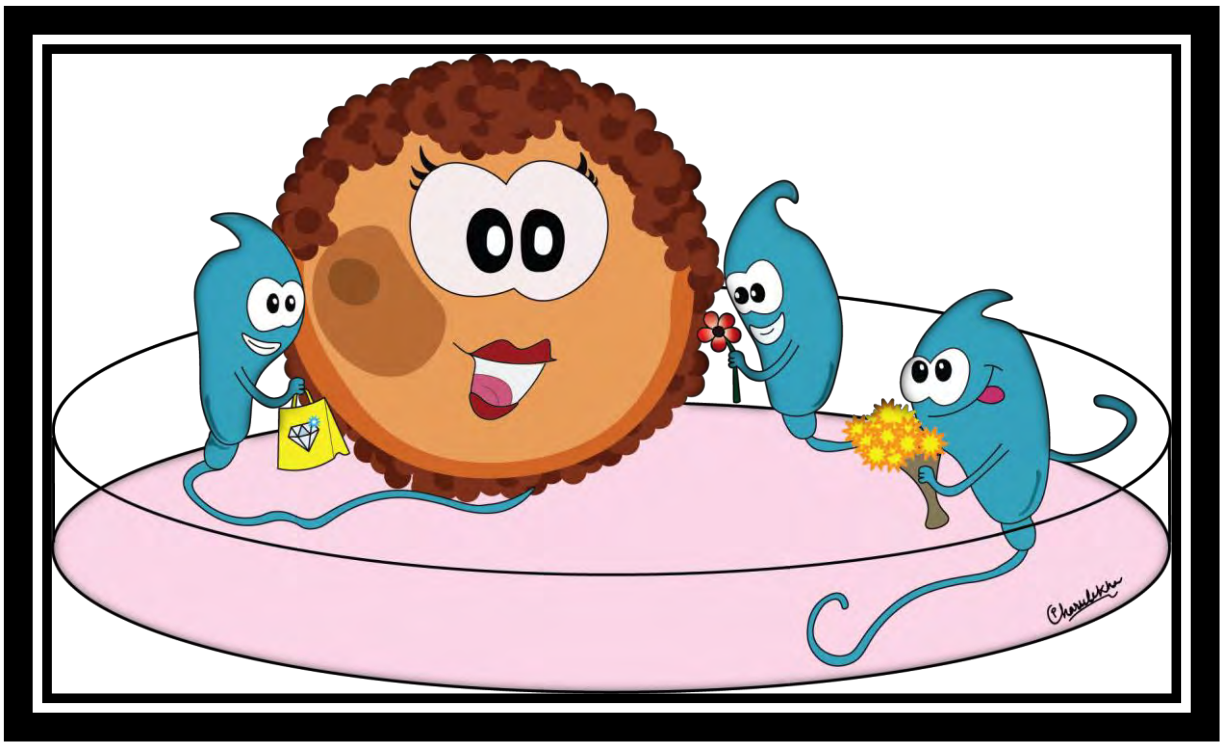
Mouse Genome Engineering Facility

Cryobiology / IVF workshop #5

December 12th – 16th, 2022

NCBS / inStem - BLiSC campus, GKVK PO, Bellary Road, Bengaluru 560065, INDIA

COURSE PROGRAM



Sunday, December 11th

Travel / Arrival / guest house Check-IN @ Campus Naidile Guest House or Parijata Hostel.

Day 1 - Monday, December 12th

Time	Venue	Topic	Speaker / Team
8.15 – 9 AM		Breakfast @ NCBS main Canteen – ground floor	Hospitality Team
9 – 9.30 AM	Raspuri	Workshop Registration	Charu – MGEF staff
9.30 – 10 AM	Raspuri	Welcome & workshop overview	Dr Aurelie Jory – Lily [MGEF]
10 – 11 AM	Raspuri	Introduction to MGEF activities & ARTs	Dr Mahesh Sahare [MGEF]
11 AM – 11.30 AM	Raspuri	BREAK – Coffee / Tea / snacks	Hospitality Team
11.30 AM – 12.30 PM	Raspuri	5 days of ART – Beginning of Human Embryology	Vakil Jagveer Singh [Cooper Surgical]
12.30 PM - 2 PM		LUNCH @ NCBS main Canteen – ground floor	Hospitality Team
2 PM - 4 PM	MGEF lab	Lab Practicals : Sperm Cryopreservation	MGEF Team
4 PM – 4.30 PM	Raspuri	BREAK – Coffee / Tea / snacks	Rajesh / Hospitality Team
4.30 PM 5.30 PM	MGEF lab	Lab Practicals : embryo handling pipette design demo & embryo handling training	MGEF Team
5.30 PM	MGEF lab	1st day wrap up discussion	

Day 2 – Tuesday, December 13th

Time	Venue	Topic	Speaker / Team
8.15 – 9 AM		Breakfast @ NCBS main Canteen – ground floor	Hospitality Team
9 – 10 AM	Raspuri / zoom	IVF generated embryos to both improve workflow in CRISPR-mediated genome editing in mice and reduce animal production numbers.	Mitra Cowen [McGill University – Canada]
10 – 11 AM	Raspuri	Cryo Repository & data Management @ MGEF	Dr Aurelie Jory – Lily [MGEF]
11 AM – 11.30 AM	Raspuri	BREAK – Coffee / Tea / snacks	Hospitality Team
11.30 AM – 12.30 PM	Raspuri	Introduction to the different types of Germplasm storage & Transport procedures	Dr Mahesh Sahare [MGEF]
12.30 PM - 2 PM		LUNCH @ NCBS main Canteen – ground floor	Hospitality Team
2 PM - 3 PM	MGEF lab	Lab Demos & Practicals : male vasectomy	MGEF Team
3 PM – 4 PM	MGEF lab	Lab Demos & Discussions : Media preparation & quality control	MGEF Team
4 PM – 4.30 PM	Raspuri	BREAK – Coffee / Tea / snacks	Hospitality Team
5 PM – 6 PM	Raspuri / zoom	Introduction to reproduction and cryopreservation	Dr Robert Taft [JAX – USA]

Day 3 - Wednesday, December 14th

Time	Venue	Topic	Speaker / Team
8.15 AM - 8.30 AM		Breakfast @ NCBS main Canteen – ground floor	Hospitality Team
8.30 AM – 11 AM	MGEF lab	Lab Practical: Sperm Cryo- recovery, oocyte collection & IVF.	MGEF Team
11 AM – 11.30 AM	Raspuri	BREAK – Coffee / Tea / snacks	Hospitality Team
11.30 AM – 12.30 PM	Raspuri	Introduction to gene editing & updates on the latest mouse genome engineering tool kit.	Dr Aurelie Jory – Lily [MGEF]
12.30 PM - 2 PM		LUNCH @ NCBS main Canteen – ground floor	Hospitality Team
2 PM - 3 PM	MGEF lab	Lab Practicals : IVF washes & embryo culture	MGEF Team
3 PM – 4 PM	MGEF lab	Lab Demos & Discussions : Cryo / IVF lab essentials & troubleshooting tips	MGEF staff
4 PM – 4.30 PM	Raspuri	BREAK – Coffee / Tea / snacks	Hospitality Team
5 PM – 6 PM	Raspuri / zoom	IVF – Common protocols and strain performance expectations.	Dr Robert Taft [JAX – USA]
7 PM – 11 PM	TBD	Course Gala Diner – Sponsored by ATNT Laboratories	

Day 4 – Thursday, December 15th

Time	Venue	Topic	Speaker / Team
8.15 AM – 9 AM		Breakfast @ NCBS main Canteen – ground floor	Hospitality Team
9 AM - 10 AM	Raspuri / zoom	Assisted Reproduction Techniques in the Rat.	Dr Barbara Stone [ParaTechs – USA]
10 AM – 10.30 AM	MGEF lab	Lab Practical: Post IVF embryo culture assessment & results	MGEF Team
10 AM – 10.30 AM	MGEF lab	Embryo vitrification protocol overview	Lily
11 AM – 11.30 AM	Raspuri	BREAK – Coffee / Tea / snacks	Hospitality Team
11.30 AM – 12.30 PM	MGEF lab	Lab Practicals : embryo vitrification	MGEF Team
12.30 PM - 2 PM		LUNCH @ NCBS main Canteen – ground floor	Hospitality Team
2 PM - 3 PM	MGEF lab	Lab Practicals : embryo Cryo-recovery & embryo culture	MGEF Team
3 PM – 4 PM	MGEF lab	Lab Demos & Discussions : Embryo electroporation & delivery of Crispr/Cas solutions	Lily
4 PM – 4.30 PM	Raspuri	BREAK – Coffee / Tea / snacks	Hospitality Team
5 PM – 6 PM	Raspuri / zoom	Reproducibility in research – common sources of variation and strategies to improve reproducibility of research that relies on mice.	Dr Robert Taft [JAX – USA]

Day 5 – Friday, December 16th

Time	Venue	Topic	Speaker / Team
8.15 AM – 9 AM		Breakfast @ NCBS main Canteen – ground floor	Hospitality Team
9 AM - 10 AM	Raspuri / zoom	3Rs Improvements for Assisted Reproduction in the Mouse.	Dr Barbara Stone [ParaTechs – USA]
10 AM – 11 AM	Raspuri	The Animal Care and Resource Center (ACRC): "A National Resource for Laboratory Mice and Rats"	Dr Yogesh Chandra [ACRC – BLiSC]
11 AM – 11.30 AM	Raspuri	BREAK – Coffee / Tea / snacks	Hospitality Team
11.30 AM – 12.30 PM	V2 & MGEF lab	Batch 1 = ACRC SPF facility Tour Batch 2 = Surgical & Non Surgical embryo transfers demos & practicals	Dr Yogesh Chandra Lily & MGEF staff
12.30 PM - 2 PM		LUNCH @ NCBS main Canteen – ground floor	Hospitality Team
2 PM - 3.30 PM	V2 & MGEF lab	Batch 2 = ACRC SPF facility Tour Batch 1 = Surgical & Non Surgical embryo transfers demos & practicals	Dr Yogesh Chandra Lily & MGEF staff
3.30 PM – 4 PM	Raspuri	Group Photo & Feed Back forms	Charu / Abhishek
4 PM – 4.30 PM	Raspuri	BREAK – Coffee / Tea / snacks	Hospitality Team
4.30 PM – 5 PM	Raspuri	Course Wrap Up & Certificate Ceremony	

Saturday, December 17th

Travel – Departure – guest house / hostel Check-OUT.

Abstracts & Bios of external guest Speakers:

Mitra Cowan, McGill University – Canada.

Mitra Cowan's expertise is in the transgenesis field and is trained in reproductive biology and embryonic stem cell technologies. She started her career running the Johns Hopkins School of Medicine Transgenic core until 2004 before developing a new Transgenic Core at the Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM) in Montréal, Canada. In 2017 Mitra accepted the position of Associate Director of the McGill Integrated Core for Animal Modeling of McGill University. In the last 5 and a half years the MICAM platform has now created now over 300 new animal models using Crispr technology and continues to expand its expertise to be a leader of creation of animal models in Quebec.

Tuesday 13th December – 9AM:

IVF generated embryos to both improve workflow in CRISPR-mediated genome editing in mice and reduce animal production numbers.

Efficient generation of high quality fertilized mouse embryos is one of the keys to success to generate CRISPR generated gene targeted mice. Our lab recently changed our workflow to generate all embryos for microinjection by IVF. This change in our workflow has had several positive impacts, these include: improving recombination efficiency several fold, allow for a more flexible microinjection schedule, create stock embryos and reduce the number of mice used to generate fertilized embryos. Here I will discuss our workflow and protocols and how they have improved our labs productivity and efficiency.

Dr Robert Taft, JAX - Jackson Laboratories – USA.

Dr. Taft received a Ph.D. in Reproductive Physiology from West Virginia University in 1999 before coming to the Jackson Laboratory to pursue postdoctoral training with Dr. John Eppig. In addition to his research experience, Dr. Taft has considerable leadership and administrative experience as PI on multiple grants as well as from developing and leading a 74 person unit supporting repository and service activities. Dr. Taft's work has led to publication, patents and the development of products and services provided by the Jackson Laboratory, demonstrating his interest is seeing basic research findings translated into solutions that help the research community operate more efficiently and effectively.

Tuesday 13th December – 5 PM:

Introduction to reproduction and cryopreservation.

Embryo cryopreservation has been used successfully for more than 40 years in mice, in this talk we will briefly cover reproductive anatomy and physiology relevant to embryo production. We will also briefly review fundamental concepts related to cryopreservation as a background to practical lab sections.

Wednesday 14th December – 5 PM:

IVF – Common protocols and strain performance expectations.

In vitro fertilization has become an essential tool, but there is no universal protocol. In this talk we will explore similarities and differences between commonly used protocols including an explanation of why key steps are included. We will also review causes of variation and differences in performance among commonly used inbred strains.

Thursday 15th December – 5 PM:

Reproducibility in research – common sources of variation and strategies to improve reproducibility of research that relies on mice.

Some say Science is experiencing a crisis in reproducibility. Careful management of laboratory animals can reduce the risk of producing irreproducible data. In this talk we will discuss practices that can be used at all phases of the life cycle of the projects.

Dr Barbara Stone, ParaTechs - USA:

Thursday 15th December – 9AM:

Assisted Reproduction Techniques in the Rat.
This lecture covers basic anatomy and considerations unique to rat assisted reproduction techniques. First, the steps required for estrous cycle synchronization, superovulation, and embryo isolation in the rat are outlined. As many of the techniques available for mouse assisted reproduction are now being used successfully in the rat, the practical use of *in vitro* fertilization (IVF), embryo culture, non-surgical embryo transfer (NSET), and cryopreservation are highlighted.

Friday 16th December – 9AM:

3Rs Improvements for Assisted Reproduction in the Mouse.
This lecture focuses on the use of techniques in mouse assisted reproduction that provide refinements and reductions in keeping with the 3Rs of animal experimentation. Non-surgical embryo transfer and artificial insemination techniques are compared to surgical procedures and the protocols discussed in depth. The use of cervical manipulation to induce pseudopregnancy in female mice without the need for vasectomized males will also be presented.

Barbara Stone, PhD

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Research Interests

Dr. Barbara Stone is the Director of Animal Research and the Chair of the Animal Care and Use Committee at ParaTechs Corporation. As a senior research scientist at ParaTechs since 2009, her primary responsibility is for technology development and verification for assisted reproductive technologies including the Non-Surgical Embryo Transfer (NSET) devices for both mice and rats. As Principal Investigator for multiple funding awards from the National Institutes of Health and state agencies, she both authors and executes research projects. Dr. Stone is also involved in technology transfer and has taught over 40 training workshops at international meetings and research institutions. She regularly lectures regarding the use of NSET as an example of technique refinement for the humane use of laboratory animals in biomedical applications. She is currently contributing to invention, development, and marketing for several products in the ParaTechs portfolio.

Education

Dr. Stone received her bachelor's degree from the University of Illinois and her PhD in Microbiology and Molecular Genetics from the University of California, Los Angeles in the United States.

Select Project Support

A non-surgical embryo transfer device (mNSET) for producing gene modified mice.

- NIH/NCRR: 1R43RR025737-01A1, SBIR Phase I, 09/03/09-09/2/2010
- NIH/OD/ORIP: 8R44OD010958-03; 3R44OD010958-03S1, SBIR Phase II and administrative supplement, 03/15/11-02/28/14
- KSTC: 184-512-11-115, SBIR Phase II match through Kentucky Science and Technology Corporation and the Kentucky Cabinet for Economic Development

A non-surgical embryo transfer device (rNSET) for producing gene modified rats.

- NIH/OD/ORIP: 1R43RR030684-01, SBIR Phase I, 7/15/2010-7/14/2011
- NIH/OD/ORIP: 2R44OD018231-02A1; 3R44OD018231-03S1, SBIR Phase II and administrative supplement, 9/01/2015-8/31/2018
- KSTC: 184-512-10-096, SBIR Phase I match through Kentucky Science and Technology Corporation and the Kentucky Cabinet for Economic Development

An innovative device integrating cryopreservation, storage, and artificial insemination of mouse sperm (mC&I device).

- KSEF: 14 8-502-51- 359, RDE, Research Development Excellence Award from the Kentucky Science and Engineering Foundation, 7/1/2015-6/30/2016
- NIH/OD/ORIP: SBIR Phase I, 4/15/2016-04/14/2018
- NIH/MH: 9R44MH122117-02A1, SBIR Phase II, 08/01/2019-07/31/2021

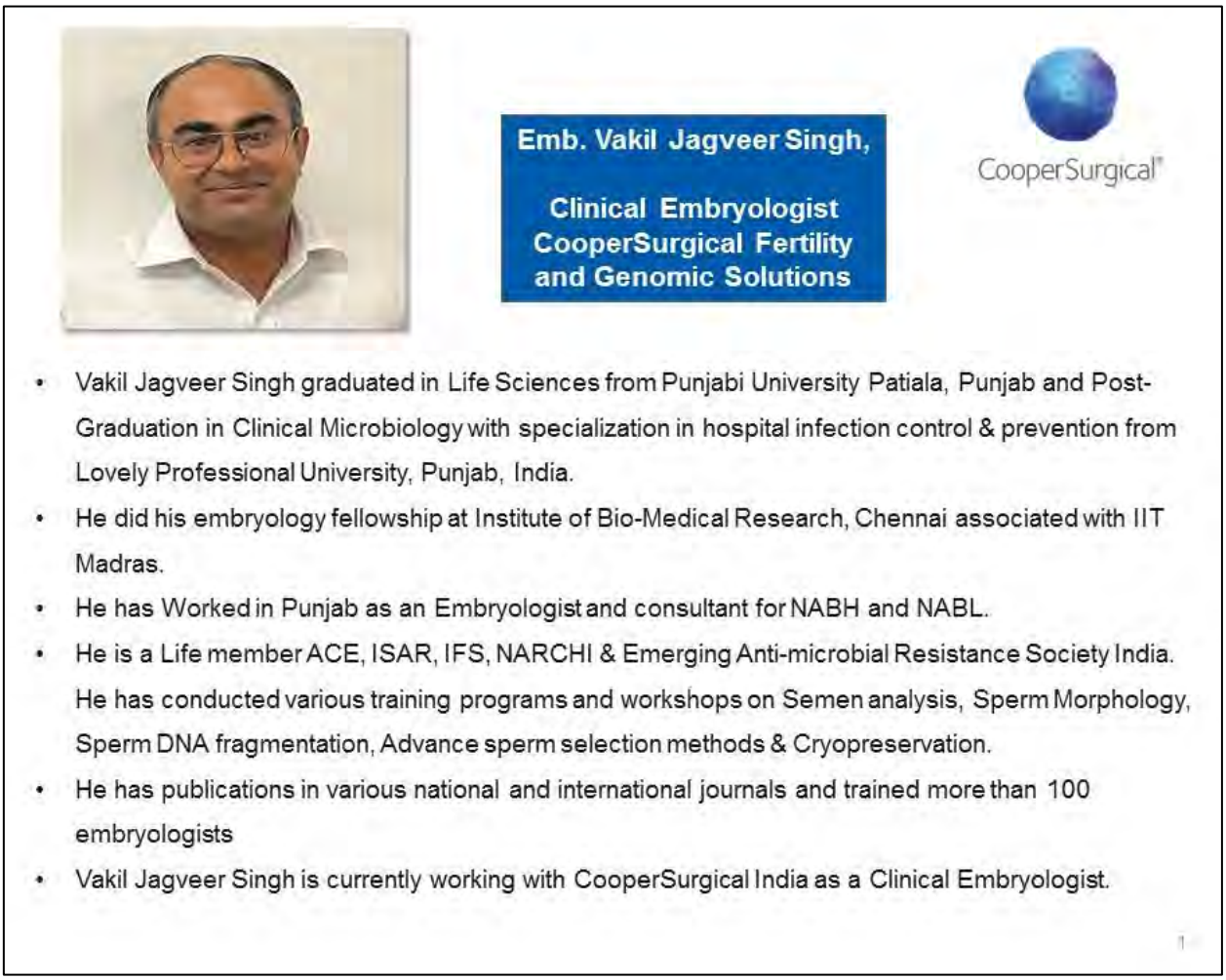
Facilitation of vitrification in animal model systems using a cryovial insert device (Cryofork/Cryosword).

- NIH/OD/ORIP: 1R43OD024887-01, SBIR Phase I, 8/1/2017-7/31/2019

Select Publications

- Men H, Stone BJ, Bryda EC. Media optimization to promote rat embryonic development to the blastocyst stage in vitro. Theriogenology. 2020;151:81-85. doi:10.1016/j.theriogenology.2020.03.007. PMID 32311604 .
- Stone BJ. Nonsurgical Embryo Transfer Protocol for Use with the NSET™ Device. Methods Mol Biol. 2020;2066:107-111. doi: 10.1007/978-1-4939-9837-1_9. PMID 31512211.
- Stone BJ, Steele KH, Fath-Goodin A. A rapid and effective non-surgical artificial insemination protocol using the NSET™ device for sperm transfer in mice without anesthesia. Transgenic Res. 2015 Aug;24(4):775-81. PubMed PMID: 26065409; PubMed Central PMCID: PMC4504984.
- Steele KH, Hester JM, Stone BJ, Carrico KM, Spear BT, Fath-Goodin A. Non-surgical embryo transfer device compared with surgery for embryo transfer in mice. J Am Assoc Lab Anim Sci. 2013 Jan;52(1):17-21. PubMed PMID: 23562028; PubMed Central PMCID: PMC3548196.

<u>Vakil Jagveer Singh</u>, Cooper Surgical, India.



Monday 12th December – 11.30 AM:

5 days of ART – Beginning of Human Embryology.

- Understanding the Indian infertility scenario.
- Common causes of infertility in males and female.
- Assisted reproduction techniques used to treat infertility
- An IVF cycle from day -1 to day 5
- Role of an embryologist in an ART lab and what does it take to become an embryologist in India
- Pre genetic testing in ART
- Cryopreservation program in ART and how it eased our lives
- Success rates in human ART
- What new we have to increase our success rate in ART
- New rules and regulations in human ART