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Cryopreservation and Other Preservation Approaches for Animal Models Workshop

Session IV. Cryopreservation and Other Preservation Methods for Rodent Models in Biomedical Research

October 16, 2024
Virtual Meeting

Final Report

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Executive Summary

The Cryopreservation and Other Preservation Approaches for Animal Models Workshop was held in six sessions to address topics related to cryopreservation and other preservation methods, including, but not limited to, (1) the needs and scientific status of cryopreservation and other preservation of gametes (sperm, oocytes, and embryos), reproductive tissues, larvae, and whole animals and their production of live offspring after revival; (2) emerging cryopreservation and other preservation methods and technologies, as well as how to optimize and implement them; (3) methods, technologies, and infrastructure to assess the impact of intrinsic and extrinsic factors on the quality, efficiency, and success of cryopreservation and other preservation protocols and revival, including scalability and reproducibility; (4) the sharing of technologies, including hands-on training for cryopreservation best practices and training of next-generation scientists; and (5) the preservation and management of samples, from collection to utilization.

Session IV addressed cryopreservation and other preservation methods for rodent models in biomedical research. Mice and rats are the most commonly used species in biomedical research to uncover the genetic origins of human diseases. The presentations covered advances in rodent cryopreservation technology, high-temperature sample storage, and new modalities for shipping and long-term storage. The workshop panelists and participants identified needs related to disseminating rodent cryopreservation techniques and raising awareness of local resources and repositories, and the group discussed challenges related to the logistics of shipping and storing frozen samples and mitigating genomic and epigenomic alterations caused by cryopreservation and rederivation-induced interventions.

Session Chair
Yuksel Agca, D.V.M., Ph.D., M.Sc., University of Missouri

Presenters
Yuksel Agca, D.V.M., Ph.D., M.Sc., University of Missouri
Ali Eroglu, D.V.M., Ph.D., Augusta University
Marcello Raspa, Ph.D., European Mouse Mutant Archive
Thomas Saunders, Ph.D., University of Michigan
Robert Taft, Ph.D., The Jackson Laboratory
Toru Takeo, Ph.D., Kumamoto University
Simon Tröder, Ph.D., University of Cologne
Willem Wolkers, Ph.D., University of Veterinary Medicine Hannover

Session IV Organizing Committee

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Workshop Report

Opening Remarks

Yuksel Agca, D.V.M., Ph.D., M.Sc., University of Missouri
Oleg Mirochnitchenko, Ph.D., Office of Research Infrastructure Programs (ORIP), Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), Office of the Director (OD), National Institutes of Health (NIH)

Dr. Oleg Mirochnitchenko welcomed the participants to the meeting. He explained that this session is one of six sessions of the Cryopreservation and Other Preservation Approaches for Animal Models Workshop sponsored by ORIP. The workshop is providing a forum for experts to discuss needs related to animal model preservation and emerging preservation technologies to enhance the maintenance and sharing of animal models. Previous sessions focused on invertebrate models, aquatic models, and technologies and resources. An upcoming session will cover nonhuman primates and other large animal models.

Dr. Mirochnitchenko provided an overview of ORIP's mission, "Infrastructure for Innovation." ORIP comprises two divisions: the Division of Comparative Medicine (which supports research centers and resources, research project grants, and training programs for veterinary scientists) and the Division of Construction and Instruments (which sustains research infrastructure through construction and instrumentation awards and an equipment program). ORIP also supports small businesses through Small Business Innovation Research and Small Business Technology Transfer programs. Dr. Mirochnitchenko invited participants to visit the ORIP website and review ORIP's 2021–2025 Strategic Plan.

Dr. Mirochnitchenko noted that ORIP's Division of Comparative Medicine supports rodent resources in several ways. ORIP supports many organizations and programs involved in the maintenance and development of rodent models for biomedical research (e.g., Cre Driver Strain Resources, Mouse Resources for Comparative Mendelian Disease Genomics, Mouse Strains for Testing Regenerative Medicine Therapies, and the Special Mouse Strains Resource at The Jackson Laboratory; the Hybrid Rat Diversity Panel at the Medical College of Wisconsin; Mutant Mouse Resource and Research Centers [MMRRCs]; the National Gnotobiotic Rodent Resource Center; the Rat Resource and Research Center [RRRC]). ORIP's Division of Comparative Medicine also supports rodent research centers and programs (e.g., the Knockout Mouse Project [or KOMP], Pilot Centers for Precision Disease Modeling, Rodent Testing Centers for Development of Reporter Systems and Evaluation of Somatic Cell Genome Editing Tools), as well as traditional research project grants.

Dr. Mirochnitchenko highlighted the efforts of Dr. Peter Mazur, an investigator at Oak Ridge National Laboratory at The University of Tennessee. In an NIH-funded study that was published in *Science* in 1972, Dr. Mazur worked with Drs. Stanley Leibo and David Whittingham to pioneer technologies leading to the successful freezing and thawing of mouse embryos without resulting in cell damage. Dr. Mazur continued his research program into the late 2000s, when he studied the use of lasers when rewarming frozen samples. After being diagnosed with lung cancer in 2013, Dr. Mazur continued studying cryobiology until his death in 2015. His lifetime achievements include more than 170 publications and numerous awards, including a Distinguished Service Award from the American Association of Tissue Banks.

Dr. Mirochnitchenko introduced the Session IV Chair, Dr. Yuksel Agca. He noted that Dr. Agca is involved with the MMRRCs and RRRC and leads a research program in cellular, biophysical, and molecular aspects of the cryobiology and biology of mammalian germplasm. Dr. Agca is interested in genome resource cryobanking of scientifically important mutant rodent models for human disease. He also uses laboratory rats as models to develop reproductive therapy strategies for women via ovarian tissue cryopreservation and subsequent auto-transplantation for the prevention of infertility and bone loss.

Dr. Agca thanked ORIP for hosting the cryopreservation workshop series and expressed appreciation to the Session IV Organizing Committee and the session participants. He acknowledged the efforts of pioneer investigators in the field, including the discovery of cryoprotective agents (CPAs), the exploration of cryobiology fundamentals, developments in rodent germplasm cryopreservation and reproductive technologies, and the establishment of mutant rodent repositories and resources. Currently, more than 60,000 mutant mouse lines and approximately 2,000 rat strains have been cryobanked worldwide.

Presentations: Part 1
Moderator: Yuksel Agca, D.V.M., Ph.D., M.Sc., University of Missouri

Challenges and Opportunities Associated with Rodent Germplasm Cryobanking
Robert Taft, Ph.D., The Jackson Laboratory

Dr. Robert Taft shared an overview of gaps and opportunities in the field of rodent germplasm cryobanking. He discussed the two main challenges faced by those involved with rodent research: time and money. Time is required to generate and acquire new strains and produce cohorts for research. Animal acquisition, care and housing, genotyping, and colony management are expensive. These challenges directly impact the speed, quality, and reproducibility of research, influencing which models are used and which projects are preferred by the scientific community.

Cryobanking is one way to address these challenges. When cryopreserved, valuable mouse models become more accessible, and existing models shorten the time from concept to data. Additionally, cryopreservation minimizes the use of live animals and improves reproducibility. However, cryobanking itself is associated with many challenges. Most cryopreserved mouse strains are ordered infrequently because of the prohibitive time and cost of producing useful mice. Animal acquisition by repositories involves high levels of engagement with the scientific community and significant costs for shipping, housing, cryopreservation, curation, and quality control. Animal distribution involves costly order processing, mouse production, storage, and shipping. In the age of globalization, international shipping and regulations are additional barriers.

Cryobanking challenges present many opportunities for technical innovation and process improvements. With respect to animal deposition and acquisition, cryopreservation techniques and alternative storage technologies can be further developed. Distribution costs can be reduced with preservation at warmer temperatures and improved *in vitro* fertilization (IVF) and embryo transfer protocols. Larger cohorts can be thawed on demand to increase the available number of animals more quickly. Researchers can be encouraged to deposit strains into repositories earlier in the life cycle to reduce animal use and associated costs and increase the accessibility of the animals.

Cryopreservation of Rodent Sperm: Efficiencies, Reproducibility, and Needs
Toru Takeo, Ph.D., Kumamoto University

Dr. Toru Takeo reported on advances in rodent sperm cryopreservation and described the Center for Animal Resources and Development (CARD) at Kumamoto University in Japan. Established in 1998, CARD is a hub for biomedical research using genetically engineered mice. In addition to its mouse bank, CARD established a rat bank in 2019. The animal banking systems enable research innovation through a collaborative ecosystem of strain archiving, provision of supplies, and data sharing that prioritizes animal reduction, availability, reproducibility, sustainability, and traceability. The CARD mouse bank comprises 5,330 strains; 1,356,382 frozen embryos; and 54,405 frozen straws of sperm. Archived strains are published in the CARD R-BASE, and 2,772 such strains are available. The mouse bank requires reproductive technologies that are efficient, robust, and reproducible. From March 2023 to March 2024, CARD performed IVF on 158,120 mouse oocytes for 576 orders, with a fertilization rate of 82.4%. The

improved reproductive technologies obtained high and stable yields of two-cell embryos. The embryos were cryopreserved for archiving and transferred to recipients for animal production. The embryos produced and archived using CARD reproductive technologies stably developed into pups; the average birth rate after embryo transfer was 37.3%.

Dr. Takeo reviewed the method developed by Dr. Naomi Nakagata for mouse sperm cryopreservation, which involves freezing samples in R18S3 solution (a mixture of 18% raffinose and 3% skim milk). Cauda epididymis tissue is collected from 12-week-old male mice, and sperm are released into R18S3 solution by piercing and gently pressing the tissue. Sperm samples are collected and frozen in the liquid nitrogen vapor phase for 10 minutes before being plunged into the liquid nitrogen for long-term storage. Mouse sperm is thawed by holding the tube in the air for 5 seconds, followed by an incubation at 37 degrees Celsius (°C) for 10 minutes.

The widely used C57BL/6 mouse strain exhibited low fertility after cryopreservation using the Nakagata method, necessitating amendments to the technique, including improved CPAs (e.g., L-glutamine), sperm preincubation medium with methyl-β-cyclodextrin (methyl-β-CD or MBCD), and fertilization medium with reduced glutathione (GSH). Dr. Takeo explained that MBCD induces capacitation (a physiological change necessary for fertilization) by promoting cholesterol efflux from the sperm membrane, and GSH breaks the disulfide bonds of the zona pellucida to expand its network structure. These modifications have ensured that sperm cryopreservation and IVF protocols are applicable to all major mouse strains and that cryopreserved mouse sperm maintain high fertility and development ability for 20 years. Dr. Takeo shared an example of 1,700 mouse pups obtained from the frozen-thawed sperm of a single mouse, indicating that the technique can reduce the numbers of required sperm donors.

Dr. Takeo noted that mice and rats differ in applicable reproductive technologies. Initially, IVF using fresh sperm (which was demonstrated in 1974) was the only successful protocol in rats. Research by Dr. Takeo and his CARD colleagues resulted in successful methods for sperm cryopreservation and IVF using frozen-thawed sperm. Rat sperm cryopreservation requires a more complex CPA mixture of 8% lactose, 23% chicken egg yolk, penicillin, streptomycin, and a detergent called Equex STM. The mixture is adjusted to pH 7.4 with tris(hydroxymethyl)aminomethane. Rat straws must be incubated on a flat surface for 4 minutes before incubation at an angle with a plastic rod. The samples are then pre-cooled for 30 minutes at 0°C before being frozen using the same technique as mouse sperm. Thawing rat sperm is also complicated and involves thawing the sample at 37°C for 10 minutes before adding the sample to modified human tubal fluid (mHTF) solution (i.e., a solution that includes 4 milligram/milliliter [mg/ml] solution of bovine serum albumin [BSA]) and incubating at 37°C in a cell culture chamber for an additional 30 minutes. The tube is centrifuged, and the sperm sediment is mixed with fresh mHTF and incubated in a culture dish for 30 minutes before the dead sperm cells are removed. The live sperm suspension is preincubated for 2 hours before insemination. Before IVF, superovulation is induced in 5-week-old female rats by successive intraperitoneal injections of pregnant mare's serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG). Two-cell embryos can be observed 28–30 hours after insemination. Dr. Takeo's group has demonstrated improved IVF using fertilization with the use of fertilization medium with a high concentration of BSA and removing cumulus cells before insemination. Reproductive technologies developed at CARD support the rat bank in efficiently producing, archiving, and supplying genetically engineered rats.

Dr. Takeo emphasized that model organism resources must share reproductive technologies worldwide. He noted that since 2000, CARD has hosted 51 Mouse Reproductive Technology Workshops in Asia, Europe, and the United States involving 700 participants. The first CARD Rat Reproductive Technology Workshop was hosted in 2021, and the first Rat and Mouse Reproductive Technology Workshop was hosted jointly by CARD and the National Laboratory Animal Center (NLAC) in Taiwan in October 2024.

Discussion

- Dr. Agca asked Dr. Taft to describe the challenges associated with acquiring important new mouse strains. Dr. Taft remarked that deciding which strains are most valuable is a major challenge. Strain acquisition by repositories and community access to those strains are hindered by intellectual property issues.

- A participant suggested that repositories could advertise the availability of live strains that are recovered for an order. Dr. Taft noted that this would require increased numbers of live animals, which would delay the original order. He added that some publications arouse interest in a strain, resulting in multiple orders within a short time frame. In these cases, The Jackson Laboratory will streamline the work to complete the orders more quickly.

- Dr. Mark Woodward asked about the need for repository users to breed their strains. Dr. Taft provided the example of Cre recombinase mouse strains, which must be bred with mice harboring *loxP* sites (or "floxed" mice) to activate site-specific recombination and generate cell-specific or inducible knockout mice. Additionally, mutations or markers are sometimes bred into pre-existing strains. The Jackson Laboratory has held internal discussions about performing some of this breeding in house.

- In response to a question from Mr. Michael Durnin about The Jackson Laboratory's policies on restocking strains, Dr. Taft explained that strains are monitored, and restocking is initiated when the material banked for a particular strain can fulfill only a few orders.

- A participant asked whether generating strains *ab initio* would be more practical than cryopreservation and recovery. Dr. Taft noted that currently, it is more expensive to generate a strain than maintain cryopreserved lines.

- In response to a participant's question about the preferred method of euthanasia for successful rodent cryopreservation and IVF, Dr. Takeo responded that his group uses cervical dislocation in mice and either anesthesia or carbon dioxide for rats (depending on their size).

- Dr. Agca asked about the effect of cooling rates on rat sperm cryopreservation. Dr. Takeo noted that his group has not yet evaluated this parameter but plans to do so.

- Dr. Ghassan Yehia asked whether the addition of BSA is helpful during mouse sperm cryopreservation. Dr. Takeo explained that the effects of BSA were tested and found to be weaker than those of MBCD.

- A participant asked how many sperm straws can fit into a floating 50 ml syringe for freezing in the vapor phase of liquid nitrogen. Dr. Takeo remarked that 10 mouse straws or 15 rat straws can be frozen in each float.

- In response to a question about whether virgin males or breeders should be used for rat sperm cryopreservation, Dr. Takeo responded that no difference has been observed between the two.

Presentations: Part 2
Moderator: Thomas Saunders, Ph.D., University of Michigan

Cryopreservation of Rodent Oocytes: Efficiencies, Reproducibility, and Needs
Yuksel Agca, D.V.M., Ph.D., M.Sc., University of Missouri

Dr. Agca presented an overview of oocyte cryopreservation in mice and rats. He listed several benefits associated with mouse and rat oocyte cryopreservation, including the reduction of donor female costs, an instant supply of oocytes for mutant rodent rederivation (without prior scheduling), rapid establishment of breeder colonies with reduced expenses and turnover time, and shortened generation times for double-mutant strains from cryopreserved gametes. A method for fertilizing female mice with transplanted ovaries derived from frozen ovarian tissue was published by Dr. Delphine Parrott in 1960. Rodent ovaries produce between 8 and 20 oocytes per cycle; the discovery of superovulation (treatment with PMSG) and ultra-superovulation (treatment with PMSG and anti-inhibin serum [AIS]) increased the quantities of gametes produced per cycle twofold and tenfold, respectively. Further studies showed no significant morphological or functional differences between mouse oocytes obtained from females superovulated with either AIS and hCG or PMSG and hCG. Experiments performed by Drs. Nakagata and Takeo at CARD showed improved oocyte yield in AIS-treated mice compared with PMSG-treated mice, but no functional differences between the two methods were observed in IVF experiments.

Dr. Agca discussed the effects of intracellular ice formation during mouse oocyte cryopreservation, which can damage the integrity of the oocyte plasma membrane and zona pellucida. Under certain conditions, intracellular ice can damage subcellular organelles (e.g., microtubules, microfilaments) without altering the morphology of the oocyte, leading to oocytes that appear healthy but are developmentally incompetent. Mitochondria and cortical granules—which are critical for fertilization—can also be damaged or disrupted by ice formation.

Ultrarapid cooling by vitrification has emerged as the preferred technique for mammalian oocyte cryopreservation because it can overcome these limitations. The success of vitrification is dependent on CPA concentration and volume, cooling and warming rates, and the vessel used (e.g., copper grid, cryoloop, Cryotop®, cryovial, French straw, microcapillary, open-pulled straw). Oocyte vitrification has been shown to protect the plasma membrane and microtubules. In an experiment involving vitrification of C57BL/6 oocytes, no difference in the rate of blastocyst formation or fetal viability was observed between combinations of fresh oocytes and fresh sperm, fresh oocytes and cryopreserved sperm, cryopreserved oocytes and fresh sperm, and cryopreserved oocytes and cryopreserved sperm. No differences in fetal viability or genomic variations were observed between cryopreserved and fresh oocytes, cryopreserved and fresh sperm, or oocytes produced by AIS treatment and those produced by PMSG treatment.

Studies have demonstrated that including antioxidants during vitrification alleviates subcellular damage and results in improved blastocyst formation rates (procyanidin B2) and increased numbers of viable offspring (N-acetyl cysteine). Spontaneous activation—resulting in unaligned or separated chromosomes and fragmented oocytes—is a major challenge with rat oocyte cryopreservation. Offspring have been produced successfully using vitrified rat oocytes via IVF and intracytoplasmic sperm injection (ICSI). The inclusion of the protease inhibitor MG132 (to inhibit spontaneous activation) during ICSI resulted in a fivefold increase in blastocyst formation.

Oocyte vitrification is still associated with challenges. Osmotic shock and oxidative stress caused during the addition and removal of CPAs must be addressed, perhaps by the discovery of less or non-toxic CPAs. More effective compounds to counteract oxidative stress and F-actin depolymerization are needed. In-depth genomic- and epigenomic-level analyses must be performed to ensure embryo quality, and the ability to repeat the technique across mutant rodent strains must be evaluated.

SEcuRe: Adapting Proven Approaches for Affordable and Simplified Mouse* In Vitro *Fertilization
Simon Tröder, Ph.D., University of Cologne

Dr. Simon Tröder shared his group's improved approach to IVF with cryopreserved sperm. He first reviewed the Ostermeier et al. and CARD protocols, two standard techniques for mouse IVF. For cryoprotective medium, the Ostermeier et al. protocol recommends using one that includes monothioglycerol (e.g., JAX® CPA), whereas the CARD protocol recommends using one that includes L-glutamine (e.g., CARD FERTIUP® CPA). For capacitation medium, the Ostermeier et al. protocol recommends using the standard fertilization medium HTF (e.g., EmbryoMax® HTF) as preincubation medium (PM), and the CARD protocol recommends tailored cTYH supplemented with MBCD (e.g., CARD FERTIUP® PM). For fertilization, the Ostermeier et al. protocol again recommends HTF, whereas the CARD protocol recommends CARD's modified HTF (e.g., CARD MEDIUM®).

Dr. Tröder shared results from more than 85 IVF procedures and compared the two protocols. The CARD protocol was more efficient than the Ostermeier et al. protocol (~70% vs. ~40% average fertilization efficiency) but also was much more expensive if the medium is not laboriously prepared in-house. His group subsequently developed the Simple Economic set-up for Rederivation (SEcuRe) protocol—based on the CARD protocol—for easy, economical, and efficient mouse fertilization. The SEcuRe protocol involves novel preparation of capacitation medium (cTYH with MBCD) from concentrated stocks and fertilization medium easily prepared from commercial HTF supplemented with calcium and GSH (HTF^+ medium) that is identical to CARD MEDIUM®. Use of the SEcuRe protocol—tested in more than 340 IVF procedures with cryopreserved sperm from various transgenic lines on the C57BL/6 background—results in fertilization efficiencies of approximately 80%, validating the approach. To enhance the economic value of their protocol, the group showed that HTF remains effective even when using commercially available HTF 6 months past its expiration date, with no impact on fertilization rates. The Tröder laboratory uses SEcuRe as a one-size-fits-all IVF protocol, because it works efficiently with frozen-thawed sperm generated by either of the common protocols (CARD or Ostermeier et al.) and can be easily modified to accommodate freshly harvested sperm. Dr. Tröder recommended using the commercial CARD protocol in instances when budget is not limiting, the Ostermeier et al. protocol for researchers with limited experience in IVF, the in-house-prepared CARD protocol for laboratories skilled in complex media preparation, and the SEcuRe protocol in all other instances.

High-Temperature Storage of Rodent Sperm
Willem (Wim) Wolkers, Ph.D., University of Veterinary Medicine Hannover

Dr. Wim Wolkers shared an overview of dry preservation of sperm. Sperm are kept at a low temperature (~4°C) for short-term storage and cryopreserved for long-term storage. Sperm samples rarely are vitrified because of technical challenges. Freeze-drying is emerging as a promising technique for sperm. The process was inspired by anhydrobiotic organisms, which can survive full dehydration because they contain naturally found CPAs that also serve as lyoprotectants. CPAs have been classified as permeating or non-permeating depending on whether they can cross cell membranes. Nonpermeating sugar-based CPAs (e.g., sucrose and trehalose), in particular, are found in anhydrobiotes. These sugars are not produced in typical mammalian cells. Moreover, freeze-drying of sperm requires the addition of bulking agents (e.g., polymers and proteins). Dr. Wolkers reviewed two theories that explain the mechanism of protective agents. The preferential exclusion theory explains that, during the early stages of drying, protective agents prevent conformational changes, reduce injuries from solution effects, and increase the barrier for protein denaturation. The water replacement theory posits that, during the later stages of drying, protective agents prevent lyotropic phase transitions and structural transformations of membranes and proteins and protect via direct hydrogen bonding interactions with biomolecules in the sample. Dr. Wolkers noted that during vitrification, samples enter an amorphous state (i.e., a glassy, highly viscous fluid state with solid-state properties) in which biomolecules are immobilized. Vitrification is

achieved by cooling a sample below its glass transition temperature (T_g). Vitrification and freeze-drying can be differentiated by their T_g. Glycerol, a typical CPA, only forms the glassy state at ultralow temperatures; trehalose, a lyoprotectant, forms a glassy state at temperatures above 50°C.

Freeze-drying involves freezing samples followed by sublimation and dehydration with a vacuum. Examples of freeze-dried products include biomolecules (e.g., antibodies, drugs, enzymes, hormones, plasma proteins), macromolecular assemblies (e.g., blood cells, liposomes, sperm, vaccines, and viruses), and certain tissues (e.g., arteries, bone allografts, heart valves, skin patches). Freeze-drying therefore has many useful applications in regenerative, reproductive, and therapeutic medicine. The storage stability of freeze-dried samples depends on freeze-drying parameters (e.g., temperature and vacuum profiles) and the sample's composition, chemical and physical properties, T_g, molecular mobility, and water content. Methods to allow lyoprotectants to permeate membranes have been developed for intracellular protection during freeze-drying. For example, freezing mediates cellular uptake of membrane-impermeable solutes (e.g., trehalose) via membrane imperfections and osmotic gradients.

In 1998, Drs. Teruhiko Wakayama and Ryuzo Yanagimachi published an IVF protocol involving ICSI with freeze-dried sperm. Sperm lose mobility and membrane integrity upon full dehydration. IVF with freeze-dried sperm involves injecting the non-viable sperm with intact nuclei into oocytes (i.e., ICSI). Successful fertilization with freeze-dried sperm subsequently was described for various species, including cattle, horses, pigs, and rats. Initially, no specific lyoprotectants were used, but the protocol was improved by adding chelating agents, antioxidants, and lyoprotectants. Researchers have encountered less success with freeze-drying somatic cells and oocytes.

The stability of freeze-dried sperm is affected by storage temperature, relative humidity, sample moisture content, atmospheric conditions (e.g., oxygen and pressure levels), and T_g. Storage in the freeze-dried states promotes the accumulation of reactive oxygen species. Lyoprotectants can mitigate, but not fully prevent, this damage. Unlike somatic cells that use chromatin, sperm use protamines to condense DNA, which enables tighter packing. Chromatin stability is another major parameter affected by the storage of sperm at ambient temperatures. Chromatin stability can be assessed using several techniques, including the sperm chromatin structure assay, the sperm chromatin dispersion (or halo) test, single-cell gel electrophoresis (or comet) assay, and noninvasive spectral fingerprinting methods (e.g., Fourier-transform infrared and Raman spectroscopy).

Dry preservation enables short-term transport and storage of sperm at room temperature. Freeze-dried sperm samples currently must be kept at a low temperature for long-term storage; however, dry preservation still significantly reduces sample weight and volume in such cases. Future challenges for high-temperature storage of rodent biosamples include developing methods for combating oxidative damage in sperm and preserving nuclei in somatic cells.

Discussion

- Dr. Woodward asked whether the reduced sperm motility after freeze-drying could be overcome. Dr. Wolkers responded that cellular conditions used by anhydrobiotic organisms to survive dehydration are difficult to replicate in sperm cells; this challenge will be difficult to overcome.

Presentations: Part 3
Moderator: Robert Taft, Ph.D., The Jackson Laboratory

Cryopreservation-Induced Epigenetic Alterations: A Neglected Aspect
Ali Eroglu, D.V.M., Ph.D., Augusta University

Dr. Ali Eroglu discussed how cryopreserved samples are affected by epigenetic alterations. Epigenetics refers to heritable changes in genome function that do not involve altering the DNA sequence. Examples of epigenetic mechanisms include DNA methylation, histone modifications, chromatin remodeling, and modulation of non-coding RNAs. Genomic imprinting is a specific form of epigenetic gene regulation found primarily in mammals that results in the expression of a subset of genes from one of two parental chromosomes. Genomic imprinting was demonstrated in the 1980s with pronuclear transfer experiments, which showed that normal development could be achieved only in embryos with both female and male pronuclei; maternal and paternal genomes were complementary and required to balance the expression patterns of certain genes, and thus to form a healthy diploid zygote. Genomic imprinting is inherited from one generation to the next through a cycle of DNA methylation events involving erasure (i.e., genome-wide demethylation in the developing embryo), establishment (i.e., prenatal *de novo* methylation in sperm and postnatal *de novo* methylation in developing eggs), and maintenance (i.e., active demethylation of the paternal genome and passive demethylation of the maternal genome) of imprinting marks. Transgenerational inheritance of epigenetic marks has also been observed in nonmammalian species (e.g., *Drosophila*). Epigenetic modifications affect all aspects of development, aging, and health, and epigenetic perturbations can cause severe diseases, birth defects, and mental disorders.

Epigenetic modifications are increasingly being recognized as a form of cryopreservation-associated stresses. Animal studies have overwhelmingly shown significant epigenetic alterations in oocytes, sperm, and embryos that underwent either slow freezing or vitrification. In contrast, a small number of human studies have reported no significant epigenetic alterations after cryopreservation. This discrepancy might be due to differences in the inclusiveness of epigenetic changes studied and the detection methods used. In general, animal studies were more comprehensive and used a combination of two or more methods, whereas human studies mostly examined a few genes using a less sensitive method. Overall, mechanistic studies are largely missing, and the underlying mechanisms of cryopreservation-associated epigenetic alterations remain poorly understood. There is a need to study the lasting effects of cryopreservation-associated changes, as indicated in a mouse study in which transcriptional analysis of four-cell embryos developed from frozen-thawed metaphase II (MII) oocytes (i.e., lasting cryoinjury) and four-cell embryos after freezing-thawing (i.e., acute cryoinjury) revealed significant but largely different changes in gene expression compared with control embryos. Considering the transgenerational inheritance and severe consequences of epigenetic perturbations, comprehensive studies are needed to address the long-term effects of cryopreservation-associated epigenetic changes and to develop preventive strategies.

Distribution of Cryopreserved Mouse Spermatozoa in Dry Ice: A Multicentric Study
Marcello Raspa, Ph.D., European Mouse Mutant Archive (EMMA)

Dr. Marcello Raspa began by introducing the National Research Council (*Consiglio Nazionale delle Ricerche*; CNR), the largest multidisciplinary public research and technological development institution in Italy. CNR was founded in 1923 and is important in driving scientific advancements and fostering technological innovation. Organized into numerous research institutes across Italy and seven departments, CNR covers various scientific disciplines, including the natural sciences, engineering, medicine, humanities, and social sciences. CNR's EMMA is the European Union's repository of valuable genetic resources for researchers. EMMA provides access to a vast collection of mouse strains, each carrying a specific genetic mutation, and a comprehensive database of detailed information on the mouse strains is maintained. Each strain is carefully characterized, ensuring that researchers have access to reliable and

well-documented genetic resources. Researchers can easily order strains online, streamlining the process and expediting their research, and EMMA provides expert technical support, assisting researchers in utilizing the resources effectively.

The INFRAFRONTIER consortium is a pan-European research infrastructure initiative that leverages resources like CNR EMMA and aims to facilitate research across different institutions and countries by promoting collaboration among researchers and sharing resources and expertise. INFRAFRONTIER establishes standards for mouse models and data management, ensuring consistency and comparability across studies, and it provides researchers with access to advanced technologies and resources, empowering them to make significant scientific breakthroughs.

In collaboration with INFRAFRONTIER and five repositories in five E.U. countries, the Technical Working Group investigated methods for disseminating frozen mouse embryos and tested different shipping methods and laboratory protocols using 6 wild-type and 12 genetically engineered strains. Previously, the embryos have been shipped and exchanged at the two-cell stage, surviving up to 2.5 days at 4°C. Male germlines can be disseminated as live animals, epididymides refrigerated (4°C) for up to 2 days, or sperm frozen in a dry shipper (−190°C) for 10 days or longer. The group in Italy developed a protocol for shipping sperm frozen in dry ice (−80°C) that lasts for 7 days. Dry ice shipping is associated with the challenges of maintaining temperature, preventing dry ice sublimation, and ensuring sample integrity. Dr. Raspa recommended that shippers use leakproof and insulated containers and monitor samples during transit (e.g., via temperature sensors, location tracking, alert systems). Fertility assessments should be carried out before and after shipping.

Dr. Raspa and his colleagues also have evaluated the quality of mouse sperm after long-term storage. In C57BL/6J and C57BL/6N (but not CD1) mice, a significantly higher percentage of live versus total cells was observed in sperm stored in liquid nitrogen than in sperm stored at −80°C for 5 years. Sperm from C57BL/6 and CD1 mice maintained similar competencies to support IVF and subsequent live births when stored in liquid nitrogen or at −80°C for 5 years. After 9 years of storage at −80°C, sperm from C57BL/6N and CD1 mice maintained fertility, whereas C57BL/6J sperm showed reduced IVF percentage. Future research should focus on optimizing protocols and exploring alternative methods for long-term storage. Continued collaboration among research institutions is crucial for advancing cryopreservation technology and preserving genetic diversity.

Rodent Germplasm Cryopreservation and Assisted Reproduction: From a Transgenic Rodent Model Core Facility Perspective
Thomas Saunders, Ph.D., University of Michigan

Dr. Thomas Saunders discussed rodent cryopreservation and IVF from a transgenic rodent core facility perspective. Transgenic core facilities provide researchers with access to specialized skills (e.g., rodent embryo and sperm handling and cryopreservation, important for shipping of frozen samples), specialized equipment for cryopreservation and storage and retrieval of frozen samples, and specialized animal protocols and housing (e.g., production of pseudopregnant female recipient mice, production of embryos for IVF or cryopreservation, strict pathogen-free housing for pregnant mice). These core facilities serve as local biorepositories that are fast (e.g., simple submission process, health status and quarantines are not obstacles), flexible (e.g., frozen embryo back-up samples can be thawed and transferred within 7 days), and trustworthy (e.g., efficient services, investigator approval required for animal transfers). Core facilities routinely cryopreserve one-cell, two-cell, and eight-cell mouse embryos, mouse sperm, and mouse embryonic stem cells. Two-cell embryos and sperm from rats also are cryopreserved routinely.

Dr. Saunders described quality control measures used by the Transgenic Animal Model Core at the University of Michigan Medical School (UM Transgenic Animal Model Core) for sperm cryopreservation. Liquid nitrogen tanks with a static holding time of 201 days are used, samples are

divided between two containers in separate buildings, and the system is monitored by an in-house data logger. After an experimental sample is thawed, sperm motility is assessed, and the results of IVF procedures using experimental and control sperm are compared. DNA is extracted from mid-gestation fetuses and genotyped to confirm the presence of a transgene or mutation. If a genotype is absent, the submitting laboratory will be consulted. If IVF fails, it is repeated no more than twice before ICSI is used to produce two-cell embryos for embryo transfer. If pups do not develop, the fertilization procedure is repeated no more than twice. If no viable pups are produced, the core facility will suggest an eight-cell embryo freeze.

For embryo cryopreservation, experimental embryos are thawed, and their morphology and viability are noted. Experimental and control embryos are transferred to pseudopregnant recipients to determine whether viability was lost during cryopreservation. DNA is extracted from mid-gestation fetuses and genotyped to confirm the presence of a transgene or mutation. If a genotype is absent, the submitting laboratory will be consulted. If pups do not develop, the test thaw and transfer procedure are repeated no more than twice. If no viable pups are produced, the core facility will suggest keeping the strain with live mice.

IVF approaches have several advantages. IVF can be used to generate animal cohorts for studies or transgenesis and protect against breeding failure. Certain mouse lines can only be maintained by IVF. Cryopreservation of IVF-generated two-cell embryos is more efficient than cryopreservation of eight-cell embryos because fewer mice and fewer cryopreservation sessions are needed. Sperm cryopreservation is more efficient than egg cryopreservation, but only a haploid genome is cryopreserved. One pitfall is that not all transgenic strains are amenable to IVF or cryopreservation. Some mutant sperm lack progressive motility (i.e., they swim in circles), some males do not produce sperm (e.g., because of mutations or aging), and occasionally, cryopreserved transgenic eight-cell embryos do not develop into mice. Some strains therefore require continuous breeding for maintenance.

Dr. Saunders shared the number of cryopreserved strains stored at the UM Transgenic Animal Model Core. In the past 2 years, sperm from 108 mouse strains, embryos from 28 mouse strains, and embryos from 2 rat strains have been cryopreserved at the facility. During the same period, 12 mouse strains have been reanimated by IVF, and 9 mouse strains have been reanimated by embryo thawing. Since its establishment in 1989, sperm from 1,046 mouse strains, embryos from 593 mouse strains, and embryos from 21 rat strains have been cryopreserved at the facility.

Discussion

- Dr. Taft asked whether concerns about sperm integrity or logistical issues contributed more to the community's reluctance to ship sperm samples on dry ice. Dr. Raspa answered that the main concerns are related to the logistics of shipment.

- Dr. Taft asked Dr. Saunders for suggestions on how core facilities can raise their visibility within and beyond their organizations. Dr. Saunders shared examples from the UM Transgenic Animal Model Core, which is part of the Biomedical Core Facilities at the University of Michigan and shares information in their monthly newsletter. The UM Transgenic Animal Model Core is also part of the Rogel Cancer Center, which spreads awareness about the animal core when publicizing shared resources. Dr. Saunders noted that a lot of awareness beyond the university relies on word of mouth and remains a challenge.

- Dr. Elizabeth Bryda asked whether transgenic cores should help educate investigators about the possibility of depositing strains in repositories even if the investigators have used their core

facilities for in-house cryopreservation. Dr. Saunders explained that he often advocates for strain submissions to repositories.

- When asked whether the quality control steps are built into the pricing of cryopreservation, Dr. Saunders noted that all steps are included except for the IVF test comparing experimental and control sperm.

- In response to a participant query, Dr. Saunders explained that computer-assisted semen analysis (or CASA) is not used to evaluate post-thaw sperm motility, which can be performed by eye using a light microscope. CASA instruments are expensive, and the data are not predictive of fertilization efficiency.

Group Discussion
Moderator: Yuksel Agca, D.V.M., Ph.D., M.Sc., University of Missouri

Dr. Agca opened the discussion with a question-and-answer session involving the workshop's panelists.

- Dr. Agca noted that the RRRC often must rederive rat strains via embryo transfer after ICSI. He asked when the use of cryopreserved sperm from inbred rat lines will become more widespread. Dr. Saunders remarked that Dr. Takeo's data show that current methods enable cryopreserved sperm from inbred (e.g., Fisher 344, Brown Norway) and outbred (e.g., Sprague-Dawley) rat lines to be recovered with efficiencies that support IVF. He noted that sperm from outbred strains are recovered more efficiently than sperm from inbred strains.

- Dr. Agca commented that workshops should be organized to disseminate cryopreservation techniques for rat sperm. Dr. Takeo reminded the participants that CARD and NLAC recently cohosted such a workshop in Taipei, Taiwan. He added that a Rat and Mouse Reproductive Technology Workshop will be convened shortly at Kyoto University in Japan.

- Dr. Saunders suggested that rat resources in the United States host similar events in collaboration or after training with Japanese colleagues. The group noted that The Jackson Laboratory does not support rat resources. Dr. Mirochnitchenko stated that NIH can consider how to assist with such events.

- Dr. Agca pointed out that other rat techniques (e.g., embryo cryopreservation, IVF) should be disseminated. He emphasized that workshops on rat reproductive technology will encourage collaboration and knowledge sharing.

- Dr. Agca asked about long-term storage in dry ice and whether packaging designs could be modified to extend the life of dry ice during shipments. Dr. Raspa shared that, in collaboration with industry, his group is developing super-freezers to maintain a temperature of −80°C more stably. He added that a shift to storage at −80°C would eliminate safety concerns associated with liquid nitrogen. Dr. Taft remarked that Linde Gas & Equipment Inc. offers SECCURUS™ dry ice shippers that maintain temperature for 30 days. Shipped samples should maintain their temperature for more than a week to account for travel time and clearing international customs.

- In response to a question from Dr. Mirochnitchenko, Dr. Raspa shared that in Europe, storage at −80°C currently costs sixfold more than storage in liquid nitrogen.

- Dr. Wolkers asked whether different cryopreservation techniques should be used for different storage methods. He has seen reports describing the use of polymers to stabilize samples during −80°C shipments. Dr. Raspa shared that, so far, his group has used one cryopreservation

technique to compare different freezing methods. They are studying the use of new antioxidants during cryopreservation.

- In response to a follow-up question from Dr. Agca, Dr. Raspa clarified that samples are more stable when rapidly cooled in liquid nitrogen before storage at −80°C. His group has demonstrated, however, that samples frozen directly at −80°C are stable for at least 2 years.

- Dr. Mirochnitchenko noted that cryopreserved samples could be stored in liquid nitrogen vapor freezers that had been reset from −150°C to −80°C to reduce nitrogen consumption.

- Dr. Agca asked about comparing storage in liquid nitrogen or at −80°C for poor-fertility strains. Dr. Raspa responded that he has not performed this experiment, but, in theory, fertility would not diminish when samples are stored for 5 years using either method.

- Creatine has been reported to improve sperm motility, but none of the panelists have tested its use in cryopreservation.

- Dr. Agca mentioned a recent report on the use of automated sperm injections for human IVF. He wondered whether freeze-dried sperm could be combined with automated injections in rodent systems. Dr. Saunders stated that rodent oocyte injection is more technically challenging than injecting human eggs. Blunt needles must be used in combination with a piezoelectric-assisted micromanipulator to prevent oocyte lysis, and this technique will not be amenable to high-throughput methods. Dr. Agca noted that artificial intelligence might enable automation of rodent IVF techniques.

- A participant asked whether raising freezer temperatures from −80°C to −70°C to save energy would affect the storage of cryopreserved samples. Dr. Wolkers responded that, eventually, any change in storage temperature will affect sample stability. Dr. Raspa shared that his industry collaboration involves testing the effects of freezer temperature fluctuations. Dr. Wolkers noted that storage in isopropanol can minimize temperature fluctuations.

- Dr. Agca noted the difficulty in defining the sources of epigenetic changes (e.g., *in vitro* culture, CPA toxicity, osmotic stress, reactive oxygen species) and their relative contributions to cellular changes during cryopreservation. Dr. Eroglu emphasized the need for systematic studies and long-term follow-up studies.

- In response to a follow-up question from Dr. Mirochnitchenko, Dr. Eroglu responded that the studies he evaluated did not discuss the phenotypic effects of epigenetic changes. He added that newer studies make use of technological advances to evaluate more global changes resulting from epigenetic effects during cryopreservation. Dr. Agca mentioned a 2023 study published in the *Journal of Clinical Investigation* showing that ICSI induces transgenerational abnormalities in mice.

- Dr. Yehia asked about potential problems associated with relying on large cohorts of mice produced by IVF for research. Dr. Taft affirmed that issues are associated with these mice. He noted evidence that superovulation affects the epigenetic state of oocytes. Many maternal characteristics can directly affect offspring produced by IVF. Experiments should be designed carefully so as not to introduce IVF as a confounding factor. Dr. Agca invited the panelists to share thoughts about a potential white paper resulting from the workshop session. He shared a potential outline for the paper, including an introduction to cryobiology of rodent germplasm, the biomedical significance of rodent cryorepositories, international opportunities for resource

sharing and collaboration among rodent repositories, disaster preparedness plans for rodent germplasm repositories, rodent sperm cryopreservation, rodent oocytes cryopreservation, effective strategies for rapid rodent sperm cryorecovery, alternative strategies for storage and transportation of rodent germplasm, and biological alterations associated with rodent germplasm cryopreservation.

- Dr. Saunders suggested including a section on the recovery of live mice from cryopreserved oocytes and sperm, especially biological changes induced by germplasm cryopreservation and rederivation via IVF (e.g., uterine environment, microbiome differences between foster mothers and offspring). Dr. Taft agreed and shared that researchers often inquire about microbiome effects.

- Dr. Wolkers proposed including a chapter on quality assurance and quality control measures and their usefulness in predicting fertility.

- Dr. Agca noted that the group can discuss whether the white paper should be divided into smaller papers.

- Dr. Mirochnitchenko recommended that the paper emphasize best practices for rodent cryopreservation and address non-experts' needs.

- Dr. Agca remarked that the paper should be published in a scientific journal that covers genomics or transgenic research. A white paper from a previous workshop on cryopreservation was published in *Biology of Reproduction*. Dr. Tröder commented that *PLoS One* or *Cryobiology* might be appropriate target journals.

- The panelists agreed to develop a structure for the paper, assign sections, and develop a timeline for the draft before the end of the year. Additional investigators will be contacted if outside expertise is needed.

Concluding Remarks
Oleg Mirochnitchenko, Ph.D., ORIP, DPCPSI, OD, NIH

Dr. Mirochnitchenko expressed appreciation to the presenters and thanked the session chair, workshop organizing committee, and attendees for their contributions and participation. He adjourned the workshop.

Appendix A: Meeting Agenda

Session IV. Cryopreservation and Other Preservation Methods for Rodent Models in Biomedical Research

Virtual Meeting
October 16, 2024

1:00–1:10 p.m. **Opening Remarks**
Yuksel Agca, D.V.M., M.Sc., Ph.D., University of Missouri
Oleg Mirochnitchenko, Ph.D., Office of Research Infrastructure Programs (ORIP), Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), Office of the Director (OD), National Institutes of Health (NIH)

1:10–2:00 p.m. **Presentations: Part 1**
Moderator: Yuksel Agca, D.V.M., M.Sc., Ph.D., University of Missouri

Challenges and Opportunities Associated with Rodent Germplasm Cryobanking
Robert Taft, Ph.D., The Jackson Laboratory

Cryopreservation of Rodent Sperm: Efficiencies, Reproducibility, and Needs
Toru Takeo, Ph.D., Kumamoto University

2:00–2:05 p.m. **Break**

2:05–3:05 p.m. **Presentations: Part 2**
Moderator: Thomas Saunders, Ph.D., University of Michigan

Cryopreservation of Rodent Oocytes: Efficiencies, Reproducibility, and Needs
Yuksel Agca, D.V.M., M.Sc., Ph.D., University of Missouri

SEcuRe: Adapting Proven Approaches for Affordable and Simplified Mouse *In Vitro* Fertilization
Simon Tröder, Ph.D., University of Cologne

High-Temperature Storage of Rodent Sperm
Willem (Wim) Wolkers, Ph.D., University of Veterinary Medicine Hannover

3:05–3:10 p.m. **Break**

3:10–4:10 p.m. **Presentations: Part 3**
Moderator: Robert Taft, Ph.D., The Jackson Laboratory

Cryopreservation-Induced Epigenetic Alterations: A Neglected Aspect
Ali Eroglu, D.V.M., Ph.D., Augusta University

Distribution of Cryopreserved Mouse Spermatozoa in Dry Ice: A Multicentric Study
Marcello Raspa, Ph.D., European Mouse Mutant Archive

	Rodent Germplasm Cryopreservation and Assisted Reproduction: From a Transgenic Rodent Model Core Facility Perspective *Thomas Saunders, Ph.D., University of Michigan*
4:10–4:15 p.m.	**Break**
4:15–5:00 p.m.	**Group Discussion** *Moderator: Yuksel Agca, D.V.M., M.Sc., Ph.D., University of Missouri*
5:00 p.m.	**Concluding Remarks** *Oleg Mirochnitchenko, Ph.D., ORIP, DPCPSI, OD, NIH*
5:00 p.m.	**Adjournment**

Appendix B: Participants

Session IV. Cryopreservation and Other Preservation Methods for Rodent Models in Biomedical Research

Virtual Meeting
October 16, 2024

Yuksel Agca, University of Missouri
Monika Aggarwal, Office of Research Infrastructure Programs (ORIP), Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), Office of the Director (OD), National Institutes of Health (NIH)
James Amos-Landgraf, University of Missouri
Kristina Andersson, Karolinska University Hospital
Renee Araiza, University of California, Davis
Ravi Balijepalli, National Heart, Lung, and Blood Institute, NIH
Kelsey Barrie, University of Michigan
Antonio Murcia Belmonte, Instituto Neurociencias Alicante
Miranda Bernhardt, Washington State University
Jackie Brooks, Mutant Mouse Resource and Research Centers, The University of North Carolina
Elizabeth Bryda, University of Missouri
Bettina Buhring, ORIP, DPCPSI, OD, NIH
Laura Burger, University of Michigan
Andrea Calvillo, Fred Hutchinson Cancer Center
Alison Cash, Cleveland Clinic Lerner Research Institute
Hasan Ali Çay, Burdur Mehmet Akif Ersoy University
Susan Chandran, ORIP, DPCPSI, OD, NIH
Brooke Chang, University of California, Berkeley
Kallayanee Chawengsaksophak, Institute of Cancer Research
Hanying Chen, Indiana University
Yong Cheng, Wistar Institute
Jonathan Clayton, University of Nebraska
Miguel Contreras, ORIP, DPCPSI, OD, NIH
Jennifer Corrigan, The Jackson Laboratory
Carme Cucarella, Instituto de Biomedicina de Valencia
Eden Dulka, University of Michigan
Sarah Duponchel, GenOway
Michael Durnin, Stowers Institute for Medical Research
Melinda Dwinell, Medical College of Wisconsin
Ali Eroglu, Augusta University
Angelika Fath-Goodin, ParaTechs Corporation
Shuo Feng, University of Michigan
Julia Fernandez, Centro Nacional de Biotecnología
Victoria Gilbert, University of California, Davis
Birgit Glasmacher, Leibniz Universität Hannover
Daniel Grigsby, University of Virginia
Audra Guikema, Van Andel Research Institute
Judy Hallett, Purdue University
Gregg Homanics, University of Pittsburgh
Kristy Hood, Novartis Institute for Biomedical Research
Mayumi Isaka, Regeneron Pharmaceuticals
Tomoko Ishikawa, University of Michigan

Sivakumar Jeyarajan, University of Michigan
Katie Johnson, Boise State University
Sarah Johnson, Harvard University
Sangwon Kim, Thomas Jefferson University
Firat Korkmaz, Burdur Mehmet Akif Ersoy University
Elizabeth Kotus, University of Michigan
Melissa Larson, University of Kansas Medical Center
Jun Hee Lee, University of Michigan
P.S. Li, The University of Hong Kong
Minyan Li, University of Copenhagen
Bo Liang, University of Michigan
Kent Lloyd, Ph.D., University of California, Davis
Glenn Longenecker, National Institute of Dental and Craniofacial Research (NIDCR), NIH
Isabel Lorenzo, Baylor College of Medicine
Rachel Mann, Case Western Reserve University
Sara Leal Marin, Leibniz University Hannover
Rachel McAdoo, University of California, Davis
Elise McBurney, Novartis Institute for Biomedical Research
Geeta Mehta, University of Michigan
Oleg Mirochnitchenko, ORIP, DPCPSI, OD, NIH
Kathleen Moosbrugger, University of Pennsylvania School of Medicine
Arun Murahari, Centre for DNA Fingerprinting and Diagnostics
Henda Nabli, University of Missouri
Mylinh Nguyen, The University of Texas Southwestern Medical Center
Karolina Nitsche, Emory University
Rada Norinsky, The Rockefeller University
Kyle O'Connor, Indiana University
Harriette Oldenhof, University of Veterinary Medicine Hannover
Tuncer Onay, Northwestern University
Felipe Ongaratto, University of Wisconsin
Eleanor (EChO) Ory
Payton Oswalt, University of Missouri
Ana Rita Pereira, Champalimaud Foundation
Craig Porter, Arkansas Children's Research Institute
Marcello Raspa, European Mouse Mutant Archive
Akshaya Raajkumar, The Hospital for Sick Children
Tiffany Raatz, Medical College of Wisconsin
Laurel Rawls, University of California, Davis
René Rudat, Otto-von-Guericke University Magdeburg
Niloofar Sadeghi, Texas Biomedical Research Institute
Thomas Saunders, University of Michigan
Tina Schumaker, Van Andel Institute
William Shawlot, The University of Texas at Austin
Stephanie Slater, Seattle Children's Research Institute
Gillian Sleep, Canadian Mouse Mutant Repository, The Centre for Phenogenomics
Claudia Solbes-Godina Riera, The University of Texas MD Anderson Cancer Center
Seham Soliman, National Research Center, Egypt
Sarah Srodulski, ParaTechs Corporation
Tina St. Laurent, Van Andel Institute
Stephanie Sterling, University of Pennsylvania
Barbara Stone, ParaTechs Corporation

John Swift, St. Jude Children's Research Hospital
Roman Szabo, NIDCR, NIH
Robert Taft, The Jackson Laboratory
Toru Takeo, Kumamoto University
Ritesh Tandon, ORIP, DPCPSI, OD, NIH
Amanda Thayer, Genentech
Simon Tröder, University of Cologne
Andrea Trumbauer, Case Western Reserve University
Walter Tsark, City of Hope
Desiree von Kollmar, ORIP, DPCPSI, OD, NIH
Shuling Wang, University of California, Irvine
Priyan Weerappuli, University of Michigan
Willem Wolkers, University of Veterinary Medicine Hannover
Mark Woodward, Wake Forest University
Lin Wu, Harvard University
Xiaojun Xing, Yale University
Nobuko Yamanaka, McGill University
Yu-Ting Yang, Cold Spring Harbor Laboratory
Ghassan Yehia, Rutgers, The State University of New Jersey
Kaitlin Zawacki, University of Michigan
Branko Zevnik, University of Cologne
Sige Zou, ORIP, DPCPSI, OD, NIH
Nikolas Zuchowicz, University of Minnesota