

**CARD-RPCI Sperm and Embryo Cryopreservation Workshop**  
**September 15-19, 2014**  
**Roswell Park Cancer Institute, Buffalo NY, USA**



***Meeting Report***

The CARD-RPCI Sperm and Embryo Cryopreservation Workshop was recently held in Buffalo NY USA on the campus of Roswell Park Cancer Institute. Eighteen trainees, fifteen instructors and lecturers, and numerous vendors were hosted by Naomi Nakagata (Kumamoto University), Aimee Stablewski (Roswell Park Cancer Institute) and Jan Parker-Thornburg (MD Anderson Cancer Center) from September 15-19, 2014. Drs. Naomi Nakagata and Toru Takeo brought their team from CARD to assist the trainees in learning the latest techniques in embryo vitrification, sperm cryopreservation, in vitro fertilization, and the new method of vitrifying oocytes. In addition, Jorge Szein instructed the trainees in methods of ovary freezing and subsequent implant after thaw; Lluís Montoliu provided lectures in current methods of embryo cryopreservation and CRISPR/Cas9 methods of generating genetically engineered mice, and Barbara Stone provided training in using the NSET method of embryo implant.

This was the first time that the CARD team provided training in North America, and the team (supported by Kumamoto University and Kyudo Co., LTD.) delivered a truly memorable experience for the trainees. Hands-on sessions were intense and intended to provide trainees with the knowledge and experience of performing the exacting CARD techniques. The eighteen trainees were composed of fourteen ISTT members and four non-members. Of these, three were from Europe, one from Australia, one from New Zealand, and the remainder from all parts of North America.

Hands on training included isolating unfertilized mouse oocytes, isolating and cold storage/shipping of mouse cauda epididymis, freezing/thawing mouse sperm and IVF using CARD frozen sperm and legacy sperm, fresh mouse sperm and IVF, cold stored sperm and IVF, freezing/thawing 2-cell IVF-derived mouse embryos, vitrification of mouse oocytes and embryos, IVF of vitrified mouse oocytes, ovary transplantation/ovary freezing, and embryo transfer techniques in mice (oviduct, uterus via NSET).

Didactic lectures were given on the topics of historic and scientific perspectives of embryo and sperm cryopreservation (by Jorge Szein), comparing current embryo and sperm cryopreservation methods (by Lluís Montoliu), vitrification of oocytes and their use for IVF (by Naomi Nakagata), new US guidelines for the use of animals in research/IACUC (by Sandra Sexton), CRISPR/Cas9 and gene editing endonucleases (by Lluís Montoliu), development of database for managing mouse banking system (by Shuuji Tsuchiyama), shipping mice, frozen or refrigerated embryos/sperm across the world (by Toru Takeo) and freezing and transplantation of ovaries (by Jorge Szein).

On Wednesday of the workshop, all of the participants were treated to a trip to Niagara Falls followed by an exceptional gala dinner (arranged by Aimee Stablewski) at a local winery. This short respite enabled the participants to replenish their energies for subsequent long days of IVF, vitrifications and surgery.

In all, it proved to be an exceptional workshop, with all participants achieving exceptional results in most, if not all, of the practicals. In fact, the depth of appreciation for being taught these methods became clear both during the closing session, where all instructors, vendors, and participants thanked Dr. Nakagata with a standing ovation, and immediately afterward, where many compliments were given. Perhaps, one of the participants said it best in an e-mail immediately following the course:

*“a big thank you to Prof. Nakagata, Dr. Toru Takeo and the entire CARD team. The way they have organised is unbelievable. As we all know in biological practical experiments, how much ever we take care, they never go according to plan and there is always a blooper. I must confess, I had my doubts that we would ever stick to the time table. I am glad that I was completely proven wrong. They have given attention to every bit of details and must have put lot of rehearsals behind this. They were fantastic. They answered all the questions and made sure everyone understood plus followed it up with the practicals. The Entire Card team was simply amazing and no other way to express my gratitude than simply saying “I bow to the entire CARD team”.”*

Prasanna Kallingappa  
University of Auckland  
New Zealand

Aimee Stablewski and Jan Parker-Thornburg, as hosts, would like to acknowledge not only our lecturers, but also the CARD team who assisted the students, including Shuuji Tsuchiyama, Kiyoko Fukumoto, Yukie Haruguchi, Tomoko Kondo, Yumi Takeshita, Yuko Nakamuta, Tomoko Umeno, (all from Kumamoto University and Kyudo Co. LTD); and the CARD “adoptees”—Kristy (Kinchen) Williams (University of Florida) and Amar Dasari (Taconic). In addition, technical assistance was ably provided by Dawn Barnas (ISTT), Karstin Webber, Sandra Sexton and Leslie Curtin (all RPCI), who treated over 600 mice with hormones!

The workshop was extremely fortunate to have extremely generous vendor support, including our platinum sponsors: Charles River Laboratories (who supplied all of the mice used in the course), Leica Microsystems (who provided all of the microscopes used for the course as well as onsite support by Louise Bertrand), and Kyudo Co., LTD who provided support for the CARD instructors as well as onsite support by Nobuyuki Mikoda; our gold sponsors: the ISTT (who sponsored Lluís Montoliu’s participation and contributed to the gala dinner), Taconic (who provided instructional support from John Couse and Amar Dasari), and Regeneron (who sponsored a lecture by Lluís Montoliu); our silver sponsors: Hamilton Thorne (who provided instruction in sperm analysis and laser-assisted IVF by Nancy Mutch), IDEXX Bioresearch for course support, Paratechs for sponsoring Dr. Barbara Stone’s participation, Cell Preservation Solutions who provided course support and Lifer media for cold-storage, CosmoBio who provided course support, Transposagen who provided course support, and our bronze sponsors: Millipore EMD and *mofa* for equipment, Tokai-Hit for equipment, Eppendorf (Mike Bady) for course support and equipment, and VWR and Sarstedt for course support.

Finally, we thank our participants for their hard work and dedication to bring the newest transgenic technologies back to their institutions.

Aimee Stablewski, Roswell Park Cancer Institute, Buffalo, NY, USA  
Jan Parker-Thornburg, MD Anderson Cancer Center, Houston, TX, USA