

Transporting frozen sperm on dry-ice

Introduction

It is essential that embryos frozen using the two-step method are transported at LN₂ temperatures otherwise their viability will be severely compromised. However, it is possible to transport frozen sperm on dry-ice between laboratories. These parcels are fully disposable removing the need for the receiving facility to return a dry-shipper. It is also a safer laboratory practice as there is a reduced need to handle LN₂ (Okamoto et al., 2001).

This protocol has been validated using each of the common sperm freezing protocols (Nakagata et al, 2003; Ostermeier et al, 2008; Takeo & Nakagata, 2010)

Materials

Sperm samples frozen using conventional techniques

Dry-ice

Insulating polystyrene box (e.g., Air-Sea, Code: 800)

Cassette for holding sperm straws (e.g., Hunter Scientific: 16980/0601)

Preparing dry-ice package and samples for shipping

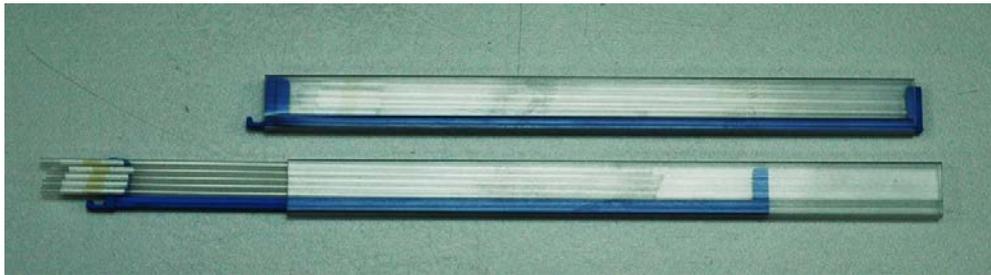
1. A large insulated polystyrene box suitable for holding dry ice (e.g., Air-Sea, Code: 800) will be required to prevent the dry-ice from completely evaporating during transport.

Dry-ice slowly evaporates (~45g/hour at 30°C) so it is important to accurately gauge the amount of dry-ice that will be required to during the expected journey time. A box holding 4Kg of dry-ice will keep the samples frozen for appropriate 3 days. Larger boxes containing 10 to 20Kg of dry-ice may be needed when sending samples between continents.

Any delay in shipment could potentially cause damage to the samples because the dry-ice levels may drop below a critical level, so ensure your package holds sufficient dry-ice for twice the expected journey time. Or ask the shipping agent to top up your package, if they can.

2. Locate the frozen samples that are going to be exported and transfer them to a small LN₂ dewar.
3. Cryo-vials can be buried directly in the dry-ice but it is advisable to load the straws into a cassette or similar container, which will hold the samples in place during transit preventing damage or difficulty finding the straws following shipment (Fig. 1).

When transporting straws it is important to submerge the cassette in LN₂ to cool it and then load the straws, making sure the straws and the cassette are always in contact with LN₂.



4. Quickly transfer the sperm samples to a pre-prepared box that has been half-filled with dry-ice. Then cover with dry-ice and seal the lid of the box with packing tapes and add the appropriate shipping labels
5. Send the samples via a regular delivery service.
6. When the dry-ice package arrives at its destination the recipient should retrieve the sperm samples using a pair of pre-cooled forceps, and then quickly transfer the samples to a dewar filled with LN₂.

Note: On arrival the sperm samples can either be thawed directly for an IVF recovery or they can be returned to LN₂ storage and used at a later date.

Anticipated results

Frozen sperm can remain viable for a prolonged period when held on dry-ice. In fact, storage on dry-ice for 730 days has been reported (M. Raspa: personnel communication) without an associated decline in fertility. Even though sperm viability may be strain/male dependent, a competent IVF laboratory should be able to achieve >40% fertilisation rates when using sperm transported on dry-ice.

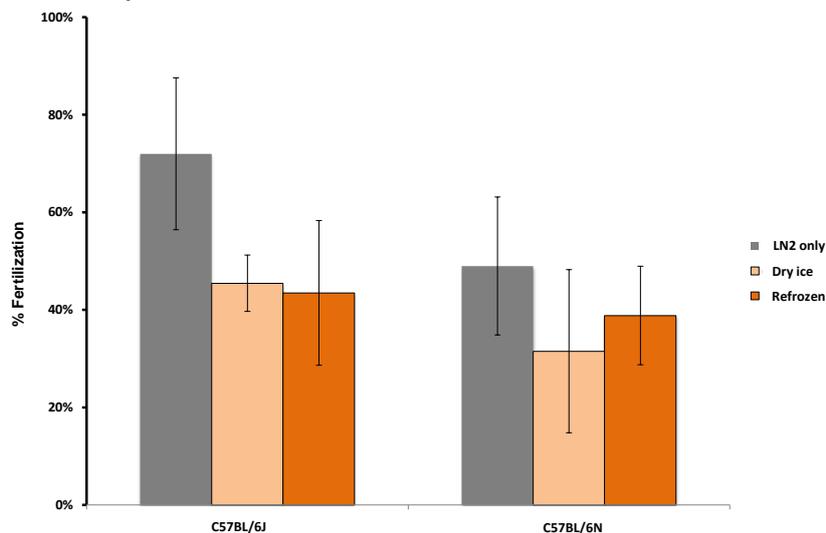


Figure showing IVF fertilisation rates performed using frozen sperm held a) in LN₂ only or b) on dry-ice for 3 days or c) returned to LN₂ storage after being held for 3 days on dry-ice. The experiment was run using two different mouse backgrounds C57BL6/J and C57BL/6N. The sperm was frozen using a standard method comprising 18% raffinose, 3% skimmed milk supplemented with 477µM mono-thioglycerol.