Breeding Times
- Zebrafish

CRYOGENETICS NEWSLETTER

May 2016

Volume 3 Issue 1

TEST OF CRYOGENETICS' CRYOPRESERVATION PROTOCOL AT BOSTON CHILDREN'S HOSPITAL

Cryogenetics' protocol for cryopreserving zebrafish sperm has shown high and stable post thaw fertilization results in all internally conducted tests. To further prove the reproducibility of the protocol, a trial was run in collaboration with Christian Lawrence, the manager of the Aquatic Resource Program at Boston Children's Hospital. The trial was completed October 2015.

In the trial, *in vitro* fertilization (IVF) results were compared using fresh and frozen sperm samples from 3 different zebrafish strains: casper, AB wild-type and a triple mutation.
All sperm, either fresh or cryopreserved, was collected from the same males within each strain. For each fertilization, 4-5 clutches of AB WT eggs were pooled and split in half. Each half of the clutch was fertilized with either fresh or cryopreserved sperm from the same strain. Sperm concentrations were standardized for fresh and cryopreserved samples to ensure comparable results. A total of 3282 eggs were used in the trial. Of these, 1581 eggs were fertilized with fresh sperm and 1701 eggs fertilized with cryopreserved sperm.

Figure 1 shows the overall results (% fertilized) of fresh sperm and cryopreserved sperm IVF, averaged by strain. Fertilization was evaluated at both 4 and 24 hours post-fertilization.
In Figure 2 the results are combined across all strains and compared at the two time points.

The cryopreserved samples resulted in fertilization rates comparable to the fresh samples.
It is worth noting that none of the fertilization events using cryopreserved sperm resulted in 0% fertilization. This supports our view that line preservation using cryopreserved sperm is highly reliable and lines are not at risk of loss due to poor fertilization outcomes when using techniques developed at Cryogenetics.

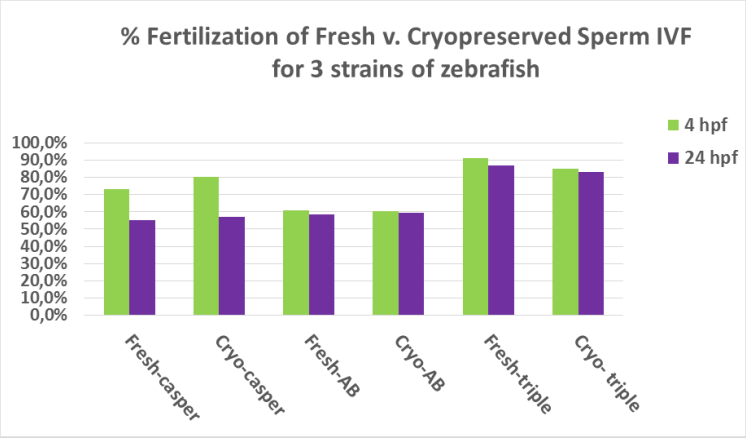


Figure 1:
Average fertilization percentage within strains casper, AB and triple using fresh and cryopreserved zebrafish sperm. Fertilization was estimated at 4h and 24h post-fertilization and showed no significant differences between sperm treatment.

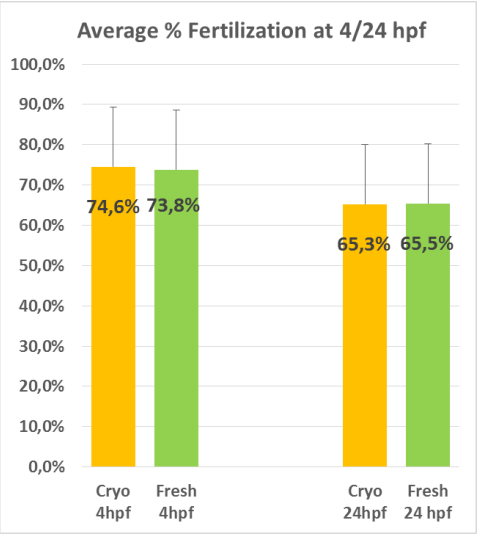


Figure 2:
Comparison of average fertilization percentage using fresh and cryopreserved zebrafish sperm (all strains combined). Fertilization was estimated at 4h and 24h post-fertilization and showed no significant difference between sperm treatment.

cryogenetics

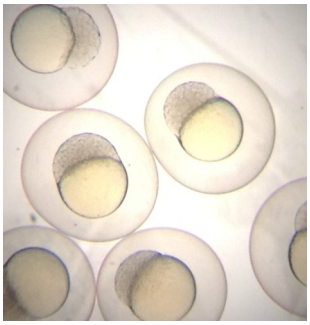
DID YOU KNOW WE OFFER IVF TRAINING AND CONSULTANCY AS A SERVICE FOR YOU?

Would you like to build staff competency in IVF at your laboratory?

IVF is an important tool to have when fish aren't breeding or when lines need to be regenerated from frozen sperm samples.

With our expertise in fish reproduction, we can offer our full support through consultancy and training.
This will enable your lab to develop the skill set needed to be confident in line propagation using fresh or cryopreserved samples.

AquaBoost® OvaCoat is a very useful tool for succesful IVF



Fertilized Zebrafish embryos

WE ALSO PROVIDE OFF-SITE STORAGE OF YOUR ALREADY EXISTING CRYOPRESERVED SAMPLES

Some of our clients already have cryopreserved samples stored in their own facility. To have a failsafe back-up program it is recommended to split storage and have samples stored at a secondary off-site location. Cryogenetics offers a secure storage space with a high attention given to biosecurity.
This includes daily inspection and regular nitrogen addition to storage tanks.

For prices and more information please contact us:

Carrie Carmichael
carrie.r.carmichael@cryogenetics.com
Tel:+1 (541) 729-9143

Katarina Nordtun Ruud
katarina.ruud@cryogenetics.com
Tel:+47 952 18 241

www.cryogenetics.com/zebrafish