Curriculum Vitae

Neelima Dubey, Ph.D.

Associate Principal Research Scientist
Dr. Reddy's Institute of Life Sciences (DRILS), Hyderabad 500046, Telangana, India
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Professional Career:

- **Associate Principal Research Scientist (**August 2022- till date**)** at Dr. Reddy's Institute of Life Sciences, Hyderabad 500046, Telangana, India
- **Assistant Professor (**April 2018-June 2022), at Dr. D.Y. Patil Biotechnology & Bioinformatics Institute, Tathawade, Pune, MH- 411033
- **Project Scientist** (October 2017-March 2018), at National Centre for Cell Science (NCCS), Pune University campus, Pune, MH-411007
- **Research Associate** (January 2017-September 2017), at National Centre for Cell Science (NCCS), Pune University campus, Pune, MH-411007
- **Special Volunteer (Foreign)** (August 2014 – August 2017), at National Institute of Health, Section on Behavioral Endocrinology, National Institute of Mental Health, National Institutes of Health (NIH), Bethesda, MD 20892
- **Research fellow** (Full Time Employee) (October 2012 – April 2014), at National Institute of Health, Section on Behavioral Endocrinology, National Institute of Mental Health, National Institutes of Health (NIH), Bethesda, MD 20892
- **Postdoctoral fellow** (January 2011 – Sep. 2012), at National Institute of Health, Section on Behavioral Endocrinology, National Institute of Mental Health, National Institutes of Health (NIH), Bethesda, MD 20892
- **Senior Research Fellow** (April 2008 – December 2009), Department of Zoology, Banaras Hindu University, Varanasi, India
- **Junior Research Fellow** (April 2004 – March 2008), Department of Zoology, Banaras Hindu University, Varanasi, India

Academic Career:

Course	Years	Institution
Ph.D.	2003-2009	Department of Zoology, Banaras Hindu University, Varanasi, India.
M.Sc. (Zoology)	2000-2002	Department of Zoology, Banaras Hindu University, Varanasi, India.
B.Sc (Zoology Hons.)	1997-2000	Banaras Hindu University, Varanasi, India
10+2	1996	Council for the Indian School Certificate Examination, New Delhi, India
10	1994	Council for the Indian School Certificate Examination, New Delhi, India

Research Skills and Experience:

Induced Pluripotent Stem Cell Culture: I have experience in generating human induced pluripotent stem cells (IPSCs) from tissues such as blood and fibroblast from human samples. I established human iPSC facility at my laboratory at National Institute of Health (NIH) and National Centre for Cell Science (NCCS), Pune while working as a post-doctoral fellow and Project Scientist. We generated 8 -10 iPS cell lines from patients suffering with premenstrual dysphoria, PMDD and approximately equal number of iPS cell lines from well matched control (asymptomatic women) at NIH. I also initiated differentiation of these iPSC lines into various lineages particularly neuronal lineage after extensive characterization.

At NCCS I was involved in establishing iPSC bio-bank from various diseases of Indian origin.

3D organoid culture: I have experience with generating fully functional human 3D skin model using dermal and epidermal cells such as fibroblast, melanocytes and keratinocytes.

Immortalized cell line culture: During my post-doc I developed several immortalized human cells lines from blood samples, diseased (PMDD) and controls. These cells served as continuous *in vitro* source of patient specific cells which were used for genetic and molecular biology studies.

Testicular cell culture. During my Ph.D. I worked with testicular cell (such as Leydig cells, sertoli cells and testicular macrophages) isolated from mammalian, reptilian and piscine model, splenic macrophage isolated from mammalian and reptilian model. I have experience in isolating and culturing these cells which were used as *in vitro* model system for various studies.

***Next Generation Sequencing*.** I have performed RNA sequencing multiple times for my postdoctoral project as well as recently for my ongoing project on Major Depressive Disorders.

I have much experience in all routine molecular biology techniques such as DNA/ RNA isolation, and whole transcriptome library construction, Real Time qPCR, TLC, ELISA, RIA, Immunocytochemistry (ICC or IFC) ,Gel electrophoresis, Spectrophotometric methods, Nitric Oxide estimation, Western Immuno blotting, confocal microscopy, FACS. I have experience with bioinformatics, some of the tools such as DAVID and GSEA, RECTOME, Gene ontology etc I used these for next-generation sequencing (NGS) data.

Teaching Experience/ Engagement (Classroom Lecture and Practical Classes):

Title	Course Name	Semester
Developmental Biology	B.Tech. (Biotechnology) and B. Tech. (Medical Biotechnology) M. Tech (Biotechnology)	Spring
Genetics	M.Sc. (Biotechnology)	Autumn
Animal Tissue Culture	B. Tech (Biotechnology)	Spring
Developmental Biology and Stem Cells	M. Tech (Biotechnology)	Spring
Biomimetics	B. Tech.(Medical Biotechnology)	Autumn
HealthCare Law Management	B. Tech.(Medical Biotechnology)	Spring
Disease Biology	B. Tech.(Medical Biotechnology)	Autumn

Human Diseases and Pathobiology	B. Tech (Biotechnology), M. Tech (Biotechnology)	Autumn
Epidemiology & Public Health	B. Tech.(Medical Biotechnology)	Autumn
Seminar Presentation Skills	M.Sc. (Biotechnology)	Autumn

Student Name	Dissertation Topics
Ms. Ananya Sharma	"Design of multigene baculovirus system for generating induced pluripotent stem cells"
Ms. Shrutika Kavali	"Design of multigene baculovirus system for generating induced pluripotent stem cells from blood cells"
Ms. Reena Shedge	"Generation of Lymphoblastoid cell lines from women with and without post-partum depression"
Ms. Nikita Bhor	"Learning cell culture techniques using different cell lines with focus on differentiation of SH-SY5Y cell line"
Ms. Stuti Mazgaonkar	Identification of pertinent pathways in Pre-menstrual Dysphoric Disorder applying Bioinformatics.
Ms. Shravani Shankar	Potential role of proteasome complex in Pre-menstrual Dysphoric Disorder.
Ms. Pratiksha Pawar	Collection of blood samples from women with Major Depressive Disorder (MDD) for RNA Sequencing.

B. Tech, M. Tech and M.Sc. Projects supervised: Seven

Students Mentored for Summer Internship: Eight

1. Ms. Nikita Bhor (B. Tech (Medical) Biotech)
2. Ms. Pratiksha Pawar (M. Tech (Biotechnology))
3. Ms. Shweta Abhimanyu Dongare (B. Tech (Medical) Biotech)
4. Mr. Anshuman Sand (B. Tech Biotechnology)
5. Ms. Debadrita Ghosh (B. Tech Biotechnology)
6. Maitreyee Mukhopadhyay (M. Tech (Biotechnology))
7. Ms. Ayushi Singh (M. Tech (Biotechnology)

Intramural Grant from Dr. D. Y. Patil Vidyapeeth, Pune for the Project Entitled "Development of *in vitro* cellular model for postpartum depression in Indian population"

List of Publications in Referred Journals:

1. Pradhan RN, Panda SK, Torres J, Kremer C, Kavali S, **Dubey N**, Naik S, Singh AK, (**2024**). A mono-aquated di-pyridine-based Gd(III) complex as T1-weighted MRI probe with high relaxivity and stability. **Inorganica Chimica Acta. 561, 121845**.

2. Buruda AP, Vinnakota R, Bharti P, Dutta P, **Dubey N**, Kumar J, (**2022**). Emerging insights into the structure and function of ionotropic glutamate delta receptors. **British Journal of Pharmacology**. **179, 3612-3627**.

3. Li JH, Goff A, Rudzinskas S A , Jung Y, **Dubey N**, Hoffman J, Hipolito Dion, Mazzu M, Rubinow DA, Schmidt PJ, Goldman D, (**2021**). Altered estradiol-dependent cellular Ca^{2+} homeostasis and endoplasmic reticulum stress response in Premenstrual Dysphoric Disorder. **Molecular Psychiatry. 26, 6963- 6974**.

4. Marrocco J, Einhorn N R, Petty G H, Li H, **Dubey N**, Hoffman J, Berman K F, Goldman D, Lee F S, Schmidt P J, McEwen B S. (**2020**). Epigenetic intersection of BDNF Val66Met genotype with premenstrual dysphoric disorder transcriptome in a cross-species model of estradiol add-back. **Molecular Psychiatry 25: 572-583**

5. * **Dubey N**, Hoffman JF, Schuebel K, Yuan Q, Martinez PE, Nieman LK, Rubinow DR, Schmidt PJ and Goldman D, (**2017**). The ESC/E(Z) complex, an effector of response to ovarian steroids, manifests an intrinsic difference in cells from women with Premenstrual Dysphoric Disorder. **Molecular Psychiatry. 22(8): 1172–1184**. doi:10.1038/mp.2016.229.

   (Extensively covered by both International and National news reports in The Telegraph, The Sun, FoxNews, New York Magazine, Zee News, Times of India, The Tribune, Indian Express etc.)*

6. **Dubey, N**, Kumar, P, Lal, B (**2015**) Endocrine regulation of testosterone production by Leydig cells in the catfish, Clariasbatrachus: Probable mediators of growth hormone. **Animal Reproduction Science. 154, 158-165.**

7. Lal, B &**Dubey, N** (**2013**) Existence of a nitric oxide synthase /nitric oxide system in fish testis and its role in modulation of androgenesis. **Fish. Physiol. Biochem**. **39:65–69**.

8. **Dubey, N** & Lal, B (**2010**).Seasonality in expression and distribution of nitric oxide synthase isoforms in the testis of the catfish, *Clariasbatrachus*: Role of nitric oxide in testosterone production. **Comp. Biochem. Physiol. Part C. 151, 286-293**.

9. **Dubey, N** & Lal, B (**2009**).Paracrine role of macrophage produced-nitric oxide (NO) in Leydig cell steroidogenesis in a teleost, *Clariasbatrachus*: Impact of gonadotropin, growth hormone and insulin on NO production by testicular macrophages. **Gen. Comp. Endocrinol**. **160, 12-18**.

10. **Dubey, N** & Lal, B (**2008**). Nitric oxide: An autocrine regulator of Leydig cell steroidogenesis in the Asian catfish, *Clariasbatrachus*. **Gen. Comp. Endocrinol. 158, 161-167**.

Manuscripts under preparation:

- Shrutika K, Mazgaonkar S, Bhor N, Saldanha D and **Dubey N** (2024). Dysregulated Glutamate Trafficking as a Result of Neuroinflammation in Major Depressive Disorder.

- Shrutika K and **Dubey N** (2024). Ionotropic Glutamate Receptors and their Role in Neuropsychiatric Disorders.

List of Publications in International and National Conferences:

1. Li H, **Dubey N**, Man JFH, Rubinow DR, Schmidt PJ, and Goldman D (2020). Premenstrual Dysphoric Disorder (PMDD) is Associated with Estradiol-dependent Aberrations in Cellular Ca^{2+} Homeostasis and the Endoplasmic Reticulum Stress Response. **REPRODUCTIVE SCIENCES** 27 (SUPPL 1), 58A-58A.

2. Jordan M, Nathan E, Li H, **Dubey N,** and McEwen, B (2019). Epigenetic Intersection of BDNF Val66Met Genotype with Premenstrual Dysphoric Disorder Transcriptome in a Cross-Species Model of Estradiol Add-Back. **BIOLOGICAL PSYCHIATRY** DOI: 10.1016/J.BIOPSYCH.2019.03.197.

3. Goff A, Hoffman J, **Dubey N**, Schuebel K, Marrieta C, Yuan Q, Martinez P, Nieman L, Rubinow D, Schmidt P, Goldman D (2017) Lymphoblastoid cell lines from women with premenstrual dysphoric disorder differ in genetic mRNA & protein expression profiles compared with asymptomatic controls. **BIOLOGICAL PSYCHIATRY** Vol 81, issue 10, page S205

4. Marrocco J, Petty GH, **Dubey N**, Hoffman JF, Berman KF, Goldman D, Schmidt PJ and McEwen BS (2017). Estradiol add-back in BDNF Val66Met mice mimics the behavioral and transcriptional phenotype of premenstrual dysphoric disorder **SOCIETY FOR NEUROSCIENCE** 2017, 159.14/OO3.

5. Hoffman JF, **Dubey N**, Schuebel K, Marietta C, Yuan Q, Martinez PE, Nieman LK, Rubinow DR, Schmidt PJ and Goldman D, (2016). Women with Premenstrual Dysphoric disorder (PMDD) differ in baseline and steroid hormone response expression profiles of the ESC/E(Z) pathway compared with asymptomatic controls. **SOCIETY FOR NEUROSCIENCE** 2016, 174.18/GGG9.

6. Hoffman JF, **Dubey N**, Schuebel K, Marietta C, Yuan Q, Martinez PE, Nieman LK, Rubinow DR, Schmidt PJ and Goldman D, (2015). Whole Transcriptome Expression Profiles in Lymphoblastoid Cells from Women with Pmdd and Controls: Diagnostic Differences and Differential Response to Ovarian Steroids. **THE ENDOCRINE SOCIETY MEETING**; General Female Reproductive Endocrinology**,** FRI-092.

7. Hoffman, JF, **Dubey N**, Schuebel, K, Marietta, C, Yuan,Qiaoping, Martinez, P, Nieman, L, Rubinow, DR, Schmidt, PJ Goldman, D.(2015) Lymphoblastoid Cell Lines from Women with Premenstrual Dysphoric Disorder (PMDD) Differ in mRNA and Protein Expression Profiles of the ESC/E(Z) Pathway Compared with Asymptomatic Controls. National Institute of Mental Health, Rockville, Maryland, USA. **NEUROPSYCHOPHARMACOLOGY (**2015) 40, S106–S271; doi:10.1038/npp.2015.325

8. Hoffman JF, **Dubey N**, Schuebel K, Marietta C, Yuan Q, Martinez PE, Nieman LK, Rubinow DR, Schmidt PJ and Goldman D, (2014). Whole transcriptome expression profiles in lymphoblastoid cells from women with PMDD and controls: diagnostic differences and differential response to ovarian steroids. **NIH Research Festival,** CLIN-8

9. Lal B, **Dubey N** (2011). Intra-testicular Nitric Oxide Regulates Steroidogenesis in Fish. **Indian J. Sci. Technol.** 4 (S8), 138-136.

10. **Dubey N,** Lal, B (2011). Possible mediator of growth hormone action on testicular testosterone production in the Asian catfish, *Clariasbatrachus*. **Indian J. Sci. Technol.** 4 (S8), 153-154.

Awards, Academic Assets, Fellowships and Grants:

- **Selected as Full-Time Employee (September 2012)** at National Institute of Health, Section on Behavioral Endocrinology, National Institute of Mental Health, National Institutes of Health (NIH), Bethesda, MD 20892

- **Selected for Post-doctoral Program at NIH, Bethesda (January 2011)** at National Institute of Health, Section on Behavioral Endocrinology, National Institute of Mental Health, National Institutes of Health (NIH), Bethesda, MD 20892
- **University Grants Commission (UGC)-Senior Research Fellow** (April 2008 – November 2009), Department of Zoology, Banaras Hindu University, Varanasi, India
- **University Grants Commission (UGC)-Junior Research Fellow** (April 2004 – March 2008), Department of Zoology, Banaras Hindu University, Varanasi, India
- **National Eligibility Test (NET) for Lectureship and Junior Research Fellowship (JRF)** conducted by Council of Scientific and Industrial Research (CSIR), New Delhi in 2003.
- **Graduate Aptitude Test in Engineering (GATE**) conducted by Indian Institute of Technology in 2003.

Scientific Talks, Presentations & Conferences and workshops Attended:

- **Participation**: International e- Conference on the theme 'Recent Adances in Life Sciences with Reference to Diseases, Disorders and Adaptations', Organised by University Department of Zoology, L. N. Mithila University, Darbhanga, Bihar, India. July 2021.
- **Participation**: International Webinar **'**Bioethics in COVID-19 Pandemic' organised by Dr. D.Y. Patil Vidyapeeth (DPU), Pune, Unit of Bioethics in Association with Education Department UNESCO Chairin Bioethics, University of Haifa & UNESCO chair in Bioethics, Melbourne, Australia. September 2020.
- **Invited Talk**: "Differential expression of an enzyme HDAC2 in the lymphoblastoid cell lines derived from women with and without premenstrual dysphoric disorder" at an International Conference on "Current perspectives of Biochemistry in Health and Diseases" at Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi, India. January 2020.
- **Oral presentation**: "Generation of patient derived Lymphoblastoid Cell Lines (LCLs) as a disease model for Postpartum Depression in Indian Women" at "ICBR 2020" Indo-US Conference on Bioengineering & Regenerative Medicine at Indian Institute of Technology, Banaras Hindu University, Varanasi, India. February 2020.
- **Oral presentation**: Importance of Ethics in Biotechnology Research at "Bioethicon 2019" International Conference of Bioethics at Chennai, India. December 2019.
- **Participation:** The Biennial conference of the International "MARCE SOCIETY FOR PERINATAL MENTAL HEALTH" at the National Institute of Mental Health and Neurosciences (NIMHANS) in Bengaluru India September 2018.
- **Poster presentation**: Differential expression of mRNA & protein in the lymphoblastoid cell lines derived from women with and without premenstrual dysphoric disorder (PMDD) at International Congress of Cell Biology, Hydrabad, India. January 2018.
- **Poster presentation:** "Whole transcriptome expression profiles in lymphoblastoid cells from women with PMDD and controls: diagnostic differences and differential response to ovarian steroids" at National Institutes of Health (NIH), Bethesda, MD, USA. September 2014
- **Poster presentation: The Society for Neuroscience** 2011 Annual Meeting in Washington, DC, November 2011
- **Workshop** on "Epigenetics" FAES Graduate School, National Institutes of Health (NIH), Bethesda, MD, USA December 2013
- **Workshop** on "Using Stem Cells for Biological and Therapeutics Discovery in Mental illness" at Bethesda, MD, USA from April 2012.
- **Workshop** on "iPS Cells: Principles and Methods" at National Institute of Health, Bethesda, MD, USA.

April 2011.

- **Oral presentation: "**Seasonality in expression of nitric oxide synthase (NOS) isoforms in the testis and testicular nitric oxide (NO) and testosetrone in the catfish, *Clariasbatrachus"* at **XX National Symposium on Chronobiology** at School of Life Sciences, Pt. Ravishankar Shukla University, Raipur, India. December 2008.
- **Poster presentation: "**Effect of nitric oxide on steroidogenesis in Asian catfish, *Clariasbatrachus*: an *in vitro* study" at **International Structural Neuroscience Conference on Peptides** at Department of Pharmaceutical Sciences, Nagpur University Campus, Amravati Road, Nagpur, India. February 2008.
- **Oral presentation: "***In vitro* effect of piscine growth hormone and insulin like growth factor- I on steroidogenic activity of Leydig cells in an Asian catfish *Clariasbatrachus"* at **National Symposium on An Update of Reproductive Endocrinology: Novel and Applied Strategies** at Centre of Advanced Study, Department of Zoology, Banaras Hindu University, Varanasi, India. February 2007.
- Participated in scientific deliberations at **National Symposium on Comparative Endocrinology and Reproductive Physiology: Retrospect and Prospect** at Department of Zoology, University of Delhi, Delhi. November 2005.
- **Oral Presentation: "**Agonistic Behavior in Animals**"** during the Seminar series at Department of Zoology, Banaras Hindu University, Varanasi, India. October 2001**.**

Membership of Scientific/ Societies/ other Professional bodies:

Society for Neurosciences (SFN)
Indian Society for Comparative Endocrinology (ISCE)
International Federation of Comparative Endocrine Society (IFCES)
Indian Academy of Neuroscience (IAN)
International Marce Society for Perinatal Mental Health

References:

Name / Designation	Institute and Address	E-mail/Phone
Dr. Peter J. Schmidt Chief Investigator	Behavioral Endocrinology Branch (SBE), National Institute of Mental Health Building, 10-CRC, Room 2-5330, 10 Center Drive, MSC 1277 Bethesda, MD 20892-1277	Email:peterschmidt@mail.nih.gov Telephone: +1-(301) 496-6120 Fax : +1-(301) 496-2588
Dr. David Goldman Chief	Laboratory of Neurogenetics National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, 5625 Fishers Lane, Room 3S-32C:MSC 9412 Rockville, MD 20851	E-mail: davidgoldman@mail.nih.gov Telephone: +1-301-443-0059 Fax : +1-301-480-2839
Prof. B. Lal Professor	Vice-Chancellor, Cluster University of Jammu, Canal Road, Jammu, Jammu and Kashmir, India; Pin : 180001	Email : lalbhu@yahoo.co.in (Mobile): +91 9336938955

Details of my Research Experience

(I) Current Research Work at Dr. Reddy's Institute of Life Science (August 2022- till date)

My research interest is in understanding the role of reproductive steroids in brain functioning and associated mood disorders. I am also interested in generating *in vitro* disease model and utilize it to understand the molecular and cellular basis of endocrine related mood disorders in women. We are developing *in vitro* model for some of the rare diseases with neurological phenotypes. These cellular models are good to study the genetic predisposition of CNS diseases/ disorders and for performing functional studies to get an in-depth understanding of diseases. These cellular models have the potential to be utilized for drug testing in the future.

My current projects involve getting insights into the pertinent pathways that might be involved in the pathophysiology of Major Depressive Disorder in the Indian population. I am using the multi-omics approach for determining the molecular and cellular basis of depression. Blood sample from women suffering with Major Depressive Disorder (MDD) and asymptomatic control have been sequenced (RNA- sequencing), data analysis is in progress and we are currently working on manuscripts based on our current findings.

(II) Teaching & Research at Dr. D.Y. Patil Biotechnology & Bioinformatics Institute, constituent institute if Dr. D Y Patil Vidyapeeth, Pune (April 2018- June 2022)

I joined Dr. DY Patil Biotechnology Institute in April 2018 as an Assistant Professor. I got the opportunity to teach a variety of subjects at the Institute such as Developmental Biology, Stem cells, Biology biology, Genetics, Health care Law Management and Biomimetic to B. Tech. Biotechnology, M.Sc & MIT (Masters in Technology) students .

(III) Postdoctoral Research at National Centre for Cell Science, NCCS (January 2017- March 2018)

My postdoctoral research at National Centre for Cell Science (NCCS), Pune, India was focused on development of 3D skin equivalent model system to understand the pathophysiology of "Melasma" which is characterized by hyper pigmentation of facial skin. We used cellular model system to understand the involvement of SERPIN B3, SERPIN B4 and COX 2 in hyperpigmentation. Essentially, understand the cross-talk between the epidermal and dermal cells in the skin. 3D skin equivalent model system was generated in my host laboratory. The project was successfully completed before I moved to another project in the same lab as a Project Scientist.

As Project Scientist, I worked in a project focused on the development of depository of human induced pluripotent stem cells (iPSC) at the institute. This involved comprehensive characterization of stem cells and quality control of the iPS cell lines of Indian origin. With my extensive experience in generating iPSCs at National Institute of Health (NIH), I could set up separate lab for stem cell culture and establish different SOPs and protocol for generating iPSCs and characterization of the same in my host laboratory at NCCS in a very short time of 7 months approximately. The protocol and SOPs for generating iPSCs were successfully established before I switched my job and joined Dr. D.Y. Patil University, Pune as an Assistant Professor.

(IV) Postdoctoral Research at National Institute of Health, NIH (January 2011- April 2014)

My postdoctoral research at National Institutes of Health (NIH), Bethesda, USA involved understanding the molecular and genetic basis of Premenstrual Dysphoric Disorder (PMDD) which is an affective disorder in women of reproductive age. My research at NIH in the laboratories of Dr. Peter J Schmidt and Dr. David Goldman aimed to elucidate the mechanism of the disease and genetic/epigenetic factors responsible for this condition.

Premenstrual Dysphoric Disorder (PMDD) is a prevalent and serious condition affecting 5-15% of women worldwide and is a major cause of depression resulting in impaired relationships, diminished overall quality of life at par with other major psychiatric disorders. Despite of serious implications, the neurobiological mechanisms underlying the disease remains largely unknown until now. My research at NIH aimed at examining the effects of gonadal steroids on affective adaptation and stress responsivity related to hormonal transitions and experimentally induced hormonal states using the cellular model generated from the human subjects. The objectives of my study were: first, to identify the sources of vulnerability to the development of PMDD reproductive hormonal transitions; second, to determine the mechanisms underlying the mood regulating effects of gonadal steroids and finally, to evaluate the potential role of hormones in PMDD.

To address the problem, our first approach was to do functional genomic study. Thus the unbiased method was adapted and we did Whole Transcriptome Sequencing of the RNA from Women with and without Premenstrual Dysphoric Disorder (Figure 1.).

We had access to a very clean patient cohort carefully selected over the years based on symptomatic and experimental screening at the clinical center (NIMH), NIH. Using EBV (Epstein Barr Virus), we generated transformed cell lines from the blood obtained from women who went through the screening procedure at the clinics at NIH. The lymphoblastoid cell lines from these women with and without PMDD were selected retrospectively, based on the observed behavioral response to the hormone manipulation in the clinic i.e., occurrence of mood symptoms proximate to hormone manipulation in women with PMDD or no changes in mood in the matched controls served as a cellular *in vitro* model to perform functional and exposure studies.

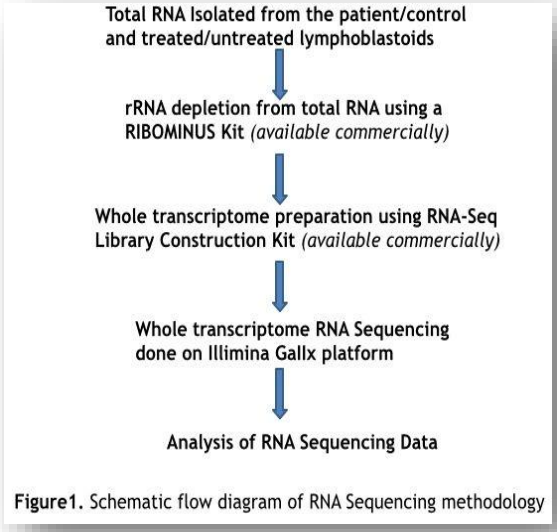

Figure1. Schematic flow diagram of RNA Sequencing methodology

Our experimental strategy was to recapitulate the endocrine events that trigger mood symptoms in women with PMDD in a study done by Peter Schmidt and his group (Schmidt et al., 1998). To achieve this, lymphoblastoid cell lines from the patients and asymptomatic control group were used to examine potential differences in cellular function between the two groups. The cells were exposed to the same endocrine manipulations as those performed in the clinical research protocol, and gene expression profiles were examined before and after each hormone exposure. First, presence of steroid hormone receptors, estrogen receptor (ER), and progesterone receptor was explored making use of RTqPCR technique and Protein Simple assay in the selected representative lymphoblastoid cells from each diagnostic group (best matched for age and race). After we identified significant difference between the mRNA expression levels of nuclear estrogen receptor (ER), and membrane progesterone receptors (mPR) between the two diagnostic groups, next, we performed whole transcriptome sequencing (RNA- Sequencing) to investigate the genome wide differential gene expression profile between the symptomatic and asymptomatic individuals.

In another experiment, same set of lymphoblastoid cell lines were exposed to physiologic levels of estradiol and progesterone (separately) and RNA extracted from these were analyzed by whole transcriptome RNA analysis to determine the RNA content, expression difference, and presence of post-transcriptional modifications in genes which are either steroid responsive or implicated in the neurobiology of affective disorders.

Pathway analysis of LCL transcriptome implicated ESC/E(Z) module, an ovarian steroid-regulated gene silencing complex. RNA and protein expression of the 13 ESC/E(Z) complex genes were individually quantitated. Pathway analysis of transcriptome revealed over-expression of ESC/E(Z) complex genes in PMDD LCLs at baseline, with more than half of these genes over-expressed as compared to controls, and

with significant effects for *MTF2*, *PHF19*, and *SIRT1* ($p<0.05$). This pattern of increased ESC/E(Z) mRNA expression was confirmed in a larger cohort by qRT-PCR. In contrast, protein expression of ESC/E(Z) genes was decreased at baseline in PMDD LCLs with MTF2, PHF19, and SIRT1 all significantly decreased ($p<0.05$). We showed that PMDD LCLs manifest an intrinsic cellular difference in ESC/E(Z) complex function both at baseline and in response to ovarian hormones. Our results have given first insights into the possible pathways contributing to PMDD. The study has been published in *Molecular Psychiatry* (Nature Publishing group), 2017 and the follow- up study in the model animal (mouse model of PMDD) was published in *Molecular Psychiatry* (Nature Publishing group) 2019. Further, we found that more than 10 genes in the topmost hits are part of physically-interacting network (*NUCB1*, *DST*, *GCC2*, *GOLGB1*) involved in endoplasmic reticulum (ER)-Golgi function. We found during series of experiments that *NUCB1*, regulator of cellular Ca^{2+} and ER stress is an important gene with strong association with estrogen signaling . We, thus believe that the hormone-dependent aberrations in cellular Ca^{2+} dynamics and ER stress may contribute to the pathophysiology of PMDD. This finding has been submitted to the Molecular Psychiatry (2021) and will hopefully get accepted soon.

Advancement on the molecular mechanism underlying such neurological disease like PMDD have been limited to immortalized cell lines blood or other tissue which do not allow the full recapitulation of key events involved in the initiation and progression of disease. So, our next approach was to develop induced pluripotent stem cell (iPSC) model from the women with and without PMDD to get closer look into the pertinent pathways that might be involved in the manifestation of PMDD (Figure 2).

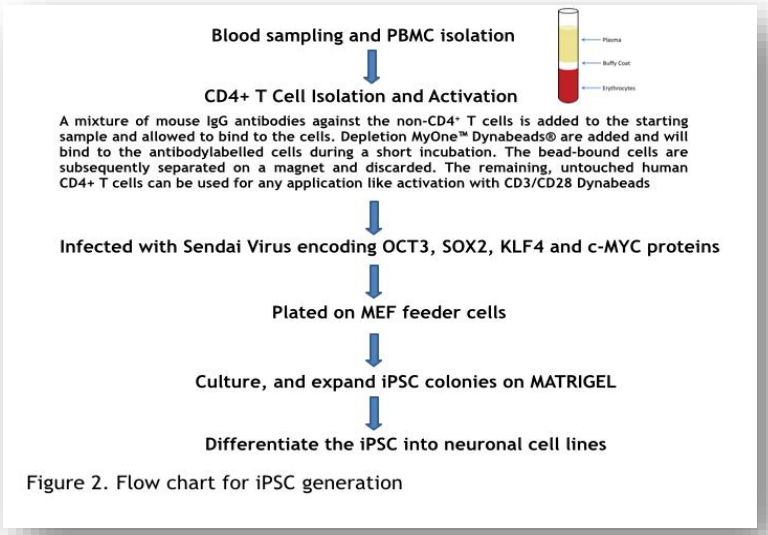

Figure 2. Flow chart for iPSC generation

These patient specific iPSCs are to be differentiated into neuronal cells thus serving as a more relevant cellular model system to study and analyze the molecular basis of PMDD with various molecular and biochemical techniques.

Before I returned back to India in 2014, I successfully established the induced pluripotent Stem Cell (iPSC) technique in my laboratory at NIH. I used less than 1ml blood (PBMCs) from women with PMDD and the asymptomatic individuals, induced it to the pluripotent stem cell stage and further differentiated the iPSC thus generated into neuronal cell (dopaminergic/glial cell) lineage.

The neuronal cell lines differentiated from these iPSC are being used as a cellular model for PMDD to study the molecular details of the altered pathway in the patients (PMDD) *vs* controls and other variability in the gene structure and functioning. This part of the project is in progress with very promising results and the manuscript from this part is under preparation for publication.

(V) Doctoral Research (September 2003- December 2009)

My Ph.D work in the Laboratory of Reproductive Endocrinology, Department of Zoology, Institute of Science, Banaras Hindu University (BHU), Varanasi was on understanding the role of nitric oxide in regulation of Leydig cell steroidogenesis in catfish, *Clarias batrachus* as a model system. During my doctoral research my mentor, Prof. Bechan Lal at Banaras Hindu University, Varanasi, introduced me to the concept of reproductive endocrinology & cell biology. While working on testicular cells in piscine model, I developed interest in cell biology and gradually in stem cell biology as I enjoyed working with cells and *in vitro* cellular model during my Ph.D. course.

I have worked on three different types of testicular cells isolated from fish testes; Leydig cells, Testicular macrophages and Sertoli cells. My main objective was to study regulation of steroidogenesis from Leydig cells (Fig 3).

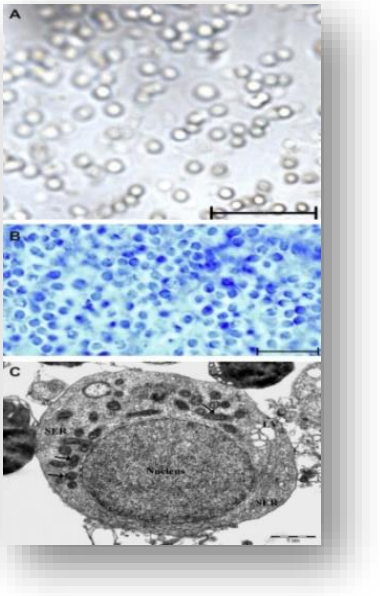

Figure 3. (A) DIC (differential interference contrast) image of live Leydig cells of the catfish, *Clarias batrachus* in representative well after 24 h culture in medium. (B) Light microscopic photograph of Leydig cells showing Δ^5-3β-hydroxysteroid dehydrogenase enzyme activity. (C) Electron micrograph of freshly isolated Leydig cells showing the tubular mitochondria (arrow head), elaborate smooth rough endoplasmic reticulum (SER) and lipid vacuoles (LV) representing characteristic features of steroid producing cell. (Scale bar A and B = 20 µm and C = 1 µm.)

Regulation of Leydig cells needs to be elaborately understood as these are the primary source of male hormones, mainly testosterone that is critical at all stages of reproductive function and health of the male. My research work involved isolation, purification, characterization and culture of Leydig cells and testicular macrophages as the precise sites of production of testosterone and nitric oxide respectively and their manipulation using different hormones in an *in vitro* system **(**Figure 3).

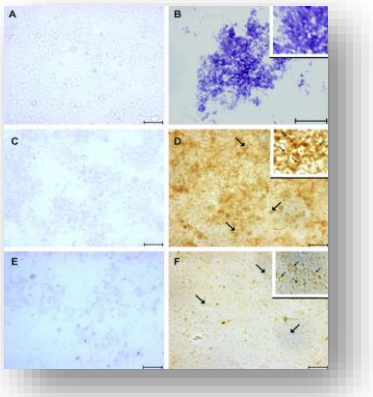

Figure 4. Immunocytochemical localization of nNOS and iNOS in cultured Leydig cells of the catfish, *Clarias batrachus*. (A) Leydig cells incubated without β-NADPH showing no reaction; (B) Leydig cells incubated with β-NADPH showing diaphorase activity; (D) Leydig cells exhibiting immunoreactivity with nNOS like molecule. (F) Leydig cells exhibiting iNOS activity.

We demonstrated for the first time the existence of nitric oxide synthase (NOS) like activity in Leydig cells in the catfish, *C. batrachus*, and showed that NO produced by these cells inhibits its own steroidogenic activity in an autocrine manner (4). We extended our work further to show that Leydig cell steroidogenesis in fish involves multiple interacting factors that act through paracrine and endocrine mechanisms. In particular, it appears to be highly sensitive to paracrine nitric oxide (NO). For the first time, we reported that testicular macrophage derived NO inhibited testosterone production by Leydig cells in ectothermic vertebrate and production of NO by testicular macrophage is under inhibitory endocrine control in vertebrates. Testicular macrophages picture formed the cover page of the journal (**General and Comparative Endocrinology; *Volume 160, Issue 1, 1 January 2009***) in that same issue (**Figure 5**.). We also went on to demonstrate the existence of two isoforms of nitric oxide synthase nNOS and iNOS like molecules in the fish testis, which are expressed differentially in the seminiferous tubules and interstitium in the recrudescing and mature fish testis. Our study also showed that testicular (Nitric oxide) NO appears to play significant role in regulation of spermatogenesis, and inhibits testosterone

production, in particular. The results of this work were published in form of five research articles and a review in journals of high repute.

Figure 5. Figure from my research article made the cover page of the journal (General and Comparative Endocrinology *Volume 160, Issue 1, 1 January 2009, Pages 12-18*)

Medium to long-term research focus and strategy

My primary research interest is in understanding the endocrine regulation of brain functions (Fig 6) and in understanding how modulations in the neuroendocrine system might be involved in the manifestation and development of "Reproductive Mood Disorders" in men and women. It is very well known that these hormones are involved not only in reproduction but also affect the brain functioning of both males and females. Many neural and behavioral functions are affected by these hormones such as mood, cognitive function, motor coordination etc. These hormones protect the brain from injury, stress; regulate brain aging and are also involved in pathophysiology of certain brain diseases such as depressive disorders in both men and women.

However, women throughout their life are exposed to changing levels of hormones such as during menstrual cycle, pregnancy and so on. During these fluctuations some women respond differently and develop depression or dysphoria and other mood symptoms which could be devastating. Fundamental understanding of various mechanisms by which reproductive hormones regulate different brain function could help us in understanding the underlying mechanisms involved in some of these brain diseases.

I wish to use an interdisciplinary approach to investigate how reproductive hormones act on the brain and modulate various changes in brain function including changes due to ageing, damage and trauma etc. This line of research could help in the identification of important involvement of reproductive steroids in brain functions and could help in the identification of cause of various neurological diseases particularly in women during their reproductive age.

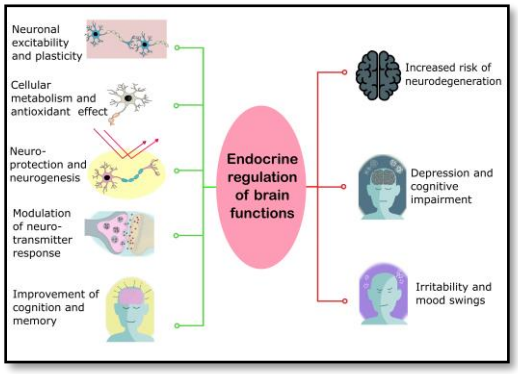

Figure 6. Endocrine regulation of brain functions

Current research