Prognosis for clinical pregnancy and birth after transferring embryos derived from a cohort of incompletely mature oocytes at retrieval time

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SUMMARY

The purpose of this retrospective study was to establish a prognosis for implantation, pregnancy and live birth rates in stimulated IVF cycles after transferring embryos derived from: 1/ retrieved immature oocytes that matured overnight in vitro (late mature group: LM); 2/ retrieved immature oocytes that matured overnight in vitro and were added to the embryos derived from retrieved mature oocytes (mixed embryos group: MX); and 3/ retrieved mature oocytes (mature group: M).

The obtained implantation, clinical pregnancy and live birth rates for the LM group were: 5.6%, 11.4%, 11.4%; for the MX group were:

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4.2%, 14.6%, 11.6%; and for the M group were: 14.6%, 45.2% and 33.3%, respectively. These measurements were significantly lower p<0.05 for the LM and MX groups in comparison to the M group. The number of oocytes retrieved and the number of embryos transferred were the lowest (p<0.001–0.05) for the LM group. It is concluded, that the retrieved immature oocytes are able to mature during overnight culture in vitro, be fertilized and provide developmentally competent embryos with the prognosis of 11% for the successful delivery. Reproductive Biology 2012 12 2: 219–229.

Keywords: immature oocytes, next day ICSI, implantation, pregnancy, birth

INTRODUCTION

The presence of a considerable number of immature oocytes at the time of egg retrieval is frequent in poor responders and in patients with unsynchronized cohort of recruited follicles [6]. However, it is very rare that all retrieved oocytes are immature after treatment of patients with stimulation protocol tailored to their needs. To “rescue” these in vitro fertilization (IVF) cycles, in vitro maturation of retrieved immature oocytes (GV: germinal vesicle - the enlarged nucleus of an oocyte before I meiotic division is completed or MI: metaphase I) can be performed followed by delayed intracytoplasmic sperm injection (ICSI) insemination. The resulted embryo(s) might be transferred alone or added to the embryos derived from the retrieved mature oocytes [5, 6, 8, 12, 13, 15]. Based on sporadic single case reports and/or limited number of analyzed IVF cycles concerning application of immature oocytes for embryo transfer, it is impossible to generate reliable prognosis for pregnancy and successful baby delivery. Retrospective analysis of substantial number of such IVF cases from our IVF program allowed us to establish statistically meaningful rates of implantation, pregnancy and birth after transferring embryos originated from retrieved immature oocytes (GV, MI) of stimulated IVF cycles. Also, we intended to determine whether the use of in vitro matured GV and MI oocytes can be beneficial in patient’s cycle management and/
or may guide the reproductive endocrinologists in the counseling patients regarding patient’s further treatment. It is also relevant in situations where the patients are financially responsible for the treatment.

**MATERIALS AND METHODS**

The Institutional Review Board of the Abington Memorial Hospital, PA approved this retrospective study. The controlled ovarian stimulation of the IVF patients consisted of commonly used IVF protocols (GnRH down-regulation followed by FSH/hMG and hCG injection or GnRH microdose flare or GnRH antagonist). Oocyte retrieval (day 0) was performed by transvaginal ultrasound-guided follicle aspiration 34–36 hours after hCG (5 000–10 000 IU) administration. To determine the nuclear status of oocytes, the oocytes were denuded approximately 1 to 2 hours after oocytes collection by a brief exposure of the cumulus-oocyte complexes to hyaluronidase (80 IU/ml; Conception Technology, San Diego, CA, USA) followed by mechanical denudation. An insemination (ICSI) of all matured (MII) oocytes was performed on the day of egg retrieval (day 0). The immature oocytes (GV and MI) were cultured overnight in fertilization medium (Cooper Surgical Inc., Trumbull, CT, USA) and after reaching maturity (MII stage) were subjected to ICSI (day 1). The sperm of patient’s husband/partner was used to inseminate oocytes. Sperm for ICSI procedure was prepared using density gradient (90–45%; PureCeption, Conception Technologies, CA, USA) and wash protocol [2]. After injection, oocytes were cultured in fertilization media followed by transfer to cleavage media (both from Cooper Surgical Inc., Trumbull, CT, USA). An assessment of fertilization (appearance of two distinct pronuclei and two polar bodies) was performed between 16 to 20 hours after ICSI (day 1 or day 2). Embryonic development was evaluated based on the number and regularity of blastomeres and degree of embryo fragmentation. Ultrasound guided embryo transfer (ET) was carried out by the transcervical route on day 2 or 3 after eggs retrieval. In our IVF program day 2 vs. day 3 of ET is not a factor that affects the clinical outcome. The number of embryos transferred was
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Generally based on the American Society Reproductive Medicine (ASRM) guidelines [1], and/or on the number of embryos available. Pregnancy was diagnosed by the detection of hCG (>5 mIU/ml) in serum at least 12 days after ET and by a rise in hCG serum concentration thereafter. A transvaginal ultrasound scan was performed approximately five to six weeks later to detect the presence of an intrauterine gestational sac with/without fetal heart beat or to diagnose ectopic implantation.

Total of 135 IVF cycles were extracted from our data base where ICSI was performed on the day of egg retrieval or day after. These cycles were divided into three groups:

1) late mature oocyte group (LM) - all transferred embryos originated from the retrieved immature (GV and MI) oocytes that matured during an overnight in vitro culture and were inseminated (ICSI) on the next day after egg retrieval (n=50);

2) mixed group (MX) - all transferred embryos originated from the retrieved mature (MII) oocytes and inseminated (ICSI) on the day of egg retrieval as well as from immature oocytes that reached maturity during overnight culture and were inseminated (ICSI) on the next day after egg retrieval (n=43);

3) mature oocyte group (M) - all transferred embryos originated from the retrieved mature oocytes and were inseminated (ICSI) on the day of egg retrieval (n=42).

Definitions of main outcome measure(s) and statistical analysis

Clinical pregnancy rate (%) was defined as a number of gestational sacs/number of cycles with embryo transfers ×100. Implantation rate (%) was defined as a number of gestational sacs/number of embryos transferred ×100. Birth rate (%) was defined as a number of babies born/number of embryo transfers ×100. Data were analyzed using the general linear model of ANOVA, and Chi-square tests where appropriate (SAS Inst. Inc., Cary, NC, USA). When the F-test was significant (p<0.05), differences among means were evaluated by using the least square means procedure [10]. For every analysis, a p value of at least <0.05 was considered statistically
significant. Numerical data (tab. 1) are expressed per cycle and were analyzed by ANOVA. Percentage data (tab. 1) are expressed per group and were analyzed using Chi-square analysis.

RESULTS

The number of retrieved oocytes was the highest (p<0.05) in the M group, lower in the MX group and the lowest in the LM group on the day of egg retrieval. Fertilization rate was higher in the M group than in the MX group, but cleavage rate between the groups did not differ (tab. 1). There were no difference among the groups in the number of oocytes that matured overnight and were inseminated (ICSI) on the next day. The fertilization rate was similar in all of the examined groups. However, the cleavage rate was lower (p<0.001) in the M group than in the other groups. The number of transferred embryos was the lowest (p<0.001) in the LM group. The rates of implantation, clinical pregnancy and birth were the highest (p<0.05) in the M group.

The age of patients, the number of days on stimulation, endometrial thickness and sperm parameters were similar for all groups (tab. 2). However, FSH baseline concentration was higher (p<0.05) in the MX group then in other groups, and concentration of estradiol-17β at the time of hCG injection was higher (p<0.05) in the M group then in the LM and MX groups.

DISCUSSION

Our study has established the statistical prognosis for clinical pregnancy (11.4%), implantation (5.65%) and birth rates (11.4%) after transferring embryos derived from retrieved immature oocytes. Out of 44 embryo transfers in the LM group, five clinical pregnancies have been achieved and five singleton babies were born. In the study reported by Shu et al. [15] two singleton pregnancies were achieved that later ended up in spontaneous abortions. De Vos et al. [4] reported only one delivery out of 15 embryo transfers where transferred embryos derived from immature oocytes on
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The day of egg retrieval. The immature oocytes in these studies were cultured only 4–6 hours and after an extrusion of the first polar body the ICSI insemination was performed. In our study, immature oocytes were cultured overnight before ICSI insemination. It has been reported that an extrusion of first polar body does not represent completed oocyte maturation process because at least 115–150 min are required to form MII meiotic spindles [11] from the time of the first polar body extrusion. Also, in published individual IVF cases when patient’s immature GV oocytes were cultured in vitro for 30 h [12] or MI oocytes for 8–22 h [3, 5, 16] before the ICSI insemination, the delivery of single baby was reported. This clearly indicates that extended culture time of the retrieved immature oocytes is an important factor in improving the embryo developmental competence. This is probably due to the gaining of cytoplasmic maturity [13, 14].

On the other hand, Jones et al. [9] have demonstrated, that immature oocytes recovered from gonadotropin-stimulated patients and matured

<table>
<thead>
<tr>
<th>Groups Measurements</th>
<th>LM (mean±SEM or %)</th>
<th>MX (mean±SEM or %)</th>
<th>M (mean±SEM or %)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrieved oocytes (n)</td>
<td>6.3±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.6 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.6 ± 0.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>–</td>
<td>34.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Cleavage rate (%)</td>
<td>–</td>
<td>95</td>
<td>100</td>
<td>NS</td>
</tr>
<tr>
<td>Inseminated oocytes (n)</td>
<td>4.5 ± 0.6</td>
<td>4.0 ± 0.5</td>
<td>3.7 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>46.2</td>
<td>52.6</td>
<td>42.3</td>
<td>NS</td>
</tr>
<tr>
<td>Cleavage rate (%)</td>
<td>93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Embryos transferred (n)</td>
<td>1.8 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.1 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>5.6 (5/90)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.2 (6/144)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.6 (19/130)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>11.4 (5/44)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.6 (6/43)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.2 (19/42)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Birth rate (%)</td>
<td>11.4 (5/44)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.6 (5/43)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.3 (14/42)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

Values within a row with different letters are statistically different; NS: not significant
Table 2. Characteristics of the IVF cycles

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Unit</th>
<th>Group LM</th>
<th>Group MX</th>
<th>Group M</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age</td>
<td>year</td>
<td>35.8±3.7</td>
<td>36.4±4.0</td>
<td>34.7±4.7</td>
<td>NS</td>
</tr>
<tr>
<td>FSH baseline</td>
<td>IU/ml</td>
<td>10.7±4.6</td>
<td>16.2±18.3</td>
<td>10.6±3.4</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Days on stimulation</td>
<td>n</td>
<td>9.2±1.6</td>
<td>9.2±2.3</td>
<td>8.8±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>$E_2$ at hCG*</td>
<td>pg/ml</td>
<td>1470±854</td>
<td>1918±962</td>
<td>2498±1772</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Endometrium thickness</td>
<td>mm</td>
<td>11.5±1.9</td>
<td>10.5±2.0</td>
<td>10.3±2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Sperm volume</td>
<td>ml</td>
<td>2.9±1.5</td>
<td>2.48±1.5</td>
<td>2.4±1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Sperm count</td>
<td>M/ml</td>
<td>48.6±43.8</td>
<td>54.0±54.6</td>
<td>37.0±42.4</td>
<td>NS</td>
</tr>
<tr>
<td>Sperm motility</td>
<td>%</td>
<td>56.8±23.3</td>
<td>56.7±22.3</td>
<td>53.5±20.4</td>
<td>NS</td>
</tr>
<tr>
<td>Normal forms **</td>
<td>%</td>
<td>10.1±4.8</td>
<td>7.6±4.0</td>
<td>9.3±1.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data were presented as mean±SEM; values with different letters within a row are statistically different; NS: not significant; * estradiol ($E_2$) plasma concentration at the time of hCG injection; ** Kruger criteria were used to assess morphologically normal sperm.
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in vitro have a large number of genes expressed at more than 2-fold higher level than oocytes matured in vivo. This suggests deregulation of transcription or post-transcriptional modification of genes which results in developmental incompetence of embryos derived from these oocytes. Nevertheless, a delivery of healthy baby from these “incompetent embryos” at the rate of 11% in our study and reported in individual clinical cases [5, 6, 8, 12, 13, 16] suggests that regulatory molecular mechanisms may have a wide range of plasticity and perhaps a self-correcting ability. Future studies should be focused on defining the regulatory mechanisms for achieving developmental competency of retrieved immature oocytes. This may help to obtain fully competent embryos.

In our study, the clinical pregnancy, implantation and birth rates for embryos originated from late matured oocytes were lower than those for embryos originated from the retrieved mature oocytes. The poor clinical outcome in the LM group may result from transferring a low number of embryos (1.8 embryos/transfer) in comparison to the MX (3.3 embryos/transfer) and M (3.1 embryos/transfer) groups. The twenty two cycles (approximately 50% cycles, data not shown) in the LM group had only one embryo transferred due to a low number of retrieved oocytes.

Pooling embryos that derived from retrieved mature oocytes with those from immature oocytes (MX group) did not significantly improve the clinical outcome in our study. In spite of transferring more embryos, the clinical outcome in the MX group was the same as in the LM group. Similar results in regards to clinical pregnancy and implantation rates were found by Vanhoute et al [16] and in regards to birth rate by Chen et al [3]. However, in the MX group more biochemical pregnancies (an initial rise of hCG in the serum) occurred, compared to other groups (5 vs. 1, data not shown). The greater incidence of biochemical pregnancies in the MX group may be related to elevated FSH level. The majority of pregnancies and births in the MX group occurred when four embryos were transferred, whereas seven pregnancies including two sets of twins occurred in the M group after transferring only three embryos (data not shown). There was no distinct predictable pattern in the occurrence of pregnancies in the LM
group considering the number of transferred embryos. It seems that supplementing the pool of transferred embryos with embryos derived from immature oocytes did not significantly affect the clinical outcome in analyzed IVF cycles.

The highest fertilization rate and the lowest incidence of multinucleation have been reported when ICSI of the oocytes was performed between 3 to 6 hours after extrusion of the first polar body [2, 7]. Our and others [15] data have shown similar fertilization rates for both *in vitro* and *in vivo* matured oocytes within each group. However, a decreased fertilization rate was also reported [2, 4, 16]. Thus, a normal fertilization can be obtained in *in vitro* matured oocytes but the further developmental competence can be compromised and may affect clinical outcome [9, 15, 16]. Further studies are needed to define optimal *in vitro* maturation conditions and the ICSI timing for human immature oocytes retrieved from stimulated cycles.

In conclusion, the present study has shown that retrieved immature oocytes can be matured *in vitro*, fertilized by ICSI, develop to embryos and produce pregnancies with live delivery - albeit at a diminished rate compared to embryos originated from retrieved mature oocytes. *In vitro* maturation of GV and MI oocytes followed by insemination may be worthwhile for the patients who do not produce sufficient number of mature oocytes or have no mature oocytes present at the time of follicle aspiration. Maturing these oocytes *in vitro* as an alternative for cancelling further patient treatment may provide opportunity for successful clinical outcome in situation when only a few immature oocytes are retrieved (older or cancer patients, difficult clinical cases). Therefore, our study provides a guideline for reproductive endocrinologists in counseling patients on the prognosis of pregnancy and delivery in situation where no mature oocytes or a limited number of mature oocytes are retrieved. Furthermore, any remaining embryos originated from immature oocytes can be cryopreserved, which may increase the cumulative pregnancy rate. However, enhancement of the competency of immature oocytes and its regulatory mechanisms remain to be elucidated.
REFERENCES

3. Chen SU, Chen HF, Lien YR, Ho GN, Chang HC, Yang YS 2000 Schedule to inject in vitro matured oocytes may increase pregnancy after intracytoplasmic sperm injection. *Archives of Andrology* 44 197-205.