Survival of Mouse Embryos after Freezing in Medium Containing Dimethylsulphoxide and Yolk Extract

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ABSTRACT

The ability of the cryoprotective solution containing dimethylsulphoxide in combination with yolk extract (DYG 550) to protect mouse embryos at ultra low temperatures is reported. Of the embryos 46.1% frozen to -196 C at various rates formed blastocysts in vitro compared to 41.2% and 23.2% survivals protected by 1M dimethylsulphoxide and 1M glycerol.

INTRODUCTION

Long preservation of mammalian embryos can be accomplished by freezing and storage at ultra low temperatures where cellular functions are reduced greatly or suspended. Rabbit embryos frozen to -79 C in saline containing 10% glycerol developed into viable young after transfer (1). In vitro and in uteri development of eight-celled mouse embryos previously frozen and stored for a short period at -79 C in modified phosphate buffered salt solution with 7.5% polyvinylpyrrolidone (PVP) as the cryoprotective agent was also successful (7). Mouse embryos survived long storage at -196 C when cooled slowly in a medium containing dimethylsulfoxide (DMSO) or glycerol as the cryoprotective agent (4). The use of yolk extract in combination with DMSO and glycerol (DYG 352) was adequate for preservation of two cell mouse embryos (4). Various ratios of DMSO to glycerol were tested with progressively increasing survival in media containing a higher proportion of DMSO. The purpose of this study was to evaluate the effect of a freezing medium containing yolk extract upon survival of eight-cell stage mouse embryos.

MATERIALS AND METHODS

Embryos were from random bred Swiss-Webster albino mice which were superovulated by the method in (3). On the second morning following the vaginal plug identification, females were killed by cervical dislocation, and the embryos were recovered by flushing the excised reproductive tracts with Brinsters medium BMOC-3 (Grand Island Biological Company). Eight-cell stage embryos were pooled and washed in Dulbecco's phosphate-buffered salt solution. PBS (Grand Island Biological Company). Groups of 25 embryos in .001 ml of PBS were pipetted into tubes 50 x 6 mm which in the case of DMSO and glycerol contained .1 ml of PBS and in the case of DYG 550 contained .1 ml of yolk extract. The cooling, thawing, and washing procedures were basically those reported by Whittingham et al. (8). Volumes of .1 ml of the following cryoprotective solutions were added at 0 C into each tube:

1. 2M DMSO in PBS
2. 2M Glycerol in PBS
3. 2M DMSO in yolk extract

Yolk extract was prepared from the equal parts of PBS and fresh hen's egg yolk, which were mixed thoroughly, centrifuged at 5,000 rpm (RCF 3,015) for 15 min, and millipore filtered (AA .8μ-Millipore, Bedford, MA, USA). Sodium bicarbonate was used for adjusting pH between 7.3 to 7.4. Split samples in three solutions were cooled to -196 C by four cooling rates .5, 1, 2, and 5 C/min. Cooling was in liquid nitrogen vapor in Linde freezing apparatus with or without the small alcohol bath depending on the cooling rate. After the temperature had reached -80 C, the cooling rate was increased to approximately 10 C/min. At -196 C the tubes were transferred directly into a liquid nitrogen tank, stored there for 3 days, and then thawed in air room temperature (approx. rate of 25 C/min). After dilution and washing, the
recovered embryos were incubated in Brinster's medium (BMOC-3) in an embryonic watch glass in a French square bottle in an atmosphere of 5% CO₂ and 95% air. Survival was defined as the number of blastocysts which developed from the embryos recovered after washing (Fig. 1). Each interaction of cryoprotective solution and temperature was tested on four groups of 50 embryos per group. The arcsin transformed data were analyzed by analysis of variance (2). The groups of three transformed individual means at .5, 1, 2, and 5 C/min cooling rates were compared by Tukey's test (6).

RESULTS AND CONCLUSIONS

The number of embryos recovered after treatment was 92% of those frozen. At .5 C/min cooling rate 133 blastocysts developed from 184 embryos as compared to 121 blastocysts formed by 185 embryos protected by DMSO only. At 1 C/min cooling rate 109 out of 179 embryos survived freezing in DYG 550 compared to 102 blastocysts out of 186 embryos recovered after freezing with 1M DMSO in PBS. Freezing at 2 C/min yielded similar

TABLE 1. Variance analysis of the survival of mouse embryos (arcsin transformation).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicates</td>
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<td>.0070</td>
</tr>
<tr>
<td>Cryoprotective solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DYG 550 = A, DMSO = B, GLYC = C</td>
<td>2</td>
<td>.7358***</td>
</tr>
<tr>
<td>A + B vs. C</td>
<td>1</td>
<td>.0266*</td>
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<tr>
<td>A vs. B</td>
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</tr>
<tr>
<td>Cooling rate</td>
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<td></td>
</tr>
<tr>
<td>Linear</td>
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<td>4.4849***</td>
</tr>
<tr>
<td>Quadratic</td>
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<td>.0095</td>
</tr>
<tr>
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<td>.0031</td>
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<tr>
<td>Cryoprotective solution × cooling rate</td>
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<td>.0433</td>
</tr>
<tr>
<td>Error</td>
<td>33</td>
<td>.0741</td>
</tr>
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</table>

*P=.05.

***P=.001.
survival values for embryos protected by DYG 550 and DMSO where approximately one-half of the recovered embryos formed blastocysts in vitro (Fig. 2). Glycerol provided some protection for mouse embryos; however, only 47.5% and 39.4% formed blastocysts after cooling at .5 C and 1 C/min. Only 11 blastocysts developed from 193 embryos protected by 1M glycerol at 2 C/min cooling rate. The 5 C/min cooling rate yielded only a few survivals for DYG 550 and DMSO and was completely lethal when the embryos were protected by 1M glycerol. The results indicated the dependence for survival on the cooling rate (Table 1) and confirms the findings of other laboratories (5, 8, 9). The cryoprotective potencies of both solutions with DMSO expressed as survival percent at various cooling rates were significantly higher than those of glycerol, the last exhibiting a biphasic curve (Fig. 2). Tukey’s test on individual means at various cooling rates revealed no significant differences between DMSO and DYG 550 except at .5 C/min when the incorporation of yolk extract resulted in significantly more survival.

REFERENCES