AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE  
64th Annual Meeting

Filename: 650555 
Submission Type: Scientific Abstract (Oral/Poster) 
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Abstract Category: 4. Cryopreservation and Frozen Embryo Transfer (SART) 
Abstract Topic: 5. Cryopreservation 
Poster presentation only? No 
Are you a fellow in training in an American Board of Obstetrics and Gynecology approved program leading to certification in Reproductive Endocrinology and Infertility? No
Data is submitted for a separate scientific abstract and video presentation: No

Awards: 
IRB Approval: No human subjects or human tissue was utilized in the studies described in the abstract.
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Accept Complete Responsibility: I accept complete responsibility for the data at the time of submission.

Title: EXTENT OF APOPTOSIS INDUCTION IN BIOPSIED BLASTOCYST FOLLOWING SLOW CRYOPRESERVATION AND VITRIFICATION

Objective: We have demonstrated that vitrification and slow cryopreservation may increase
apoptotic index of blastocysts. Also assisted zonal hatching decreases the post-warming apoptosis. Our objective was to 1) assess if blastocyst biopsy can induce apoptosis and 2) evaluate if vitrification and slow cryopreservation can further increase apoptosis in biopsied blastocysts.

**Design:** prospective in vitro study

**Materials and Methods:** 35 blastocysts were biopsied after assisted hatching and trophectoderm biopsy. After biopsying, 16 blastocysts were slowly cryopreserved, 12 were vitrified, and 7 immediately fixed. Another group of 41 intact blastocysts were used. Of these 15 were slow cryopreserved, 22 were vitrified and 14 were used as fresh controls. Slow cryopreservation and vitrification was done as described in our earlier published work. After thawing, blastocysts were incubated in 10% albumin enriched HTF media at 37°C for 4 h with 5% CO₂, fixed in formalin and stained for DNA damage using TUNEL assay. Blastocysts were counterstained with DAPI and examined by confocal microscopy. DNA integrity index was calculated as percentage of TUNEL−ve to total number of blastomeres.

**Results:** There was no statistically significant difference between the DNA integrity of intact and biopsied blastocysts in fresh and slowly cryopreserved blastocysts. Biopsied vitrified blastocysts had significantly higher DNA integrity index compared with intact vitrified blastocysts (P <0.001). Among the biopsied blastocysts, there was a significant difference between the fresh and slowly cryopreserved (P = 0.001) or vitrified blastocysts (P = 0.034). No significant difference was seen between vitrification and slow cryopreservation in the biopsied group.

**Table 1.** DNA integrity Index in intact and biopsied blastocysts following slow cryopreservation and vitrification.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intact Blastocysts (n = 41)</th>
<th>Biopsied Blastocysts (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh (n = 14)</td>
<td>Slow cryopreservation (n=15)</td>
</tr>
<tr>
<td>DNA Integrity Index (%)</td>
<td>95.47 ± 4.25</td>
<td>90.87 ± 6.16</td>
</tr>
<tr>
<td></td>
<td>Fresh (n=7)</td>
<td>Slow cryopreservation (n=16)</td>
</tr>
<tr>
<td>DNA Integrity Index (%)</td>
<td>98.12 ± 1.99</td>
<td>94.03 ± 2.36</td>
</tr>
</tbody>
</table>

**Conclusions:** Blastocyst biopsy does not induce apoptosis. Biopsied blastocysts can tolerate slow cryopreservation or vitrification to the same extent Biopsied vitrified blastocysts can tolerate vitrification better than intact expanded blastocysts. This may be related to assisted hatching or blastocele puncture associated with the biopsy procedure.

**Support:** None.

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