Shipping Refrigerated Embryos

Introduction

The ability to transport unfrozen embryos over long distances has been recognised for many years although it has not become common practice and most laboratories have preferred to transport frozen embryos at LN₂ temperature. Recently, there has been a resurgence of interest in transporting embryos in the absence of LN₂ which has been focused on simplifying the exchange of mutant mouse strains. Of particular relevance to the mouse community is the ability to transport unfrozen embryos that had previously been cryopreserved. This allows laboratories that don’t have access to LN₂ or are not familiar with handling frozen embryos to take advantage of the vast numbers of mouse strains held in archives around the world without resorting to live animal transportation (Takeo et al., 2009; Takeo et al., 2010).

Two important observations underpin the success of the protocol described in the following text. Firstly, a period of *in vitro* culture before transportation promotes embryo viability and secondly, the temperature gradient used to cool the embryos prior to transportation is important for a successful outcome.

1. Media
   1.1. M2 Medium
   1.2. KSOM (e.g., Millipore/Chemicon: MR-020P-5F)
   1.3. MEM Amino Acids Solution (50X) (e.g., Life Technologies: 11130036)
   1.4. MEM Non-essential Amino Acid Solution (100X) (e.g., Sigma: M7145-100ML)

2. Equipment
   2.1. Microtube thermo hinged flat cap natural 0.5ml Thermo Scientific Abgene (e.g., Fisher: TUL-952-010W)
   2.2. Parafilm
   2.3. Dissecting microscope
   2.4. Laboratory timers
   2.5. Embryos Biotransporter Kit (Air Sea Containers: B/0472)

3. Protocol steps
   3.1. Thaw the embryos following an appropriate protocol
3.2. Transfer embryos into a 200µl drop of KSOM, plus amino acids and culture for 2-3hrs in a CO2 incubator at 37°C.

3.3. Make 3 X 150µl M2 drops in a culture dish.

3.4. After the embryos have been cultured in the incubator wash them through the 3 drops of M2.

3.5. Fill 0.5ml microfuge tube with 0.6ml M2 medium at room temperature, then load 30-40 embryos into each microfuge tube and sealed with parafilm (Fig 1).

![Fig. 1](image1.jpg)

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

![Fig. 2](image2.jpg)

3.7. Place the biotube into the aluminium lined box (Room Temperature) (Figure 3a), then place two gel cool packs (Room Temperature) into the lined box, so they surround the biotube (Figure 3b).
3.8. Seal the aluminium box with sellotape.

3.9. Then place the aluminium box into the polystyrene container following the assembly instructions. Then seal the polystyrene container with packing tape (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.
3.10. Send the samples via a regular delivery services.

3.11. When the cold package arrived, remove biotube from aluminium box, and remove the microfuge tube containing embryos from the biotube.

3.12. Allow tube to stand vertically at room temperature for 30mins avoiding directly exposure to light.

3.13. Open the microfuge tube containing the embryos, then gently re-suspend the embryos and aspirate the entire M2 medium from the tube using 1000µl pipette, and then transfer the solution to the centre of a culture dish.

3.14. Locate the embryos and transfer them to a second 200µl drop of fresh M2 medium.
3.15. The embryos are now ready for use and may be transferred into recipient females or in KSOM, plus amino acids until required.

4. Experimental results

Table 1 In vitro development of frozen/thawed 2-cell C57BL/6NTac embryos after being held for 24, 48 and 72 hours in M2 medium at 4-8 °C. The data represent the total number of embryos tested over 3 replicate experiments.

<table>
<thead>
<tr>
<th>Duration held at 8°C</th>
<th>No. embryos tested</th>
<th>No. developed into blastocysts</th>
<th>Mean blastocyst development rate (%)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>85</td>
<td>87.33</td>
<td>5.98</td>
</tr>
<tr>
<td>24hrs</td>
<td>94</td>
<td>79</td>
<td>84.35</td>
<td>2.65</td>
</tr>
<tr>
<td>48hrs</td>
<td>93</td>
<td>77</td>
<td>85.42</td>
<td>4.81</td>
</tr>
<tr>
<td>72hrs</td>
<td>95</td>
<td>58</td>
<td>66.99</td>
<td>20.05</td>
</tr>
</tbody>
</table>

Table 2 In vivo development of frozen/thawed 2-cell C57BL/6NTac embryos after being held for 24, 48 and 72 hours in M2 medium at 4-8 °C. The data represent the total number of embryos tested over 3 replicate experiments.

<table>
<thead>
<tr>
<th>Duration held at 8°C</th>
<th>No. embryos transferred</th>
<th>No. offspring produced</th>
<th>Mean Birth Rate (%)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>108</td>
<td>40</td>
<td>37.04</td>
<td>10.32</td>
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<tr>
<td>24hrs</td>
<td>108</td>
<td>51</td>
<td>47.22</td>
<td>8.50</td>
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<td>48hrs</td>
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<td>36</td>
<td>33.33</td>
<td>13.14</td>
</tr>
<tr>
<td>72hrs</td>
<td>108</td>
<td>32</td>
<td>29.63</td>
<td>13.08</td>
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</table>

5. Reference
