A Simple Method for Freezing Bovine Embryos

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Deep freezing (cryopreservation) of bovine embryos provides an efficient economical method for storing embryos until suitable recipient cows (surrogate mothers) are available, or for transporting embryos to recipient cows at a distant location (e.g., exportation or importation of embryos between continents or geographic areas). Pregnancy rates achieved with frozen bovine embryos have increased steadily during the past five years and are approaching those obtained with fresh bovine embryos. However, acceptable pregnancy rates are only achieved when Grades 1 and 2 quality (excellent and good quality) embryos are frozen; survivability of Grade 3 (fair quality) embryos is considerably higher if the embryos are transferred fresh rather than frozen. A simplified method for freezing and thawing bovine embryos is described, and pregnancy rates obtained are compared with those for fresh embryos.

Procedure

Bovine embryos at the morulae and early to expanded blastocyst stages of development were obtained nonsurgically from superovulated donor cows 7.5 days after artificial insemination. After the embryos were located in the flush medium of 2% bovine serum albumin (BSA) in Dulbecco’s phosphate buffered saline (PBS), they were transferred with a micropipette into a holding medium of 4% BSA in modified PBS (PBS-BSA), held at 75°F for 1-2 hr until all embryos were located and evaluated for quality. Subsequently, the Grades 1 and 2 embryos were transferred to the cryoprotective freezing medium of 1.5 M glycerol (11% v/v) in PBS-BSA, allowed to equilibrate for 5 min at room temperature (~75°F), and then each embryo was aspirated individually into a .25 cc plastic French semen straw in a small volume of freezing medium. Embryos were loaded one per straw with an air bubble at each end to restrict movement of the embryo within the straw as shown in Figure 1. The open end of the plastic straw was sealed with a heat sealer and the opposite end (plug end) was labelled with the identity of the embryo; heat sealing of the plugged end of the straw is optimal. The sealed straws were positioned vertically in a holding rack and submerged in a small volume of freezing medium. Embryos were loaded into a .25 cc plastic French semen straw and then each embryo was aspirated individually into a .25 cc plastic French semen straw containing the cryoprotective medium of 1.5M glycerol in PBS-BSA.

Figure 1—Schematic representation of an embryo loaded into a plastic French semen straw containing the cryoprotective medium of 1.5M glycerol in PBS-BSA.

Thawing of the embryos was achieved by holding the straw in air for 15 sec at room temperature (70°F to 75°F), followed by 30 sec in 98°F water. Both ends of the plastic straw were clipped off and the contents emptied into a small petri dish. Removal of the cryoprotectant (glycerol) was accomplished by step-wise dilution in sucrose. Upon recovery from the straw, the embryo was placed immediately in a thawing medium of .13 M glycerol and .69 M sucrose in PBS-BSA for 5-10 min at room temperature, transferred to a second thawing medium of .4 M sucrose in PBS-BSA for 5-10 min, and then rinsed twice in PBS-BSA medium. The embryo was subsequently loaded into a .25 cc plastic French semen straw and transferred nonsurgically into the uterine horn ipsilateral to the corpus luteum of a recipient cow at 7 to 8 days postestrus.

Results

Pregnancy rates for embryos transferred nonsurgically into recipient cows either fresh or after cryopreservation are presented in Table 1. Pregnancy rate was about 10% lower in recipient cows receiving deep-frozen embryos.
Table 1—Pregnancy rates for fresh and deep-frozen bovine embryos

<table>
<thead>
<tr>
<th>Status of Embryo</th>
<th>No. of Recipients</th>
<th>Pregnant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>87</td>
<td>49 (56.3)</td>
</tr>
<tr>
<td>Deep-frozen</td>
<td>50</td>
<td>23 (46.0)</td>
</tr>
</tbody>
</table>

*One embryo was transferred nonsurgically per recipient cow.

Embryos were suspended in a cryoprotectant, cooled to -33°F, and frozen and stored in liquid nitrogen.

Pregnancy rates obtained with deep-frozen (cryopreserved) embryos are about 10% lower than those obtained with fresh embryos. However, the lower fertility with deep-frozen embryos is compensated for with increased managerial, transportational, and marketing benefits or opportunities. The freezing and thawing procedures described in this report are simple, practical, and economical to perform and provide results comparable to those obtained with elaborate, complicated procedures.

Because of the previously noted differences in storage life between deep-frozen and fresh embryos, it is more economical and practical to transport deep-frozen embryos long distances. Likewise, the exportation or importation of genetic material or germ plasm among countries can be accomplished more economically by transporting deep-frozen embryos than live animals, provided proper embryo-washing and sanitation procedures are employed.

Conclusion

Pregnancy rates obtained with deep-frozen (cryopreserved) embryos are about 10% lower than those obtained with fresh embryos. However, the lower fertility with deep-frozen embryos is compensated for with increased managerial, transportational, and marketing benefits or opportunities. The freezing and thawing procedures described in this report are simple, practical, and economical to perform and provide results comparable to those obtained with elaborate, complicated procedures.