Hands-on Workshop on Cryobiology and Assisted Reproductive Technologies (ARTs) in the Laboratory Mouse

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Abstract

Animal models are important in biomedical research for validation of in vitro results, conducting proof of concept studies, and investigating the variety of disease processes and therapeutic targets that help in narrowing the gap between bench and bedside. The advent of precise genome engineering technologies has allowed the generation of an increasing number of novel and complex genetically engineered mouse models that can be made available to the scientific and research community. It is important to also provide access to efficient technologies to safely archive, protect, manage, and maintain these precious mouse models. Assisted Reproductive Technologies (ARTs) are emerging as an important tool in the strategic management of vivariums, which involves animal welfare, disaster preparedness, cost minimization, and most importantly, reproducible research.

To disseminate high-end skills of ARTs in laboratory mouse models, the MGEF team conducted a hands-on workshop on **"Cryobiology and Assisted Reproductive Technologies in the Laboratory Mouse"** held at the **National Centre for Biological Sciences and Institute for Stem Cell Science and Regenerative Medicine** from September 11th to September 14th, 2023. 20 professionals from all over India were selected and trained in this workshop by our highly expert staff. The workshop was designed to provide an in-depth understanding and practical knowledge in the field of ARTs and repository management in laboratory animals.

Key words: 3Rs, Cryopreservation, Cryorepository, ARTs, Sperm and Embryo cryopreservation, Hands-on workshop, skill India

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Introduction:

Genetically modified animal models are essential to answering questions in biology, modelling human and non-human animal diseases, and generating therapeutic recombinant proteins. The advancement of genome editing technologies such as clustered regularly interspaced short palindromic repeat (CRISPR)–Cas-associated nucleases, has greatly expedited the accurate and precise mice model generation. Maintaining these strains as breeding colonies has become financially and spatially impractical. The recent advances in mouse sperm and embryo cryopreservation, In-Vitro Fertilization, rederivation, and cold transportation of mouse germplasm can be used as very simple cost-effective solutions to preserve genetic quality, achieve more reproducible research results as well as to greatly reduce the number of animals being used in alignment with the principals of 3Rs (Replacement, Reduction and Refinement).

We have set up a well-equipped state-of-the-art functional facility at the **Mouse Genome Engineering Facility (MGEF) & Animal Care and Resource Center (ACRC)** (https://www.ncbs.res.in/research-facilities/acrc) for the generation of transgenic and gene-edited mouse models as well as harnessing the Assisted Reproductive technology (ARTs) in mice models. We have facilitated animal studies of both support to internal and external researchers whose work depends on genome editing and assisted reproductive technologies including cryo-preservation of the sperm, oocytes, embryos, mouse in-vitro fertilization, and re-derivation of stocks (either from embryos or sperms) via embryo transfer procedures. These capabilities have allowed us to emerge as a national repository for mouse models, cryo-archiving, and distribution of strains to the scientific community both, nationally and internationally. As of today, we are archiving 450 mouse strains as a part of our repository.

One of the critical activities of both the MGEF and ACRC is to offer advanced training and workshops to keep Indian scientists at the leading edge of animal biotechnology. This hands-on workshop was conducted to provide participants with practical knowledge and hands-on experience in the

field of assisted reproductive technologies and repository management in laboratory animals. Participants, including researchers, students, and veterinary professionals, had the opportunity to acquire and enable them to apply these techniques effectively in their research endeavour's. The main objective of this workshop was to elevate the standards of laboratory mouse research by equipping participants with advanced skills, fostering collaboration, and expanding the horizons of scientific exploration.

Workshop theme: The four-day workshop was conducted by a national and international faculty of leading experts in the field and assisted by trained staff at MGEF and ACRC facilities.

The program of workshop included a hands-on experience and 12 talks which were categorized into the day wise them as follows,

Day 1 - Sperm Collection & Freezing (September 11, 2023),

Day 2 – In-Vitro Fertilization (IVF) (September 12, 2023)

Day 3 - Embryo Collection and Vitrification (September 13, 2023)

Day 4 - Surgical Embryo Transfer (September 14, 2023)

Hands-On Sessions

Day 1 – Sperm cryopreservation (Monday, September 11, 2023):

The workshop commenced with participants' registration and a warm welcome by Dr. Mahesh Sahare, setting the tone for an engaging learning experience. Dr. Sahare's welcome address was followed by a captivating talk on "The Mouse Genome Engineering Facility: A National Resource to Generate, Archive, and Distribute Genetically Altered Mouse Models Globally." The morning session included an enlightening presentation by Dr. Sahare on the "Fundamentals of Cryobiology and Sperm Cryopreservation," underscoring the importance of this technique in mouse research. The hands-on session included demonstrations of sperm cryopreservation, embryo cryopreservation and in-vitro fertilization. The two primary methods are commonly known as the JAX method, developed by researchers at The Jackson Laboratory in Bar Harbor, ME, USA, and the CARD method, developed by scientists at the Center for Animal Resources and Development at Kumamoto University in Kumamoto, Japan. Both methods incorporate a fundamental mixture of raffinose and skim milk components, but they differ in their choice of cryoprotectant agent **(Longenecker et al., 2021)**. We adopted the CARD Method for mouse sperm cryopreservation and in vitro fertilization using frozen-thawed sperm. These protocols are widely used by various mouse model repositories, resulting in a stable fertilization rate of>80% in IVF **(Takeo et al., 2019).**

Day 2 - IVF:

The participants were taught hands-on IVF procedures including the preparation of fertilization dishes, thawing of sperm, oocyte collection, and performing IVF. The IVF assessment was done the next day after assessing the fertilized two-cell embryos as compared to the original oocytes. The participant's successful results of IVF are summarized in Figure 1.

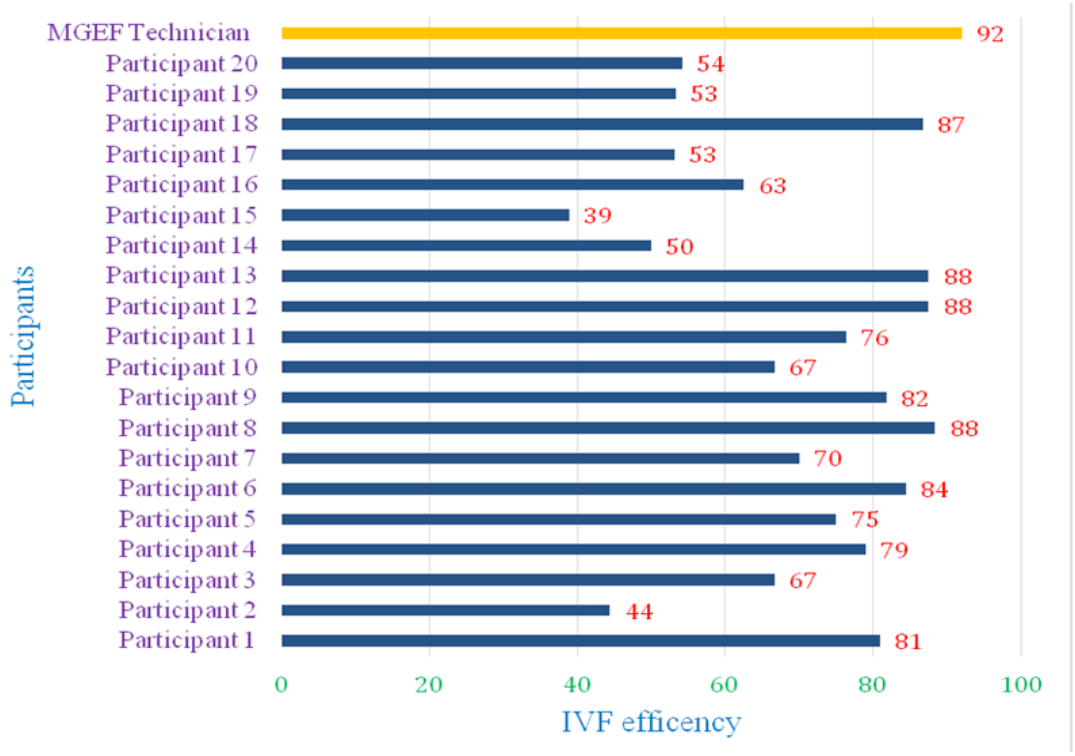

Figure 1. IVF efficiency results of the participants in the hands-on training session.

Dr. Mahesh Sahare's presentation on "In Vitro Fertilization (IVF) and Colony Management" provided crucial insights, followed by hands-on sessions covering IVF and its applications. Also, Dr. Sahare's talk, "The Art of Embryo Cryopreservation," concluded the day, providing participants with a comprehensive understanding of embryo preservation techniques.

Day 3 - Embryo Collection and Vitrification:

The third day of the workshop focused on assessing participants' IVF skills through practical demonstrations. Embryo vitrification and handling techniques were central, accompanied by an introduction to mouse embryo cryopreservation. Attendees actively engaged in the whole experience, gaining proficiency in embryo freezing and transportation techniques. Additional sessions covered the thawing of embryos and the transportation of germplasm, highlighting the necessity for the effective management of frozen sperm and embryos.

Day 4 - Surgical Embryo Transfer:

The workshop culminated with a focus on surgical embryo transfer methods. Dr. Mahesh Sahare introduced the concept of aseptic surgery and rederivation, critical for maintaining mouse colonies. Participants observed and learned from live demonstrations of vasectomy, oviduct and uterine surgical embryo transfer, and non-surgical embryo transfer (NSET). An informative tour of the ACRC SPF facility provided valuable insights into maintaining high-quality research animals. Participants were divided into batches for demonstrations of the surgical techniques and the tour.

The workshop concluded with a quiz and assessment led by the MGEF Team, followed by a feedback session and certificate distribution. A group photo commemorated the successful completion of the workshop.

Speaker Sessions

Dr. Ashwini G B, (Clinical embryologist and Director of ASPIR Fertility clinic) presented the talk on clinical and laboratory advancements in human Assisted Reproductive Technology (ART). The discussion provided a spotlight on significant clinical progress, including OHSS-free clinics, transitioning from cleavage stage embryo transfer to blastocyst transfer, the preference for frozen embryo transfer, ERA, and the potential replacement of antagonists with oral medroxyprogesterone acetate. Moreover, laboratory innovations, such as the evolution of incubators (CO2 to triple gas and time-lapse technology), LASER Assisted Hatching, advanced sperm selection techniques, PGT-A, niPGT-A, and the integration of automation and artificial intelligence in embryology laboratories, were explored in detail.

Dr. Aurélie (Lily) Jory (Head of International Operations, Janvier Labs, France), presented the talk on Introduction to Mouse Genome Engineering and Applications expanded participants' knowledge horizons while discussing the latest advances in mouse transgenesis and genome engineering and presented the key points required to carefully design the correct mouse models precisely adapted to answer a specific scientific question or research application. Additionally, mouse model nomenclature rules and their importance in data sharing, mouse model archiving, and maintenance procedures were also discussed.

Dr. Mohan G H, (Head, Animal Care Resource Centre, NCBS, Bangalore) introduced the "Animal Care and Resource Facility," emphasizing the vital importance of animal care and ethical research practices in research. This lecture focused on management of Specific pathogen-free (SPF) rodents, encompassing animal health, genetic integrity, and environmental factors, as a key strategy for achieving reproducible research results

Dr. Sachin Atole (Head of Veterinary Science, Aragen Life Sciences, Bangalore) provided a thought-provoking discussion on Enhancing Research Reproducibility Through Laboratory Animal Quality and addressed many critical points. Reproducibility is a growing concern, with estimates suggesting a high proportion of irreproducible research findings. Factors like flawed experimental design, research materials, methods, data management, and transparent reporting contribute to this issue.

Dr. Robert Taft, (Senior Services Program Manager at The Jackson Laboratory, USA) highlighted the Jackson Laboratory's rich history as a repository, spanning over 40 years, and its 30-year commitment to training students worldwide in repository establishment. Beyond protocols, he emphasized the multifaceted aspects of running a successful repository, sharing insights from their own experience and aiding others in setting up repositories. Dr. Taft stressed the critical role of quality in repository operation, both in maintaining animal model standards and optimizing operational procedures. He discussed strategies for safeguarding animal model quality and establishing quality systems, aiming to empower students in expanding the global network of mouse repositories for the scientific community.

Dr. Barbara Stone, currently serves as the Director of Animal Research and Assisted Reproduction Sciences at ParaTechs Corporation. She delved into the theme of "3Rs Improvements for Assisted Reproduction in the Mouse." The lecture prominently highlighted techniques in mouse-assisted reproduction designed to align with the principles of the 3Rs - Refinement, Reduction, and Replacement - in animal experimentation. She thoroughly explored non-surgical embryo transfer and artificial insemination methods, comparing them to traditional surgical procedures, with a detailed examination of the associated protocols. Additionally, she introduced a novel approach that obviates the need for vasectomized males by employing cervical manipulation to induce pseudopregnancy in female mice. This innovative process encompasses estrous cycle synchronization, cytological profiling, and estrous cycle determination. Furthermore, Dr. Barbara provided a concise overview of embryo and sperm cryopreservation, showcasing the "Cryodropper" technology as a valuable tool.

Concluding remarks

The reproducibility and 3Rs principles are being addressed globally at scientific, ethical, and legislative levels. Numerous assisted technologies are employed for better reproducible and translational research. We focus on providing hands-on training in the latest techniques related to sperm and embryo cryopreservation, IVF, embryo transfer, and repository management in laboratory mouse models. This workshop was a significant success, bringing together a diverse group of 20 participants from various backgrounds, including PhD students, department heads from different universities, and experienced veterinarians from across India. Finally, IVF efficiency results of the participants in the hands-on training session.is given in Figure.

Ethical approval

The animal experimental protocol for high-skill training was approved by the Institutional Animal Ethics Committee (IAEC) of The Institute for Stem Cell Biology & Regenerative Medicine (inStem).

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Competing interest

The authors declare that their no competing interest to influence the work reported in this paper.

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