Cryopreservation of equine embryos

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Introduction

Embryo cryopreservation has been successfully used in human and bovine embryos for more than twenty years.

Embryo Cryopreservation

<table>
<thead>
<tr>
<th>Study</th>
<th>Size Range (µm)</th>
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<tbody>
<tr>
<td>Slade et al. 1985</td>
<td>&lt; 300</td>
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<td>Lascombes and Pashen 2000</td>
<td>300 - 720</td>
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<td>Eldridge-Panouska WD et al. 2005</td>
<td>300 - 720</td>
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<td>Choi et al. 2011</td>
<td>448 - 1268</td>
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<tr>
<td>Diaz et al. 2015</td>
<td>180 - 571</td>
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<tr>
<td>Sanchez et al. 2017</td>
<td>180 - 571</td>
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Relative size of embryos

- Human embryos: 140 µm to 2000 µm
- Bovine embryos: 160 µm to 2300 µm
- Equine embryos: 300 µm to 1800 µm

Overcome difficulties: collect embryos on day 6.

Commercial vitrification kits

- Agtech – Equine Vitrification Kit
- Bioniche, Syngro – Equine Vitrification Kit

Successful cryopreservation of expanded equine blastocysts

Large equine embryos (>300 um) need to be collapsed before cryopreservation.

Preimplantation Genetic Diagnosis (PGD) → Embryo Cryopreservation

2011 Choi et al.
Successful cryopreservation of expanded equine blastocysts

Flushng → Embryo biopsy

Large equine embryos (>300 um) need to be collapsed before cryopreservation.
Cryopreservation of equine embryos

Materials and Methods

- Using a microscope + micromanipulator
collapse embryo by removing as much blastocoele fluid as possible

- Immediately start vitrification process:
  - Vitrification Solution 1 (VS1) for 3 to 5 minutes
  - Vitrification Solution 2 (VS2) and load in hemistraw within 45"
  - Plunge in liquid nitrogen
  - Seal, label and store

- Store blastocoele fluid for PCR
Vitrification of large embryos (> 300 µm)

Necessary materials and equipment

- Inverted microscope with micromanipulation system
- Stereomicroscope
- Small volume (<10µl) pipette and sterile tips
- Hemistraw
- Vitrification solutions
- Liquid nitrogen

Trained operator !!!
Vitrification of large embryos (> 300 µm)

Necessary materials and equipment

- Hemistraw

Vitrification solutions

Vitrification solution (VS1) (Vajta et al.)
7.5% (v/v) ethylene glycol
7.5% (v/v) dimethylsulfoxide

Vitrification solution (VS2) (Vajta et al)
16.5% (v/v) EG
16.5% (v/v) DMSO
0.5 M sucrose

Vitrification of large embryos (> 300 µm)

Necessary materials and equipment

- Vitrification

Trained operator !!!

Previous experience in vitrification
If no experience, plan proper training with experienced professional

Collapsed vitrified embryo 1000 µm

Fresh embryo  
Post warming  
72 hours IVC (650 µm)

Collapsed vitrified embryo 750 µm

Fresh embryo  
Post warming  
24 hours IVC (1100 µm)
Cryopreservation of equine embryos is now possible, although this technique is not widely used in commercial programs. The actual technique requires expensive equipment and trained personnel. Embryos can be shipped to a different location for cryopreservation.