

CARD-Texas Mouse Reproductive Technology Workshop

Hosted by Texas A&M Institute for Genomic Medicine
(TIGM)
College Station Texas, USA
14-18 November, 2017

The CARD-Texas Mouse Reproductive Technology Workshop was held on November 14th-18th 2017 at the Texas A&M Institute for Genomic Medicine (TIGM) in College Station, Texas. The workshop was organized by Naomi Nakagata and Toru Takeo of the Center for Animal Resources and Development (CARD) at Kumamoto University, Andrei Golovko and Benjamin Morpurgo of TIGM, Jan Parker-Thornburg of The University of Texas M.D. Anderson Cancer Center, and William Shawlot of The University of Texas at Austin. Nineteen students and seventeen instructors and lecturers participated in the five-day lecture and hands-on workshop.

The first day of the workshop began with welcoming remarks by Bill McCutchen, Executive Associate Director of Texas A&M AgriLife Research, and Ben Morpurgo, Executive Director of TIGM. This was followed by a series of morning lectures. Professor Nakagata gave an overview of cryopreservation in mice and a history of the cryopreservation course. Jorge Szein, Visiting Professor of CARD, Kumamoto University, followed with a presentation on the history of cryopreservation and John Stallone from Texas A&M University gave an overview of the workings and the responsibilities of the Institutional Animal Care and Use Committee. This was followed by a talk from Chuck Long of Texas A&M University on cryopreservation techniques in large animals. Those of us working in mice were fascinated to hear about the logistics of collecting cattle embryos. Professor Nakagata followed with a lecture on the vitrification of mouse oocytes and their use for *in vitro* fertilization (IVF). The lecture portion of the first day concluded after lunch with presentations by Nicola van der Walt from MilliporeSigma and Andrei Golovko of TIGM on strategies for CRISPR editing in mice and real-world results.

The laboratory portion of the workshop began on the afternoon of the first day with a session on cold storage of cauda epididymides and sperm freezing. For each laboratory session, Professor Nakagata gave an overview of the specific technique followed by a live demonstration of the technique by a member of the CARD team. Students then performed the technique at their workstation and were guided by a CARD instructor. There was one CARD instructor for each two students, so students received extensive personalized instruction and feedback.

On the morning of the second day, students learned to perform *in vitro fertilization* using frozen sperm and sperm from cold-stored cauda. While the fertilization reactions were incubating, the CARD team gave additional presentations on embryo handling, the breeding, genotyping and backing-up of genetically engineered mouse models, the organization and mission of the CARD, and a report on the CARD hyperovulation procedure. After examination of the IVF oocytes for the presence of pronuclei and a group photo outside the TIGM building, the day concluded with a presentation by Fernando Benavides from The University of Texas M.D. Anderson Cancer Center on the importance of monitoring genetic background in transgenic and mutant mice and strategies for more quickly transferring mutations on to more suitable genetic backgrounds for phenotypic analysis.

The morning of the third day of the workshop focused on vitrification techniques using oocytes and 2-cell embryos. In the afternoon, Jorge Sztejn gave a presentation on ovary freezing, which was then followed by a laboratory session on ovary freezing, thawing, and transplantation to host mice.

On the morning of the fourth day of the workshop, students learned how to obtain high IVF rates with sperm cryopreserved using legacy protocols and they also performed IVF using vitrified oocytes. In the afternoon, Shuuji Tsuchiyama from CARD, gave a presentation on his efforts to develop a database for banked mouse embryos and Ben Morpurgo presented on the history and mission of TIGM. The official course dinner was held later that evening at Calvary Court, a new boutique hotel in College Station. This was followed by after-dinner drinks around the hotel pool.

On the final day of the course, Barbara Stone from ParaTechs Corporation gave a presentation on using the NSET device for non-surgical embryo transfer in mice. Students were then able to work with the device in the lab. After a little probing, most students were able to successfully position the device and transfer their practice embryos. After scoring their IVF dishes that used legacy sperm stocks, students had a round table discussion on cryopreservation techniques. Toru Takeo and Hidetaka Yoshimoto of the CARD then gave a presentation on new protocols for shipping frozen and refrigerated embryos and sperm. Toru then presented the compiled data for embryo viability and IVF rates that the students had obtained over the five days of laboratory work. The overall rates were uniformly high and were similar to the results obtained last year in the CARD workshop held in Paris. The workshop concluded with Professor Nakagata presenting each student a certificate for their successful completion of the course.

In summary, the workshop was very successful. All participants, including the organizers and the instructors, learned something new, caught up with old friends, and made new friends. One thing that all the students commented on was the amazing organization of the individual lab sessions. Reagents appeared magically and trays

loaded with incubation dishes were shuttled to and from lab benches and incubators with flawless precision. The CARD instructors responsible for this amazing organization were: Toru Takeo, Shuuji Tsuchiyama, Tomoko Kondo, Yuko Nakamuta, Eri Ishida, Hidetaka Yoshimoto, Satohiro Nakao, Ayumi Mukunoki, Jorge Sztejn, and Kristy Williams. The organizers also acknowledge Nobuyuki Mikoda from Kyudo Corporation, who photo-documented the entire workshop.

In addition to support from the ISTT, a number of companies were instrumental in the success of the workshop. Charles River Laboratories donated all the mice used in the workshop and we thank Pat Sikes for her help in organizing the mouse logistics. Leica Microsystems arranged for the shipment and set up of the microscopes used by the students and we thank Louise Bertrand for her help in overseeing this and Ross Warrington for setting the microscopes up. Other key sponsors of the CARD-Texas workshop were IDT (Ric Sugarek), MilliporeSigma (Nicola van der Walt), Cosmo Bio, and Regeneron Pharmaceuticals.

On a final note, the organizers would like to also thank Stephanie King and Amy Gonzales of TIGM. Stephanie designed the course website and made all the administrative arrangements both large and small that enabled the workshop to run so smoothly. Amy let the workshop borrow microscopes from her lab when we found out that we were short several microscopes due to a shipping error. Amy also saved the day on several occasions by replacing bulbs on several of the course's microscopes from her own supply. She also helped Stephanie to make sure critical administrative details, like lunches for hungry students and organizers, were on time and set up. We owe both Stephanie and Amy a big thank you for all their efforts, both seen and unseen.