Successful transport of frozen cattle embryos from New Zealand to Australia

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Several laboratories have reported the birth of young from frozen-thawed mouse embryos transferred to recipient females (Whittingham, Leibo & Mazur, 1972; Wilmot, 1972) and frozen mouse embryos have been successfully transported from North America to the United Kingdom (Whittingham & Whitten, 1974). Of the domestic species, continued development in vivo and in vitro has been achieved with cattle (Wilmot & Rowson, 1973; Bilton & Moore, 1976a), sheep (Moore & Bilton, 1976; Willadsen, Polge, Rowson, & Moor, 1976) and goat (Bilton & Moore, 1976b) embryos stored in liquid N₂, and the potential value of frozen embryos has been noted.

During March 1976, cattle embryos were collected at Christchurch, New Zealand, from mature parous cows of mixed dairy breeds 7–8 days after A.I. with semen from a Simmental bull. All embryos were morulae or early blastocysts and after collection they were frozen in Dulbecco's phosphate buffer enriched with 25% bovine blood serum (DB+S) and containing 1·5 m-dimethylsulphoxide (DMSO) or 1·0 m-glycerol. DMSO was added at 30°C and the embryos were moved through a series of dishes containing increasing concentrations of DMSO (0·25, 0·5, 0·75, 1·0, 1·25, 1·5m) in DB+S, remaining in each dish for 4·5 min. Glycerol, when used, was added at 37°C by a more direct procedure. DB+S containing 2·0 m-glycerol was added over a period of 20 min to the embryos held in an equal volume of DB+S devoid of glycerol. After the addition of DMSO or glycerol, the embryos together with 2–3 ml of their respective medium were transferred to Pyrex glass freezing tubes (75 × 10 mm) and cooled to 0°C at a rate of 0·7°C/min. The tubes were then cooled to −50°C at rates of 0·13 or 0·3°C/min and crystallization was initiated at −3°C by the addition of crystals of frozen DB+S. Once the tubes had reached −50°C they were cooled more rapidly (1°C/min) to −105°C and then transferred to liquid N₂. The embryos were then transported in liquid N₂ containers by air to Sydney, Australia, and then by road (120 km) to Mittagong. After storage in liquid N₂ for 2–3 months the embryos were thawed as described by Bilton & Moore (1976a) at one of three rates (Table 1) measured over the range −50°C to 0°C. The tubes were then warmed to 30°C (0·7°C/min) and the DMSO and glycerol were removed by dilution. DMSO was removed by the reverse of the procedures used for its addition, whilst glycerol was diluted by the slow addition over 15 min of three volumes of DB+S. After dilution the embryos were washed twice in fresh DB+S and then cultured in DB+S at 37·5°C for 12 h (Bilton & Moore, 1976a).

The embryos were thawed in two batches. In the first, 11 embryos stored in DMSO were thawed but none showed any development in culture. It was subsequently found that the serum used in the dilution procedures and culture medium was at fault: DB+S prepared from this serum failed to

<table>
<thead>
<tr>
<th>Cryoprotectant</th>
<th>Freezing Rate (°C/min)</th>
<th>Thawing Rate (°C/min)</th>
<th>Stored and cultured</th>
<th>Developed in culture</th>
<th>Transferred to recipients</th>
<th>Developed to calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO (1·5 m)</td>
<td>0·13</td>
<td>1·2</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0·13</td>
<td>2·2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0·30</td>
<td>4·6</td>
<td>6</td>
<td>2</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Glycerol (1·0 m)</td>
<td>0·13</td>
<td>1·2</td>
<td>9</td>
<td>6</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

* Measured over the range 0°C to −50°C.
support development in culture of freshly collected Day-7 cattle embryos. In the second thawing, 24 embryos were thawed and cultured in medium containing serum known to support development in culture. Ten of the 24 embryos developed in culture to expanded blastocysts, the remaining 14 showing little or no development. The 10 developed embryos and 6 of those which showed little or no development were transferred to the uterine horns of 11 recipient cows which had been in oestrus 7½–8½ days earlier. Nine recipients each received one embryo which had developed in culture, one received one embryo which had and two which had not developed after freezing in glycerol, and the remaining cow received four embryos which had failed to develop following freezing in DMSO (Table 1). Uterine palpation per rectum at 8 and 12 weeks and 5 months after transfer indicated that 5 of the 11 recipients were each carrying a single fetus and they subsequently gave birth to 5 live and apparently normal calves. All five had received one embryo which had developed in culture.

The experiment clearly shows that frozen cattle embryos can be transported over considerable distances without marked loss of viability. However, too few embryos were involved to make valid comparisons between different freezing and thawing rates, or between the effectiveness of the two cryoprotectants used, but the overall success rate (5/24 embryos) compares quite well with the rates reported for mice and sheep.

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References


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