020	2023

26.	Role of toll-like receptors and TLR agonists in modulating response to chemotherapy in TNBC patients	Nupur Mukherjee	DBT-BIOCARE	2019	2022
27.	Delineating pathogenesis of obese and lean PCOS phenotype using integrated transcriptomics and proteomics approach	Pallavi Shukla	DHR	2023	2026
28.	Study of maternally inherited mitochondrial DNA variants in women with polycystic ovarian syndrome	Pallavi Shukla	ICMR	2021	2023
29.	Design and development of a microfluidic chip for sperm selection/sorting based on chemotaxis	Priyanka Parte	DBT	2021	2024
30.	Functional significance of Testis Specific Histone H2B variant (TH2B) in sperm and early embryonic development	Priyanka Parte	DBT	2021	2024
31.	Development of primary health care models for palliative care, elderly care and mental health in Maharashtra	Ragini Kulkarni	DHR	2023	2024
32.	Community based screening and management of latent TB among under-five children from urban slums in Mumbai: Phase 2 - Screening TB contacts of index case in age group of 5-12 years	Suchitra Surve	DHR	2019	2023
33.	Exploring clinical and therapeutic relevance of novel biomarkers among the children presenting with idiopathic and incomplete precocious puberty at tertiary hospital Mumbai	Suchitra Surve	ICMR	2021	2024
34.	Population based birth defect surveillance in linkage with Rashtriya Bal Swasthya Karyakram (RBSK) programme in rural blocks of Palghar district in Maharashtra	Suchitra Surve	DHR	2020	2023
35.	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
36.	Identification of differentially expressed genes in lean and obese PCOS and validation using rat animal models	Susan Thomas	DHR	2019	2023
37.	Identification of enriched biochemical networks and polypharmacological targets in metabolic syndrome	Susan Thomas	SERB (STAR)	2020	2023

38.	Integrated analyses of genomic scale metanolic models and omics profiles to capture the host-pathogen-environment interplay of Candida sp	Susan Thomas	SERB	2021	2024
39.	Machine learning algorithms trained on voice to predict psychological distress and postpartum depression	Susan Thomas	ICMR	2023	2025
40.	Establishment of Centre for Maternal & Child Genetics at ICMR-NIRRCH, Mumbai	Shailesh Pande	DBT	2023	2025
41.	Mission program on paediatric rare genetic disorders (Mumbai Chapter)	Shailesh Pande	DBT	2022	2027
42.	Validation of a novel assay for detection of Y chromosome microdeletions and development of an algorithm to predict clinical outcomes in male infertility	Stacy Colaco	DHR	2020	2023
43.	Evaluation of therapeutic potential of PVF-VEGF in trophoblast differentiation and placental spiral artery remodelling in murine models of preeclampsia	Taruna Madan	DBT	2021	2024
44.	Role of Receptor for Advanced Glycation End product (RAGE) and high mobility group B protein in endometrial repair	Uddhav Chaudhari	SERB	2019	2022
45.	An integrated approach towards characterizing the Treg reservoir in HIV-1 infection.	Vainav Patel	DST-SERB	2023	2026
46.	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
47.	Immune response to precautionary third dose of Covishield/Covaxin among healthy adult population: an ICMR Cohort study, India	Vainav Patel (Site PI)	ICMR	2022	2024
48.	Identification and evaluation of novel metabolites with the potential of prenatal diagnosis of fetal congenital heart diseases - a pilot study	Venkanna Bhanothu	DST-SERB	2022	2025
49.	Molecular profiling of common clinical phenotypes associated with congenital hypothyroidism - a pliot study	Venkanna Bhanothu	ICMR	2023	2026
50.	Evaluation of drugs-cytochrome P450 enzyme interaction through fluorometric high throughput screening assays	Vikas Dighe	CCRAS	2021	2024

51.	Evaluation of synergistic impact of oral nano-curcumin and Alpha-Linolenic Acid on maternal and fetal health in a rat model of preeclampsia	Vikas Dighe	ICMR	2021	2024
52.	Exploring the therapeutic potential of peptides Targeting Lysophosphatidic Acid (LPA) Receptors in Ovarian Cancer	Vikas Dighe	DBT	2021	2024
53.	Evaluation of immunomodulatory and anti-cancer properties of Hydroxychavicol, a major constituent of *Piper betel*	Vikas Dighe	DHR	2023	2026
54.	Preclinical study on efficacy, safety and toxicity of Swarna Prashan regimen as adjuvant therapy in pediatric acute lymphoblastic leukemia	Vikas Dighe	CCRAS	2023	2026
55.	Sertoli and Leydig cell homing peptides as molecular steering for testicular targeted drug delivery	Vikas Dighe	DHR	2021	2024
56.	Intranasal mucosal vaccine for COVID-19	Vikrant Bhor	DBT-BIRAC	2020	2023
57.	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	2025

AYUSH *Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy*
BioCARe *Biotechnology Career Advancement and Re-orientation Programme*
BIRAC *Biotechnology Industry Research Assistance Council*
BRNS *Board of Research in Nuclear Sciences*
CCRAS *Central Council for Research in Ayurvedic Sciences*
DAE *Department of Atomic Energy*
DBT *Department of Biotechnology*
DHR *Department of Health Research*
DST *Department of Science and Technology*
HMSC *Health Ministry Screening Committee*
HTAIn *Health Technology Assessment in India*
IAVI *International AIDS Vaccine Initiative*
ICMR *Indian Council of Medical Research*
NACO *National AIDS Control Organization*
SERB *Science and Engineering Research Board*
STAR *Science and Technology Award for Research*
WHO *World Health Organization*

INTRAMURALLY FUNDED ONGOING AND NEW PROJECTS

17. INTRAMURALLY FUNDED ONGOING AND NEW PROJECTS

S.No.	Title	PI	Start year	End year
1.	Developing indigenous diagnostics for evaluating the levels of kisspeptin	Antara Banerjee	2022	2025
2.	Implementation of multidisciplinary model of care for PCOS	Anushree Patil	2016	2024
3.	Androgen receptor –glucocorticoid receptor crosstalk in ovarian cancer cells and its impact on chemosensitivity	Bhakti Pathak	2023	2028
4.	Deciphering the role of PSP94 and CRISP family proteins in ion channel modulation	Bhakti Pathak	2019	2024
5.	Developing an immunochromatography based strip test for analysing P_LGF concentration for prediction of risk for developing preeclampsia	Bhakti Pathak	2017	2024
6.	Analysis of molecular cargo and paracrine effects of extracellular vesicles secreted by ovarian cancer cells	Bhakti Pathak	2021	2026
7.	Assessing Trop2 expression and its correlation with the anti-Trop2 immune status in ovarian cancer patients	Bhakti Pathak	2017	2014
8.	Determining the role of HOXA10 in the pathogenesis of endometriosis	Deepak Modi	2021	2024
9.	Development of a microfluidic-based tool for assessing placental functions and evaluating its potential application in pregnancy-related disorders	Deepak Modi	2023	2026
10.	Investigating the role of epithelial to mesenchymal transition in the process of embryo implantation	Deepak Modi	2020	2024
11.	Study on placenta of women with coronavirus disease 2019 (COVID-19) and its correlation with pregnancy and neonatal outcomes	Deepak Modi	2022	2025
12.	Comprehensive assessment of women diagnosed with Spontaneous premature ovarian insufficiency-Multicentric study	Deepti Tandon	2023	2026
13.	Three dimensions of *Mycoplasma genitalium* infection detectioncure rate and co-infections in women attending STI clinics	Deepti Tandon	2020	2024
14.	Genetic aberrations and their functional analysis in patients with intellectual disability: implications of genetic defect in iPSCs derived neurons	Dhanjit Kumar Das	2023	2028
15.	Genetic and biochemical characterization of mitochondrial oxidative phosphorylation (OXPHOS) disorders in children	Dhanjit Kumar Das	2021	2026

16.	Investigating the role of microdeletion syndrome in neuronal functions using induced pluripotent stem cells	Dhanjit Kumar Das	2022	2027
17.	Effect of maternal gestational micronutrient deficiency on offspring's fertility and its underlying epigenetic mechanisms in germline	Dipty Singh	2021	2026
18.	Idiopathic recurrent pregnancy loss: Possible association with paternal exposure to endocrine disruptors and epigenetic modifications in sperm	Dipty Singh	2018	2023
19.	Unravelling the sperm epigenetic landscape in infertile men with clinical varicocele	Dipty Singh	2020	2025
20.	Identification and characterization of genetic factors associated with multiple morphological abnormalities of sperm flagella (MMAF)	DVS Sudhakar	2022	2025
21.	Investigating the contribution of DNA damage, repair and demethylation in pathogenesis of endometriosis	Geetanjali Sachdeva	2023	2026
22.	Investigating the key elements in estrogen signalling in the context of prostate cancer	Geetanjali Sachdeva	2021	2024
23.	Uterine alarmins and their relevance in implantation	Geetanjali Sachdeva	2023	2026
24.	Investigating sperm 5hmC landscape in male infertility and recurrent pregnancy loss	Kushaan Khambata	2023	2028
25.	Unravelling sperm epigenetic landscape regulated by estrogen receptors in adult male rats	Kushaan Khambata	2020	2025
26.	Exploring the association of cervicovaginal microbiome with transient and persistent high-risk HPV infection	Kiran Munne	2023	2026
27.	Evaluating utility of molecular workflow for establishing microbial profile and antimicrobial resistance for neonatal sepsis in a tertiary care NICU, Mumbai.	Kiran Munne	2023	2026
28.	Transgenerational effects of paternal hypertension on fertility and pregnancy outcome: An epigenetic approach	Kumari Nishi	2021	2026
29.	Deciphering the trophoblast-breast epithelial cell cross talk in pregnancy associated breast cancer (PABC)	Nupur Mukherjee	2021	2026
30.	Role of TLR4 signaling in driving crosstalk between tumor-associated macrophages (TAMs) and tumor epithelial cells in TNBC	Nupur Mukherjee	2022	2025
31.	Role of Toll-like receptors and TLR agonists in modulating response to chemotherapy in TNBC patients	Nupur Mukherjee	2020	2025
32.	Study of epigenetic factors involved in mitochondrial dysfunction in obese women with PCOS	Pallavi Shukla	2019	2025

33.	Implications of gonadotropin and their receptor gene variants in male infertility	Periyasamy Kuppusamy	2022	2026
34.	Design and development of a microfluidic chip for sperm selection/sorting based on chemotaxis (partly funded by DBT)	Priyanka Parte	2021	2024
35.	Functional significance of testis specific histone H2B variant (TH2B) in sperm and early embryonic development (partly funded by DBT)	Priyanka Parte	2018	2024
36.	Investigation of potential chemotactic metabolites in the follicular fluid	Priyanka Parte	2020	2024
37.	Deciphering Gut Microbial Signatures in Breast Cancer and Impact of their Modulation on Immune Response Following Therapy	Sadhana Gupta	2023	2027
38.	Deciphering the functional significance of novel genes associated with polycystic ovary syndrome identified from network analysis	Shaini Joseph	2021	2024
39.	Assessing the coagulation and fibrinolytic system as contributors of thrombotic state in polycystic ovary syndrome	Srabani Mukherjee	2022	2025
40.	Exploring the epigenetic alterations regulating miRNA expression in women with polycystic ovary syndrome	Srabani Mukherjee	2021	2026
41.	Investigation of epigenetic alterations in murine model of cystic ovary	Srabani Mukherjee	2022	2026
42.	Unravelling the metabolic nexus in the granulosa cells of women with PCOS (DHR sanctioned)	Srabani Mukherjee	2023	2026
43.	Unravelling the redox status and glucose metabolism dynamics in oocyte microenvironment in women with PCOS	Srabani Mukherjee	2021	2024
44.	Determining the prevalence and risk factors associated with Precocious puberty among pre-pubertal girls in Mumbai	Suchitra Surve	2023	2025
45.	Electronic capture and machine learning analysis of data pertaining to childhood tuberculosis from tertiary hospitals	Susan Thomas	2019	2025
46.	Establishment of Bioinformatics and Computational Biology Centre	Susan Thomas	2020	2026
47.	Host pathogen signatures associated with congenital transmission and pathogenesis of human cytomegalovirus Part A: Immune correlates and viral signatures associated with congenital transmission and pathogenesis of human cytomegalovirus	Vainav Patel	2019	2024

48.	Studies on HIV Latency and Reactivation in Cellular Reservoirs	Vainav Patel	2018	2023
49.	Molecular profiling of cases with congenital hypothyroidism	Venkanna Bhanothu	2022	2025
50.	Establishment and validation of the assays for the detection of inborn errors of metabolism and endocrine diseases- a pilot newborn screening (NBS) program	Venkanna Bhanothu	2021	2024
51.	Characterization of the human gut microbiome immune-axis in pregnancy and functional assessment in an animal model	Vikrant Bhor	2021	2026
52.	(A) Host-Pathogen signatures associated with congenital transmission and pathogenesis of human cytomegalovirus (B) Exploring the gut and breast milk microbiome associated with congenital transmission and pathogenesis of human cytomegalovirus	Vikrant Bhor	2021	2024
53.	Investigation of the role of *Gardnerella vaginalis* membrane vesicles in pathogenesis of bacterial vaginosis	Vikrant Bhor	2023	2028
54.	Heterogeneity of gestational diabetes mellitus based on insulin resistance	Uddhav Chaudhari	2023	2026
55.	Role of HMGB1 and RAGE in endometrial repair	Uddhav Chaudhari	2022	2025

STAFF AND STUDENTS

18. STAFF AND STUDENTS

DIRECTOR'S OFFICE

Dr. Geetanjali Sachdeva, *Director*
Mr. M. P. Chabukswar, *Personal Assistant*
Mr. K. N. Kadam, *Attendant*

BIOMEDICAL INFORMATICS CENTRE

Dr. Susan Thomas, *Scientist 'E'*
Mr. R. S. Barai, *Technical Officer*
Mr. N. B. Shelar, *Laboratory Assistant*
Ms. Shuvechha Mukherjee, *Ph. D. student*
Ms. Indra Kundu, *Ph. D. student*
Ms. Kshitija S. Rahate, *Ph. D. student*
Ms. Karishma Desai, *Ph.D. student*
Ms. Ulka Gawde, *Ph.D. student*

BIOSTATISTICS DEPARTMENT

Dr. Shahina Begum, *Scientist 'E'*
Dr. R. K. Prusty, *Scientist 'C'*
Dr. Mahadev Bhise, *Scientist 'C'* **Appointed on 16.02.2023*

CELL PHYSIOLOGY AND PATHOLOGY LABORATORY

Dr. Geetanjali Sachdeva, *Scientist 'G'*
Dr. U. K. Chaudhari, *Scientist 'E'*
Dr. R. R. Katkam, *Principal Technical Officer*
Ms. Sushma Gadkar, *Sr. Technical Officer-2 and Ph. D. student*
Mr. B. P. Mayekar, *Laboratory Assistant*
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. Arif Hussain, *Ph. D student*
Mr. M. I. F. J. Shaikh, *Ph. D. student*
Mr. A. Khandvilkar, *Ph. D. student*
Mr. G. R. Paswan, *Ph. D. student*
Ms. Nikita Sharma,*Ph. D. student*

CELLULAR AND STRUCTURAL BIOLOGY LABORATORY

Dr. Bhakti Pathak, *Scientist 'F'*
Dr. Dhanashree Jagtap, *Scientist 'D'*
Dr. Antara Banerjee, *Scientist 'C'*
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*
Ms. Jyoti Vishwkarma, *Ph. D. student*

CHILD HEALTH RESEARCH DEPARTMENT
Dr. Suchitra Surve, *Scientist 'D'*
Dr. Kiran Munne, *Scientist 'C'*
Ms. Sharmila Kamat, *Technician 'A'*

CLINICAL RESEARCH LABORATORY
Dr. R. Gajbhiye, *Scientist 'E'*
Dr. P. Kuppusamy, *Scientist 'C'*
Mr. A. Vadigopulla, *Principal Technical Officer* **Retired on 31.03.2023*
Ms. Shagufta A. Khan, *Sr. Technical Officer-2*
Ms. Vaishali Chalke, *Labortory Assistant*

GAMETE IMMUNOBIOLOGY LABORATORY
Dr. Priyanka Parte, *Scientist 'F'*
Dr. Kushaan Khambata, *Scientist 'B'*
Ms. Smita Yevate, *Technical Officer*
Mr. M. T. More, *Laboratory Assistant*
Mr. D. G. Gaikwad, *Laboratory Assistant*
Ms. Veena Dalvi, *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 LABORATORY
Dr. Sadhana Gupta, *Scientist 'D'*
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 LABORATORY
Dr. Taruna M. Gupta, *Scientist 'F'*
Mr. Manish Ghosalkar, *Sr. Technical Officer-2* **Transferred from NIREH on 12.12.2022*
Mr. R. D. Shinde, *Laboratory Assistant*
Ms. Aishwarya Rao, *Ph. D. student*
Ms. Kasturi Ganguly, *Ph. D. student*
Ms. Hajra Gupta, *Ph. D. student*
Ms. Rutwija Athalye, *Ph. D. student*

MOLECULAR AND CELLULAR BIOLOGY LABORATORY
Dr. D. N. Modi, *Scientist 'F'*
Dr. Nupur Mukherjee, *Scientist 'C'*
Ms. Sarika Ahire, *Technical Officer*
Mr. S. G. Sakpal, *Laboratory Assistant*
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. Babita Negi, *Ph. D. student*
Ms. Pranya N, *Ph. D. student*
Ms. Rushigandha Salunke, *Ph. D. student*

MOLECULAR ENDOCRINOLOGY LABORATORY
Dr. Srabani Mukherjee, *Scientist 'G'*
Dr. Pallavi Shukla, *Scientist 'D'*
Ms. Sushma Khavle, *Technical Officer-C*
Ms. Gayatri Shinde, *Sr. Technical Officer-1*
Ms. Nanda Joshi, *Technical Officer*
Mr. P. P. More, *Technician 'C'*
Mr. V. M. Khedekar, *Laboratory Assistant*
Mr. A. Naigaonkar, *Ph. D. student*
Ms. Snehal Bhingardeve, *Ph. D. student*
Ms. Komal Khade, *Ph. D. student*
Ms. Medini Samant, *Ph. D. student*
Ms. Manisha Kumari, *Ph. D. student*

MOLECULAR IMMUNOLOGY AND MICROBIOLOGY

Dr. V. Bhor, *Scientist 'E'*
Dr. K. C. Itta, *Scientist 'C'*
Dr. Clara Aranha, *Principal Technical Officer*
Ms. Gauri Bhonde, *Sr. Technical Officer-1*
Mr. S. D'souza, *Laboratory Assistant*
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*
Mr. Rohan Pawar, *Ph. D. student*

NEUROENDOCRINOLOGY LABORATORY

Dr. Nafisa H. Balasinor, *Scientist 'G'* ** Retired On 31.05.2022*
Dr. Dipti Singh, *Scientist 'D'*
Dr. Kumari Nishi, *Scientist 'C'*
Ms. Vaishali H. Nakhawa, *Sr. Technical Officer- 2*
Ms. Shobha Sonawane, *Sr. Technical Officer- 1*
Ms. Reshma Gaonkar, *Technical Officer*
Mr. M. G. Pawar, *Technician 'C'*
Mr. S. Mandavkar, *Technician 'C'*
Mr. D. B. Shelar, *Technician 'C'*
Mr. V. V. Chavan, *Technician 'C'* ** Retired on 30.06.2022*
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*
Ms. Deepashika Arya, *Ph. D student*
Ms. Anushruti Singh, *Ph. D student*

OPERATIONAL AND IMPLEMENTATION RESEARCH DEPARTMENT

Dr. S. L. Chauhan, *Scientist 'G'* **Retired on 31.05.2022*
Dr. Beena Joshi, *Scientist 'F'*
Dr. Ragini Kulkarni, *Scientist 'F'*
Dr. G. Srimathi, *Scientist 'B'* **Resigned on 31.12.2022*
Dr. M. K. Bhavya, *Scientist 'B'*
Mr. I. S. Mashal, *Technician A*
Mr. P. S. Sanap, *Technician A*
Smt. M. S. Kumre, *Laboratory Attendant- 2*

PRECLINICAL REPRODUCTIVE AND GENETIC TOXICOLOGY CENTRE

Dr. V. D. Dighe, *Scientist 'F'*
Mr. S. V. Jadhav, *Sr. Technical Officer-2*
Ms. Shilpa C. Kerkar, *Technical Officer-C*
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*
Mr. Bipradip Saha, *Ph. D. student*

REPRODUCTIVE AND BONE HEALTH DEPARTMENT

Dr. Lalita Savardekar, *Scientist 'F'*
Ms. Neera Mehta, *Technical Officer 'B'*
Ms. Devyani Rathod, *Nursing Orderly*
Mr. K. Y. Chavan, *Laboratory Attendant-2*

REPRODUCTIVE ENDOCRINOLOGY AND INFERTILITY DEPARTMENT

Dr. S. L. Chauhan, *Scientist 'G'* **Retired on 31.05.2022*
Dr. Anushree Patil, *Scientist 'E'*
Dr. Deepti Tandon, *Scientist 'C'*
Ms Leena Tendulkar, *Sr. Technical Officer-2*
Ms. Pratibha Kokate, *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. Shilpa C. Kerkar, *Technical Officer-C*
Ms. Sunita Kharat, *Sr. Technician-1*
Ms. Anamika Akula, *Sr. Technician -1*
Ms. Sunita Kendre, *Laboratory Assistant*
Ms. Akshaya A. Rathod, *Attendant (Services)*
Ms. Shalini S. Lambade, *Attendant (Services)*
Ms. Sarita Bhange, *Attendant (Services)*

STEM CELL BIOLOGY LABORATORY

Dr. D. K. Das, *Scientist 'E'*
Dr. Shyla Ravindran, *Technical Officer -C*
Ms. Sandhya Anand, *Technical Officer - B*
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 LABORATORY

Dr. V. Patel, *Scientist E*
Dr. D. Gogoi, *Scientist 'B'* * *Transferred to RMRC, Dibrugarh on 31.05.2022*
Ms. Varsha Padwal, *Sr. Technical Officer-3*
Ms. Shilpa M. Velhal, *Sr. Technical Officer-2*
Mr. S. T. Bhagat, *Technical Officer -B*
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*
Mr. Nandan Mohite, *Ph. D. student*

EXPERIMENTAL ANIMAL FACILITY

Dr. D. K. Das, *Scientist 'E'*
Dr. S. M. Metkari, *Principal Technical Officer*
Mr. S. C. Gondhalekar, *Sr. Technician-1*
Mr. H. Raut, *UDC*
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 Attendant- 2*
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, *Attendant (Services)*
Mr. P. K. Shingare, *Attendant (Services)*
Mr. S. S. Chavan, *Attendant (Services)*
Mr. S. S. Kadam, *Attendant (Services)*
Mr. B. Koli, *Attendant (Services)*
Mr. M. S. Qureshi, *Attendant (Services)*
Mr. R. S. Merchande, *Attendant (Services)*
Mr. Y. Shinde, *Attendant (Services)*
Mr. M. V. Mali, *Attendant (Services)*

CONFOCAL FACILITY
Dr. Dipti Singh, *Scientist 'D'*
Ms. Shobha Sonawane, Sr. *Technical Officer- 1*
Ms. Reshma Gaonkar, *Technical Officer*

DNA SEQUENCING FACILITY
Dr. Srabani Mukherjee, *Scientist 'G'*
Ms. Nanda Joshi, *Technical Officer*

ELECTRON MICROSCOPY FACILITY
Dr. Dipty Singh, *Scientist 'C'*
Dr. Kumari Nishi, *Scientist 'C'*
Ms Vaishali Nakhwa, *Sr. Technical Officer-2*
Mr. M. G. Pawar, *Technician 'C'*

FLOW CYTOMETRY FACILITY
Dr. Srabani Mukherjee, *Scientist 'F'*
Ms. Sushma Khavale, *Technical Officer-C*
Ms. Gayatri Shinde, *Sr. Technical Officer-1*

ETHICS SECRETARIAT
Dr. Vikrant M. Bhor, *Scientist 'E'*
Dr. Suchitra Surve, *Scientist 'D'*
Ms. Vaishali Bhogate, *Sr. Technical Officer-1*
Ms. Zakiya Ansari, *Technical Assistant*
Mr. A. H. Hankare, *Laboratory Assistant*

ACCOUNTS
Mr. Waman Narkar, *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. Gavit, *UDC*

ADMINISTRATION
Mr. A. S. Gaikwad, *Sr. Administrative Officer*
Ms. Seema A. Deshmukh, *Administrative Officer* **Transferred from NIN on 12.12.2022*
Mr. S. D. Mate, *Administrative Officer* ** Retired on 31.08.2022*
Mrs. Sunayna L. Barde, *Sr Private Secretary*
Mr. A. Sarnaik, *Office Assistant*
Mr. K. Pawar, *UDC*

GENERAL ADMINISTRATION
Ms. Akanksha A. Dalvi, *Section Officer*
Mr. S. A. Sangelkar, *Office Assistant*

PROJECT CELL
Ms. Akanksha A. Dalvi, *Section Officer*
Mr. M. M. Shinde, *UDC*
Ms. Ruchita Veerkar, *UDC*

PAY BILL SECTION
Ms. Akanksha A. Dalvi, *Section Officer*
Mr. V. M. Satav, *Office Assistant*
Mr. H. V. Jadhav, *Office Assistant*
Mr. S. T. Chorage, *UDC*

STORES
Mr. P.K. Chavan, *Section Officer*
Mr. G. M. Darpe, *Office Assistant*
Mr. K. Rama Rao, *Office Assistant*
Ms. Mamta Jadhav, *UDC*
Mr. S. Petkar, *UDC*
Mr. S. S. Sawant, *UDC*
Mr. K. Keni, *Laboratory Assistant*
Mr. N. P. Bavdane, *Laboratory Assistant*

ESTABLISHMENT
Mr. K. T. Solanki, *Section Officer*
Ms. Kranti S. Patankar, *Personal Assistant*
Mr. S. Sasi kumar, *Personal Assistant* **Transferred to NIRT on 21.12.2022*
Ms. Harsha Kurup, *UDC*

ICMR INTERNATIONAL HOSTEL AND STAFF QUARTERS
Dr. Taruna M. Gupta, *Scientist F*
Ms. Vasanthi Rajan, *Sr. Technical Officer-3* **Retired on 31.05.2022*
Mr. G. R. Devadiga, *Sr. Technician - 3*

INSTRUMENTATION RESEARCH & MAINTENANCE/WORKSHOP
Mr. V. D. Koli, *Sr. Technical Officer-2*
Mr. J. Patharwat, *Technical Officer-C*
Mr. K. R. Sukumar, *Technical Assistant*
Mr. Joseph D. Lobo, *Sr. Technician - 3*
Mr. V. G. Rane, *Sr. Technician – 2*

Mr. A. Anglekar, *Attendant (Services)*

LIBRARY AND INFORMATION CENTER

Dr. Prabhjeet Kaur, *Library and Information Officer*
Ms. Simmy Saji, *Technical Officer-C*
Ms. Priya Menon, *Technical Officer-B*
Mr. V. L. Shinde,*Technician A*
Mr. S. A. Gavas, *Laboratory Assistant* **Expired on 05.09.2022*

SECURITY AND MAINTENANCE

Mr. J. D. Lobo, *Sr. Technician - 3*
Ms. Swaruparani Karunakaran, *Office Assistant*
Mr. N. S. Bhilare, *Sr. Technician - 2*
Mr. G. P. Narayan, *Sr. Technician - 2*
Mr. S. L. Shivtarkar, *Sr. Technician - 1*
Mr. S. S. Subramnian, *Technician- 2*
Mr. S. K. Jadhav, *Technician- 2*
Mr. A. Y. Lokhande, *Laboratory Assistant*
Mr. S. F. Cardoza, *Laboratory Assistant*
Mr. M. Palande, *Laboratory Assistant*
Mr. R. Naik, *Laboratory Assistant*
Mr. K. B. Pawar, *Laboratory Assistant* **Retired on 31.05.2022*
Mr. S Misal, *Laboratory Assistant* **Transferred fom NIREH on 19.12.2022*
Mr. A. D. Bhandwalkar, *Laboratory Attendant-2*
Mr. S. Y. Urankar, *Laboratory Attendant-2*

ACITIVITIES DURING THE YEAR 2022-2023

19. ACITIVITIES DURING THE YEAR 2022-2023

Dr Geetanjali Sachdeva, Director, felicitated the housekeeping staff maintaining cleanliness in the institute as part of Swachhata Pakhwada held during April 1-15, 2022.

A talk on "Health and hygiene" was organized demonstrating methods of washing hands for students of RM Bhat English medium Primary School, on April 12, 2022.

A 4-week, DHR sponsored training course on Paediatric Medical Genetics was organized during June 20, 2022 to July 15, 2022.

Dr Geetanjali Sachdeva, Director, hoisted flag in the presence of the staff and students on Independence Day 2022 on August 15, 2022.

Various activities related to Hindi language and literature were organized for the staff and students as part of Hindi Pakhwada during September 16-29, 2022.

An educational session was conducted at PHC Aghai, District Thane, on the occasion of International Snakebite Awareness Day on September 19, 2022.

An awareness activity on menstrual hygiene and breast cancer screening was conducted for female staff of Veermata Jijabai Bhosale Udyan on September 28, 2022.

An interactive session titled "All about growing up" was conducted for girls of class 8th to 10th at Akanksha School, Abhyudaya Nagar on September 30, 2022.

A community-based camp was organized to screen for sarcopenia, teach elders a daily exercise protocol, educate them on importance of high protein diet and prepare them to make easy and nutritious recipes on October 1, 2022.

World Osteoporosis Day was celebrated by our Bone Health Clinic by organizing a recipe contest for traditional diet foods & high protein diet on October 20, 2022.

Activities like painting, games and story telling were organized at our Child Health Clinic to celebrate Children's Day on November 14, 2022.

Dr Rajiv Bahl, Director General, ICMR, visited our institute for a strategic review meeting with our scientists on November 18, 2022.

Scientific Advisory Committee meeting was conducted during December 1-2, 2022.

International Conference on Kaleidoscopic Insights into Reproductive and Child Health (ICKIR) was organized to celebrate the birth centenary year of Founder Director, Dr Shanta S Rao during January 23-25, 2023.

CME on Genetic Testing and Counseling in Medical Practice was conducted on January 23, 2023.

As part of celebrations for our 53rd Foundation Day, cricket and badminton competitions were organized for our staff and students on February 21, 2023.

पिढी़यों की प्रेरणा

तेईस जनवरी उन्नीसौं तेईस
बड़ा ही पावन था वो दिन
गगन से धरा, उतरी थी देवी
भविष्य के लेकर सपने रंगीन ॥१॥

सुरज जैसी थी उसकी आभा
स्वभाव में न था गर्व या मान
दूरदर्शी,सकारात्मक दृष्टि से
समस्या का करती समाधान ॥२॥

दयालू, क्षमाशील उदार व्यक्तित्व
अनुकरणीय संगठन क्षमता
बनी पिढी़यों के लिए प्रेरणा
अनुसंधान प्रति दृढ़ प्रतिबद्धता ॥३॥

नींव रखी इस संस्थान की
कर्म ही थी जिसकी पूजा
शांता राव नाम उस देवी का
मां का ही वह स्वरूप दुजा ॥४॥

सीमित न था उसका आसमां
खुद के परिवार के रूप में
सुनहरा पिढी का भविष्य किया
कदम कदम पर हर एक स्वरूप में ॥५॥

जिस पौधे को सिंचा लगनसे
आज है फुलाफला वृक्ष बना
रहते छत्रछाया में इस संस्थान के
होगा न इसका अस्तित्व फना ॥६॥

- क्रांती पाटणकर
वैयक्तिक सहायक

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