Mice Sperm Cold Transportation

Introduction

In recent years, techniques for transporting fresh sperm within the epididymis at refrigerated temperatures (4-8°C) has been developed that do 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 and the sperm can be used in any standard *in vitro* fertilisation (IVF) procedure to produce embryos for freezing or subsequent embryo transfer (Takeo et al, 2012).

1. Media

1.1. LiFor preservation medium (Lifor; Lifeblood Medical Inc, NJ, USA)

1.2. Sphingosine-1-phosphate (S1P; Cambridge Bioscience, S6130)

1.3. 70% ethanol

2. Animals

Males over 12 weeks of age

3. Equipment

3.1. Microfuge tube with attached flat cap polypropylene 0.2ml Brand (Fisher Scientific, TUP-114-010Q)

3.2. Paper tissues

3.3. Parafilm

3.4. Dissecting microscope

3.5. Dissecting instruments e.g. standard dissecting forceps, fine watchmakers forceps and scissors.

3.6. Laboratory timers

3.7. Code 570 Biotransporter boxes (Air Sea Containers)

3.8. Ice packs (Fisher Scientific, ICE-910-020W)

3.9. ThermoCafé by Thermos 0.5 Litre Flask (Argos, 927/0518)

3.10. Maxim Integrated Products iButton Thermochron F5 (Digi-Key Corporation, DS1921G-F5#-ND)

3.11. 7.0ml Bijou tube or similar container

4. Protocol steps

4.1. The selected male should be at least 8 weeks old, and not have been used for mating for at least 3 days before sperm collection.

4.2. Sacrifice the male and swab the abdomen with 70% alcohol.

4.3. Cut through the abdominal skin, and then cut through the body wall, to reveal the internal organs.

4.4. Dissect the vasa deferentia and cauda epididymides from the mouse.

4.5. Fill the 0.2ml microfuge tube with 0.2ml LiFor preservation medium, supplemented with 10μM S1P (at room temperature). Then load the epididymides into microfuge tube and seal the tube with parafilm.

4.6. Place the tube containing the embryos into a 7ml Bijou or similar container.

4.7. Insert two small cold packs (Figure 1) and a thermo iButton (Figure 2) into a small thermos flask (Figure 3), and then insert the Bijou containing the epididymides and close the cap.

Figure 1 Small ice pack for inserting into thermos flask (14.5cm X 4.0cm x 2.0cm)

Figure 2 Thermo iButton and 0.2ml microfuge tube

Figure 3 Thermos Flask

4.8. Place the thermos flask into polystyrene box following the assembly instructions (Figure 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.

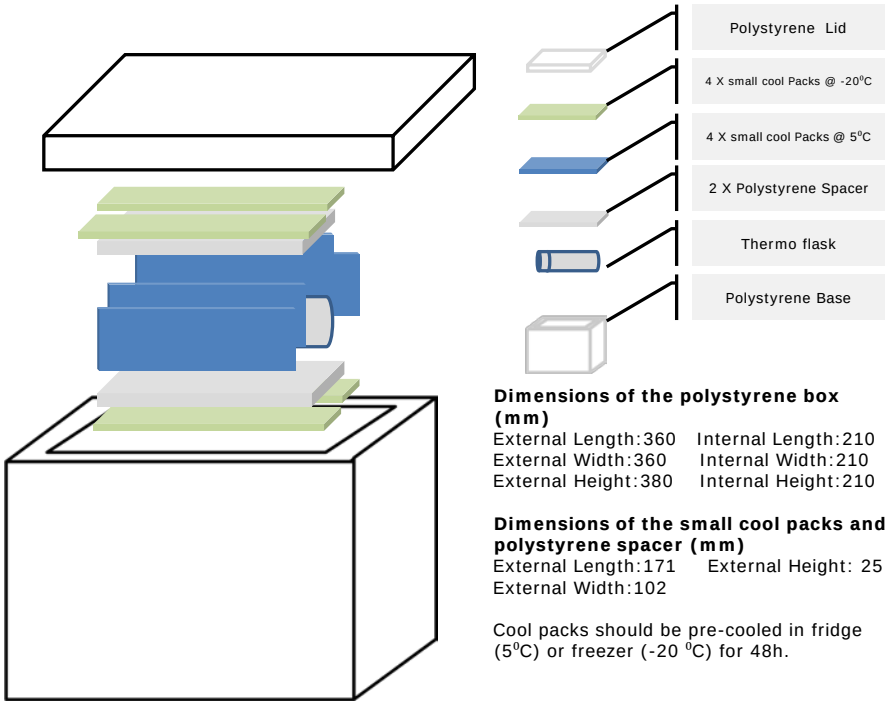


Figure 4 Cold package assembling instructions

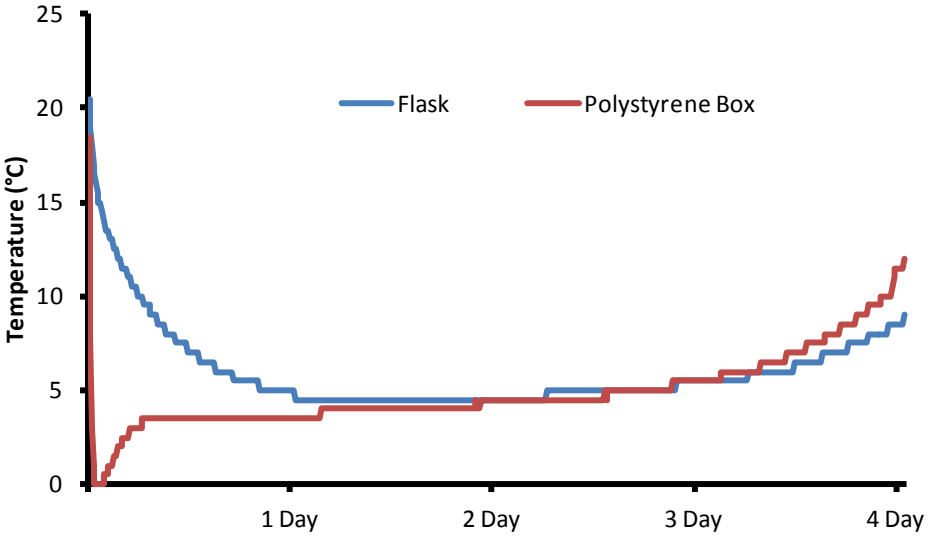


Figure 5 Temperature profile of the cold package during transportation

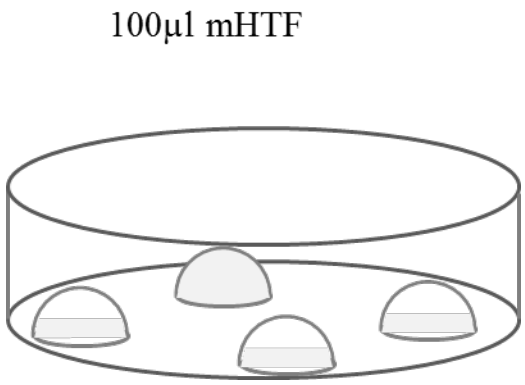
4.9. Send the samples to the client via a standard delivery services.

4.10. On arrival the recipient should remove the Bijou from the thermos flask, open the container and recover the cauda epididymides.

4.11. The cauda epididymides should be wiped free of any Lifor solution using paper tissue.

4.12. Wash the cauda epididymis through 3 x drops of IVF media e.g. human tubal fluid [mHTF]). After washing, wipe away any excess media using a paper tissue.

Comment: To make a washing dish, put 4 drops (100ul) IVF media into a 35mm Petri Dish (Falcon 351008). There is no need to overly with mineral oil



4.13. Clean off all adipose and vascular tissue. This is best achieved by placing the organs on a paper tissue and examining them under a dissecting microscope lit from above.

4.14. The sperm can be used for either sperm freezing or IVF. INFRAFRONTIER protocols are recommended as the standard IVF and sperm freezing procedure (https://www.infrafrontier.eu/knowledgebase/protocols/cryopreservation-protocols.)

5. References

5.1. 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.

5.2. 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