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Contact/Presenting Author: Amr Kader, M.D.
Department/Institution: Cleveland Clinic
Address: E 9500, Euclid Avenue, Desk A 19.1
City/State/Zip/Country: Cleveland, United States
Phone: 1-216-444-9485 Cell Phone: 1-216-445-6049 E-mail: agarwaa@ccf.org

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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: APOPTOSIS IS NOT INDUCED BY SLOW CRYOPRESERVATION OR VITRIFICATION IN BIOPSED CLEAVAGE STAGE EMBRYOS

Objective: Cleavage stage embryo biopsy makes embryos more vulnerable to development
failure. Cryopreserved biopsied embryos are at a higher risk for developmental or implantation failure. The aim of our study was to 1) examine if apoptosis is induced in cleavage stage embryos after biopsy and 2) evaluate the extent of apoptosis following vitrification or slow cryopreservation of biopsied cleavage stage embryos.

**Design:** In Vitro, prospective

**Materials and Methods:** 44 cleavage stage I embryos were biopsied and 1-2 blastomeres were removed by the aid of micromanipulator. After biopsy, 13 embryos were vitrified, 16 embryos were slowly cryopreserved and 15 embryos were immediately fixed in 4% formalin. Another group of 44 intact cleavage stage embryos were used. Of these 13 were used as fresh controls, 14 were vitrified and 17 were subjected to slow cryopreservation. Slow cryopreservation was done using Irvine propanediol based media. Vitrification was carried out using the Irvine vitrification media and cryotip loading devices. After thawing, all embryos were incubated in 10% albumin enriched HTF media for 4 h at 37° C with 5% CO₂ and fixed. Embryos were stained for DNA damage using TUNEL assay. Blastomeres were mounted in Vectashield containing DAPI for counterstaining and examined by confocal microscopy. DNA integrity index in each embryo was calculated as percentage of TUNEL −ve to total number of blastomeres.

**Results:** DNA integrity index was comparable in fresh and biopsied groups. No further increase in apoptosis was observed following slow cryopreservation or vitrification.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intact Embryos (n = 44)</th>
<th>Biopsied embryos (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Integrity Index (%)</td>
<td>100.00 ± 0.00</td>
<td>99.11 ± 3.57</td>
</tr>
<tr>
<td>Slow cryopreservation (n = 17)</td>
<td>96.17 ± 5.55</td>
<td>98.29 ± 6.16</td>
</tr>
<tr>
<td>Vitrification (n = 14)</td>
<td>100.00 ± 0.00</td>
<td>99.11 ± 3.57</td>
</tr>
</tbody>
</table>

**Conclusions:** Embryo biopsy does not induce apoptosis in cleavage stage embryos. Both slow cryopreservation and vitrification do not induce apoptosis in biopsied cleavage stage embryos.

**Support:** None.

**Author:** Amr Kader, M.D.

**Department/Institution:** Cleveland Clinic

**Address:** E 9500, Euclid Avenue, Desk A 19.1

**City/State/Zip/Country:** Cleveland, United States

**Phone:** 1-216-444-9485  **Cell Phone:**  **Fax:** 1-216-445-6049  **E-mail:** agarwaa@ccf.org

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Author: Gihan Mansour, M.D.
Department/Institution: Cleveland Clinic
Address: City/State/Zip/Country: Cleveland, United States
Phone: Cell Phone:  Fax:  E-mail: agarwaa@ccf.org
Author: Ashok Agarwal, Ph.D.
Department/Institution: Cleveland Clinic
Address:  
City/State/Zip/Country: Cleveland, United States
Phone: Cell Phone: Fax: E-mail: agarwaa@ccf.org

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Author: Rakesh Sharma, Ph.D.
Department/Institution: Cleveland Clinic
Address:  
City/State/Zip/Country: Cleveland, United States
Phone: Cell Phone: Fax: E-mail: agarwaa@ccf.org

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Author: Tommaso Falcone, M.D.
Department/Institution: Cleveland Clinic
Address:
City/State/Zip/Country: Cleveland, United States
Phone: Cell Phone: Fax: E-mail: agarwaa@ccf.org

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