charles river

Webinar Summary

Dr. Philip Damiani discussed the key aspects to consider for reliable and scalable cryopreservation solutions, including the advantages of embryo and sperm cryopreservation. He also outlined best practices to help reduce risk and secure genetic lines while saving time and money, and supporting the 3Rs.

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When setting up a cryofreezing unit from scratch, how many staff would be needed/timeline and what training courses would be best?

The number of staff needed is a factor on the type of cryopreservation that you are trying to accomplish. For example, if you are only trying to cryopreserve sperm, you need one or two people to accomplish this. You can collect the male reproductive tract and process the sample using one person. The training for sperm cryopreservation is fairly easy and there are great protocols with videos that show the individual steps.

What are the specific parameters to call a IVOS analysis of thawed frozen sperm pass/fail?

This is generally set by your laboratory. Parameters can differ depending on your live born rates following embryo transfer. For example, if you have a lower live born rate, more embryos are required to be transferred to reach your objective. That will influence the sperm parameters to ensure you can generate enough embryos following IVF.

In our laboratory, these are the following parameters:

- For fresh assessment during IVF, the parameters set for a passing sample are a minimum of 10 million sperm/ml for concentration, 40% progressive motile and 30% rapid motility.
- For frozen assessment during IVF, the parameters set for a passing sample are the minimum of 10 million sperm/ml for concentration, 15% progressive motile and 10% rapid motility. We also have the option of using laser IVF for a sample if the parameters are lower. We generally give a timeline of about 5-6 months for an embryo tech to be fully competent in all skills of embryology.

What synchronization protocol do you recommend for collecting embyros?

We use PMSG (5IU) followed by HCG (5IU) 48 hours post PMSG. We use animals between 3-5 weeks of age for most strains. However, we make modifications to the superovulation protocols based on some specific strains like BALB/c or 129s.

Click here to learn more about our rat and mouse cryopreservation services

In a 2006 paper published by RIKEN, they were able to demonstrate regeneration of a line from sperm recovered by whole bodies that was stored at 20 °C for 16 years. As an emergency backup, have you ever consulted on its viability?

We have not attempted this directly, however, we have been able to generate cell lines from animals that were deceased and placed in a freezer for long-term storage. It could be used as an alternative option collecting sperm or spermatogonia; however, the motility will not be present and will therefore require advanced assisted reproductive techniques, such as intracytoplasmic sperm injections (ICSI), for fertilization.

Should oocytes be cryopreserved? Do you think this is sufficient?

There are currently some protocols for cryopreservation of oocytes with different success rates depending on specific methods. I would not recommend that as your only option of cryopreserving material, as it requires skill in vitrification and you may potentially lose your line if you aren't highly skilled.

Usually I freeze at least 500 embryos to consider a line cryopreserved since about 20% of them become blastocysts when thawed and cultured. Do you think this is sufficient?

If you have an inbred strain, that is sufficient to reconstitute the line as you would expect a large number of offspring, even with a 20% live born rate. If this was an outbred, you may need to increase this number closer to 1,000. Ideally, you should try to reconstitute a minimum of 25 unrelated breeding pairs, but 50 is the best number when working with an outbred stock.

During this pandemic, we have chosen to freeze sperm by CARD protocol. I'm freezing 10 straws of each line. In terms of security, do you think it is safe to recover the line after?

In regard to the number of straws, 10 should be sufficient if the material has decent total sperm concentration and motility. Depending on the number of offspring required after being thawed, the material you should be able to generate sufficient number of offspring from just one straw of sperm. You should be able to fertilize a few plates with one thawed straw.

Do sperm-freezing protocols for non-B6 strains differ?

No, the current sperm protocols should work for a majority of mouse strains.

Have you tried using AI to test the quality of cryopreserved sperm?

Yes; the procedure required a surgical deposit of the cryopreserved/thawed sperm into the oviduct due to the low motility post thaw. The surgery was conducted like a standard ET procedure. I have not tried a transvaginal AI with post-thawed sperm.

What are you using to hold straws?

We are currently using CBS visotubes and goblets for their Daisy system. We find this system ideal for inventory and its ability to hold the straws in a method that allows for easy access and recovery.

Do you think that estrous stage control is necessary before superovulation?

We generally use prepuberal animals so they haven't entered a estrous cycle. This allow us to have a larger yield of oocytes/embryos per collection. We generally do not use postpuberal animals like you typically do with other species.

Do you have advice for cryopreserving rat lines?

There are protocols for rat sperm cryopreservation and just recently, a new protocol has been published which we are actively evaluating. For most rat lines we are currently recommending embryo cryopreservation.

Does the method of cryopreservation (rapid or slow) matter when revitalizing the line?

Yes. The survivability is higher using vitrification than controlled-rate cryopreservation. This is generally the best method; however, you need to be able to work very quickly with the material. As I mentioned, the cryoprotectant is at such a high concentration that it can be toxic if the embryos are exposed for too long. Most vendors offer straws using controlled-rate cryopreservation.