



Shipping Refrigerated Spermatozoa

Introduction

A technique for transporting fresh sperm within the epididymis at refrigerated temperatures (4-8 °C) has been developed in recent years. This technique does not require the use of a dry shipper. The samples can be shipped by a conventional low cost delivery service. What is more, the cold package kit doesn't need to be returned. On arrival the sperm can be quickly extracted from the epididymides and used in a regular IVF protocol to generate embryos for archiving/embryo transfer. Alternatively, the sperm can be cryopreserved and used at a later date. This protocol is based on the work published in Takeo et al., (2012 & 2014).

A. Dissection of epididymides

1. The selected male should be at least 10 weeks old, and not have been used for mating for at least 3 days before sperm collection.
2. Cull the male and swab the abdomen with 70% alcohol.
3. Cut through the abdominal skin, and then cut through the body wall, to reveal the internal organs.
4. Dissect the cauda epididymides from the mouse (Fig. 1).

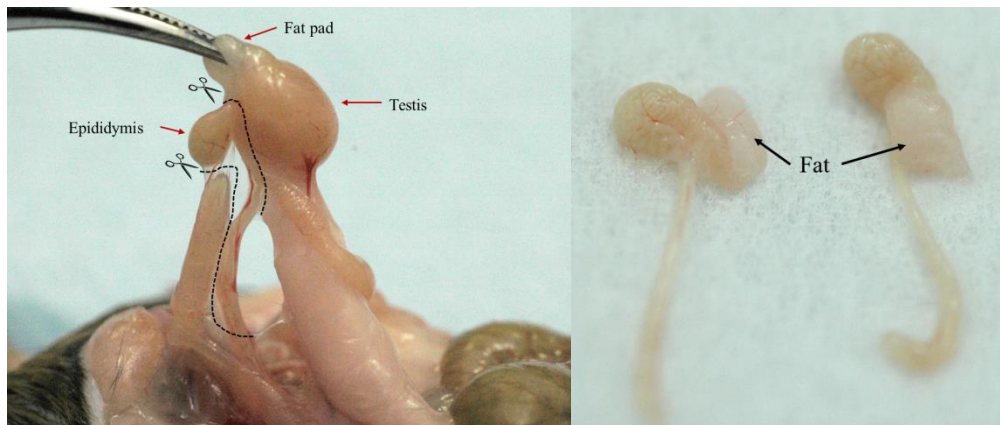


Fig. 1

5. Fill the 1.5ml Eppendorf tube with 1.5ml Lifer preservation medium, supplemented with 10 μ M Sphingosine 1-Phosphate (S1P), at room temperature.
6. Load the epididymides into the microfuge tube and seal the tube with parafilm.

B. Preparing the refrigerated package

1. Place the tube containing the epididymides into the biotube which is supplied within the cold transportation kit (Fig. 2).



Fig. 2 Biotube

NOTE: It is possible to also include an i-button in the biotube to log the temperature of the package during the shipment.

2. Place the biotube into an aluminium lined box (room temperature) (Fig. 3a), then place two gel cool packs (room temperature) into the aluminium box so they surround the biotube (Fig. 3b).



Fig. 3a Aluminium lined box

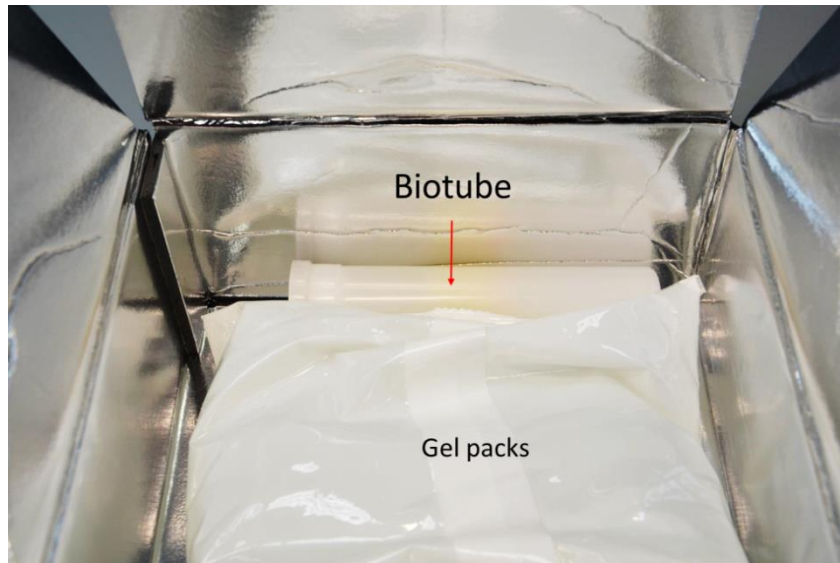


Fig. 3b Inside of the aluminium lined box

3. Seal the lining box with sellotape.
4. Place the aluminium lined box into the polystyrene container following the assembly instructions supplied with the container. Then seal the polystyrene box with packing tape (Fig. 4). This thermal control unit will maintain a temperature of 4-8°C for up to 72hrs (Figure 5). The sperm will maintain its fertility for at least 72hrs under these conditions.

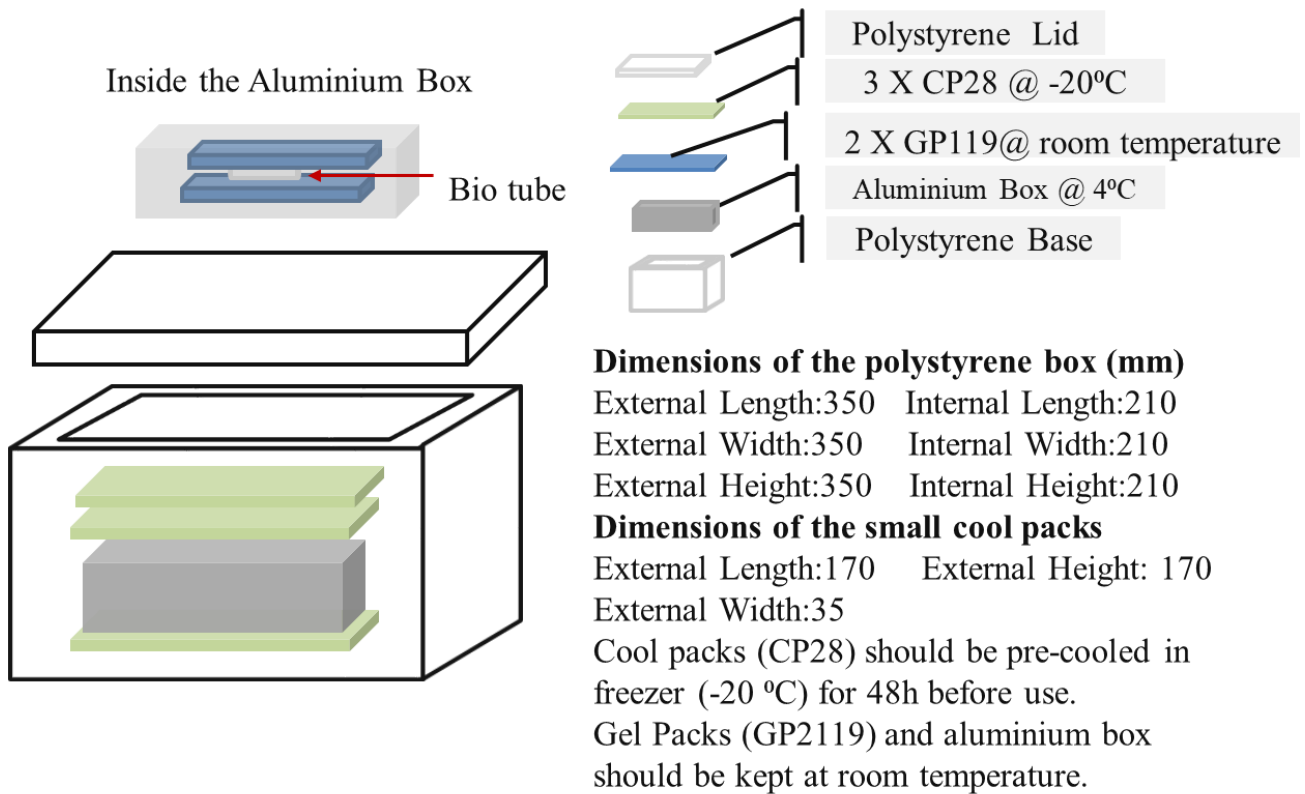


Fig. 4 Cold package assembly instructions

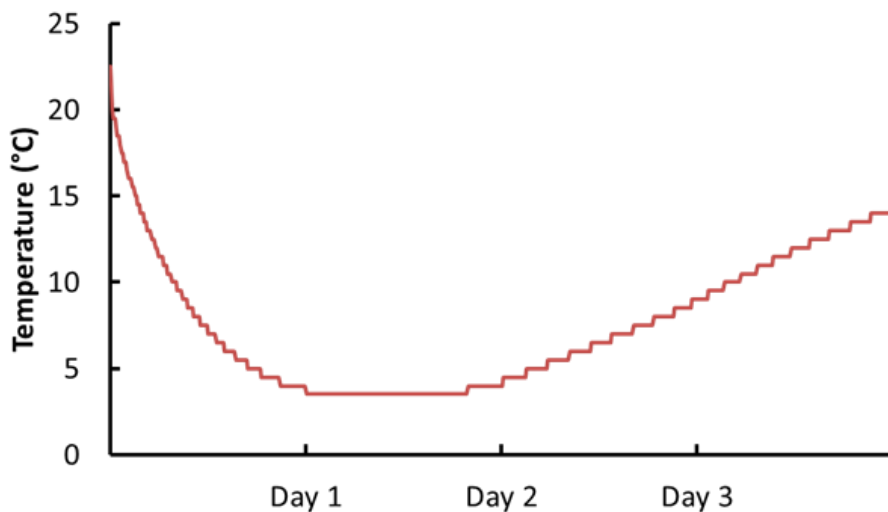


Fig. 5 Temperature profile of the cold package during transportation

5. Send the samples to the client via a standard delivery services.



C. References

Takeo T, Fukumoto K, Kondo T, Haruguchi Y, Takeshita Y, Nakamuta Y, Tsuchiyama S, Yoshimoto H, Shimizu N, Li MW, Kinchen K, Vallelunga J, Kent Lloyd KC, Nakagata N. Investigations of motility and fertilization potential in thawed cryopreserved mouse sperm from cold-stored epididymides. *Cryobiology*. 2013 Nov 4. pii: S0011-2240(13)00394-5. doi: 10.1016/j.cryobiol.2013.10.007.

Takeo T, Tsutsumi A, Omaru T, Fukumoto K, Haruguchi Y, Kondo T, Nakamuta Y, Takeshita Y, Matsunaga H, Tsuchiyama S, Sakoh K, Nakao S, Yoshimoto H, Shimizu N, Nakagata N. Establishment of a transport system for mouse epididymal sperm at refrigerated temperatures. *Cryobiology*. 2012 Dec;65(3):163-8