Title: EFFECTS OF A PROLONGED EQUILIBRATION-VITRIFICATION PROTOCOL ON DAY 5 AND DAY 6 BLASTOCYST DNA INTEGRITY

Objective: Adequate equilibration with cryoprotectants and dehydration before transfer into
liquid nitrogen is important for optimized vitrification of blastocyst. The objective of our study was to evaluate the effect of a 15 minutes equilibration - vitrification protocol versus conventional 4 minutes equilibration on day 5 and day 6 blastocysts.

**Design:** prospective in vitro study.

**Materials and Methods:** 32 day 5 and 35 day 6 blastocysts were vitrified using Irvine vitrification media and cryotip loading devices. In each group, blastocysts were exposed to equilibration media for either 4 minutes (day 5, n = 17) and day 6 (n = 22) or 15 minutes (day 5, n = 15) and day 6 (n = 13) before transferring to the vitrification media, loading and submerging into LN₂. After warming, all blastocysts were incubated in albumin enriched HTF media (10%) at 37°C for 4 h in 5% CO₂. Fresh day 5 (n = 14) and day 6 (n = 13) blastocysts were used as controls. All blastocysts were fixed in 4% formalin. Blastocysts were incubated with TUNEL stain for 1 h at 37°C to stain DNA damaged blastomeres. Blastocysts were mounted in Vectashield containing DAPI to counterstain all blastomeres and examined by confocal microscopy. DNA integrity index was assessed in each group as percentage of TUNEL⁻ve to total number of blastomeres.

**Results:** Significant improvement in DNA integrity index was seen in day 5 blastocysts following prolonged equilibration versus short equilibration-vitrification protocol (P <0.001). There was no significant difference between the 2 protocols in day 6 blastocysts vitrification. Compared to fresh controls, there was no difference in DNA integrity with day 5 prolonged equilibration-vitrification but this was significantly different in all other groups.

**Table 1.** DNA integrity indices in day 5 and day 6 blastocysts in the two protocols

<table>
<thead>
<tr>
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<th>Day 5 Blastocysts (n = 32)</th>
<th>Day 6 Blastocysts (n = 35)</th>
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<tbody>
<tr>
<td>DNA Integrity Index %</td>
<td>93.94 ± 6.64</td>
<td>77.61 ± 16.65</td>
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<td>Short equilibration (n=17)</td>
<td>Prolonged equilibration (n=13)</td>
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<tr>
<td>DNA Integrity Index %</td>
<td>84.36 ± 8.76</td>
<td>72.22 ± 11.44</td>
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<tr>
<td></td>
<td>Fresh (n=14)</td>
<td>Fresh (n=13)</td>
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**Conclusions:** Prolonged equilibration-vitrification protocol improves only day 5 blastocyst vitrification.

**Support:** None.

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