**ABSTRACT**

Study question: To determine the pregnancy outcome after vitrification of all fresh embryos produced in stimulated assisted reproduction technique cycles (ART) and replacing them in subsequent non-gonadotropin stimulated cycles.

Summary of review: Vitrification of all fresh embryos produced in stimulated ART cycles and replacing them in subsequent non-gonadotropin stimulated cycles resulted in highly successful implantation rate and individual and cumulative pregnancy rate.

What is known already: It has been proposed that supraphysiological hormone levels during ovarian stimulation may adversely affect embryo implantation in assisted reproductive treatments. Very few studies have addressed the effect of cryopreservation of all embryos as a method to avoid the undesirable effect of gonadotropin ovarian stimulation on implantation and pregnancy rate, as well to prevent the occurrence of iatrogenic events such as ovarian hyperstimulation syndrome (OHSS).

Study design, size, duration: A prospective trial of series of cases in a private fertility center (ART) clinic. 113 patients (average age 35 years) with a history of developing OHSS or with uterine factor who underwent vitrification of all fresh embryos from January 2010 to May 2013. Participants with multiple cycles were included in the analysis. Embryo survival was 98%. After thawing, 609 embryos were transferred into hormone replacement cycles in a total of 24 cycles. The average number of embryos transferred per cycle was 2.3. Main reasons for not transferring the embryos were for a successful clinical pregnancy or cycle cancellation.

Wider implications of the findings: The results motivate us to suggest vitrification of all fresh embryos produced in stimulated ART cycles and replacing them in subsequent non-gonadotropin stimulated cycles.

**INTRODUCTION**

It is well known that supraphysiological hormone levels during controlled ovarian stimulation (COS) for assisted reproduction techniques (ART) may adversely affect implantation in fresh embryos in IVF cycles (1,2). Several studies have addressed the effect of vitrification of all fresh embryos (EVA) as a method to avoid the undesirable effect of gonadotropin ovarian stimulation as well as prevent the occurrence of the ovarian hyperstimulation syndrome (OHSS) but very few have assessed pregnancy outcome in such cases. (3,4)

**MATERIALS AND METHODS**

We studied 135 patients (age range 24 to 43) who underwent vitrification of all fresh embryos in a controlled ovarian hyperstimulation (COS) cycle due to either a risk of severe OHSS or a uterine factor from January 2010 to May 2013. Ovarian stimulation was carried out with recombinant or urinary FSH and GnRH antagonistic antagonism was triggered with recombinant hCG or Leuprolide acetate for high responders (5). A total of 1106 embryos were vitrified with Kuewyan’s technique at cleavage-stage embryos (72 h) or blastocyst stage (120 h).

After thawing 693 embryos, 609 were subsequently transferred into a hormone replacement cycle in all 135 patients, in a total of 249 cycles (range 1 to 4 cycles per patient). The average number of embryos transferred per cycle was 2.3 (range 1-3). Embryo survival rate was reported as 98%. At the time of the assessment for this presentation 413 embryos remained vitrified.

**RESULTS**

We determined the pregnancy outcome after vitrification of all fresh embryos produced in stimulated ART cycles and replacing them in subsequent non-gonadotropin stimulated cycles.

**DISCUSSION**

Embryocryopreservation by vitrification is performed with increasing frequency, providing higher survival rates and minimal deleterious effects on post-thaw development quality, improving clinical outcome compared with conventional slow freezing.

Vitrification of all embryos offers patients an excellent chance of pregnancy when pregnancy is transferred in a non-gonadotropin stimulated cycle to avoid a negative influence of supraphysiological steroid level in embryo implantation as well as a strategy to prevent iatrogenic complication (3,4).

Several mechanisms for this impairment on implantation for supraphysiological steroid level have been proposed: -an asynchrony between endometrial development and embryonic age with delayed glandular maturation and advanced stromal secretion (B), -altered expression of genes and cytokines (9.10). -direct negative effect on embryo development (11).

Schoolcraft et al has found successful results when all embryos were thawed after ultra-sonic screening with delayed transfer. The author claims that such results could be due to both strategies, delayed transfer and genetic screening (12).

We compared the results in this study group of patients with all patients treated for ART in the same Institution that fulfilled similar criteria as population (age, duration and etiology of infertility) that received fresh embryo transfers and have additional embryos for vitrification. The results are shown in the following table:

<table>
<thead>
<tr>
<th>Pregnancy Outcome Per Group</th>
<th>Group</th>
<th>Vitrification group</th>
<th>Fresh group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>135</td>
<td>593</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (range)</td>
<td>33.6 ± 4.6</td>
<td>35.9 ± 4.7</td>
<td></td>
<td></td>
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<tr>
<td>Clinical pregnancies (n)</td>
<td>105</td>
<td>160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy rate per cycle (%)</td>
<td>42.1 ± 20.3</td>
<td>28.3 ± 16.2</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>33.7 ± 20.1</td>
<td>16.2 ± 16.1</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Multiple pregnancy rate (%)</td>
<td>32.8 ± 15.8</td>
<td>12.5 ± 15.4</td>
<td>0.011</td>
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</tr>
<tr>
<td>Abortion rate (%)</td>
<td>23.8 ± 15.8</td>
<td>22.6 ± 16.8</td>
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</tbody>
</table>

**CONCLUSION**

Based on the literature review it is tempting to speculate that the results of this study are the consequence of the delay in embryo transfer into the uterus in a state of more receptive milieu.

Successful outcome as high pregnancy rate and implantation rates makes us consider the option of EVA with subsequent transfer in a non-gonadotropin stimulated cycle as a strategy to apply in all cases, changing traditional thinking, improving pregnancy outcome and preventing iatrogenic complications in ART.

The high incidence of multiple pregnancies makes us rethink diminishing the number of embryos to transfer per cycle in the future.