Strategy of mild stimulation cycle
IVF combined with IVM treatment

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Bart C.J.M. Fauser
Development of in vitro maturation techniques for clinical applications

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In vitro maturation (IVM) refers to maturation in culture of immature oocytes at different stages that may or may not have been exposed to short courses of gonadotropins. The source of immature oocytes is an important feature for subsequent embryonic development, pregnancy, and healthy live births. IVM is an effective treatment that has already achieved significant outcomes of acceptable pregnancy and implantation rates and has led to the births of several thousand healthy babies. As the development of IVM treatment continues, an attractive possibility for improving the already successful outcome is to combine a natural-cycle in vitro fertilization (IVF) treatment with immature-oocyte retrieval followed by IVM of those immature oocytes. If the treatment processes can be simplified for immature-oocyte retrieval, different types of infertile women may be able to take advantage of these treatments. Mild-stimulation IVF combined with IVM treatment may represent a viable alternative to the standard treatment. Although IVM treatment is still considered to be experimental, it is now time to reconsider the IVM technology and its development. Mild-stimulation IVF combined with IVM may prove to be not just alternatives to standard treatments, but potentially first-line treatment choices. (Fertil Steril® 2017;108:577–84. ©2017 by American Society for Reproductive Medicine.)

Key Words: IVM, oocyte, immature, IVF, mild stimulation

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Follicular development in the ovary

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<table>
<thead>
<tr>
<th>Stage</th>
<th>Follicular size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primordial</td>
<td>0.03 – 0.04</td>
</tr>
<tr>
<td>Primary</td>
<td>0.05 – 0.06</td>
</tr>
<tr>
<td>Secondary</td>
<td>0.07 – 0.11</td>
</tr>
<tr>
<td>Preantral</td>
<td>0.12 – 0.20</td>
</tr>
<tr>
<td>Early antral</td>
<td>0.21 – 0.40</td>
</tr>
<tr>
<td>Antral</td>
<td>0.41 – 16.00</td>
</tr>
<tr>
<td>Preovulatory</td>
<td>16.10 – 20.00</td>
</tr>
</tbody>
</table>

LH surge induces *in vivo*

- Oocyte maturation;
- Cumulus expansion;
- Ovulation;
- Luteilization;
- Follicle’s atresia
Oocyte maturation (from GV to M-II) requires approximately 24 hours after LH surge.
Concept of oocyte maturation

GV

M-I

M-II
In vitro maturation (IVM)

- Immature oocytes can be matured *in vitro* after releasing from the antral follicles;
- Culture condition affects oocyte maturation rate and quality;
- Cumulus and granulosa cells associated with oocyte quality.
Updated IVM success rates

- Clinical pregnancy rate approximately 35-50% per ET;
- More than 10,000 live births.
• Natural cycle IVF;
• Mild stimulation with clomiphene citrate or HMG to generate more than one oocyte;
• GnRH agonists or antagonist in combination with gonadotropins to generate multiple oocytes.
Natural-cycle in vitro fertilization combined with in vitro maturation of immature oocytes is a potential approach in infertility treatment

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Department of Obstetrics and Gynecology, McGill Reproductive Center, Royal Victoria Hospital, Montreal, Quebec, Canada

Objective: To describe pregnancies and live births that resulted from IVF of mature oocytes retrieved from dominant follicles in a natural cycle combined with in vitro maturation (IVM) of immature oocytes retrieved from small follicles.

Design: Case reports.

Setting: McGill Reproductive Center, Royal Victoria Hospital, McGill University.

Patient(s): Three women with normal ovaries or polycystic ovaries who underwent infertility treatment.

Intervention(s): Administration of SC hCG (10,000 IU) 36 hours before oocyte retrieval in a natural cycle. After aspiration of all follicles, mature oocytes were inseminated immediately; immature oocytes were matured in vitro, inseminated by intracytoplasmic sperm injection (ICSI), and then the embryos transferred.

Main Outcome Measure(s): Pregnancy and live birth.

Result(s): Three pregnancies (two live births and one ongoing at time of writing) were achieved after the combination of natural-cycle IVF with IVM after transfer of the resulting embryos.

Conclusion(s): Natural-cycle IVF combined with IVM might be a new approach to IVF treatment for women with various causes of infertility. (Fertil Steril® 2004;82:1675–8. ©2004 by American Society for Reproductive Medicine.)

Key Words: Natural cycle, IVF, hCG, immature oocytes, in vivo maturation, pregnancy
From the results confirmed

- The maturational and developmental competence of immature oocytes derived from the small antral follicles is not affected by the presence of a dominant follicle and phases of folliculogenesis.
Natural cycle IVF/M protocol

- ≥ 7 antral follicles seen under baseline ultrasound scan;
- The leading follicle reached to 12-14 mm in diameter and endometrial thickness reached to at least 6.0 mm, HCG (10,000 IU) is given;
- Egg retrieval is scheduled 36 hrs later.
Commentary

New alternative to infertility treatment for women without ovarian stimulation

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Abstract

Natural cycle IVF produced the world first successful live birth, but slowly this treatment has been replaced by ovarian stimulated cycle IVF, because it has been believed ovarian stimulated cycle IVF will increase the number of available embryos for transfer. Therefore, it directly increases the chance of pregnancy from the treatment cycle. However, ovarian stimulation is always associated with side effects. The recovery of immature oocytes followed by in-vitro maturation (IVM) and IVF is an attractive alternative to stimulated cycle IVF. IVM treatment provides a successful option to infertile women with polycystic ovaries and polycystic ovary syndrome. It is now possible to combine natural cycle IVF with IVM as an alternative for a selected group of women with various causes of infertility without recourse to ovarian stimulation.

Keywords: IVM, live birth, natural cycle IVF, ovarian simulation, polycystic ovary syndrome
Mature and immature oocytes retrieved from natural cycle IVF/M
Table 1. Results of mature and immature oocyte retrieval and in-vitro maturation and fertilization, followed by embryo transfer in women with normal ovaries and regular menstrual cycles.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>82</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.9 ± 2.6</td>
</tr>
<tr>
<td><strong>Mature oocytes retrieved</strong></td>
<td></td>
</tr>
<tr>
<td>Total number</td>
<td>99</td>
</tr>
<tr>
<td>Mean</td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td><strong>Immature oocytes retrieved</strong></td>
<td></td>
</tr>
<tr>
<td>Total number</td>
<td>619</td>
</tr>
<tr>
<td>Mean</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
<td>No. of oocytes matured <em>in vitro</em> (%)</td>
<td>495 (80)</td>
</tr>
<tr>
<td>No. of oocytes fertilized (%)</td>
<td>371 (75)</td>
</tr>
<tr>
<td>No. of embryos cleaved (%)</td>
<td>356 (96)</td>
</tr>
<tr>
<td><strong>Embryos transferred</strong></td>
<td></td>
</tr>
<tr>
<td>Total number</td>
<td>205</td>
</tr>
<tr>
<td>Mean</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>No. of clinical pregnancies (%)</td>
<td>29 (35)</td>
</tr>
<tr>
<td>No. of implantation (%)</td>
<td>39 (19)</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation unless otherwise stated.
• Selected women with various types of infertility can be treated efficiently by this new approach without ovarian stimulation, namely natural cycle IVF combined with IVM;
• This new approach without stimulation will benefit many infertile women.
Selection of patients for natural cycle in vitro fertilization combined with in vitro maturation of immature oocytes

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Objective: To determine a successful method of selecting patients for natural cycle IVF combined with in vitro maturation (IVM) of immature oocytes and to determine treatment efficacy.

Design: Retrospective analysis of IVF treatments.

Setting: Hospital IVF clinic.

Patient(s): Women with infertility according to ovarian reserve and endocrine profile were selected for different treatments.

Interventions(s): According to screening criteria, infertile women were divided into three treatments: [1] natural cycle IVF combined with IVM (IVF/M), [2] IVM alone, and [3] controlled ovarian hyperstimulation (COH) with gonadotropin.

Main Outcome Measure(s): The distribution of completed IVF-ET cycles in each treatment; clinical pregnancy and implantation rates in each treatment.

Result(s): A total of 417 cycles were started, and 410 cycles (98.3%) were completed. Of 410 cycles, 151 (36.8%) were treated by natural cycle IVF/M, 63 (15.4%) underwent IVM alone, and 196 (47.8%) underwent COH. With increasing age fewer cycles can be treated by natural cycle IVF/M or IVM. Clinical pregnancy rates were 40.4% (61 of 151) for natural cycle IVF/M, 41.3% (26 of 63) for IVM alone, and 37.8% (74 of 196) for COH. There were no differences in implantation rate in the three groups (17.8% [82 of 462], 16.7% [35 of 210], and 20.1% [103 of 513]).

Conclusion(s): Natural cycle IVF/M together with IVM-alone treatment can offer more than 50% of infertile women with an acceptable pregnancy and implantations rates. (Fertil Steril® 2009;91:1050–5. ©2009 by American Society for Reproductive Medicine.)

Key Words: Natural cycle, IVF, IVM, oocytes, pregnancy
Detailed protocol for natural cycle IVF combined with IVM.

Natural Cycle IVF/M
(Lim-Chian Protocol)

Baseline ultrasound scan on day 3-5 of menstrual cycle to ensure ≥7 follicles in both ovaries

Subsequent ultrasound scan to ensure the size of leading follicles ≥12 mm in diameter and endometrial thickness ≥6 mm and then 10,000IU HCG administered

36 hours

The mature oocytes are inseminated by ICSI

Mature and immature oocyte retrieval.
Collected oocytes are identified for the maturity.
Mature oocytes are inseminated on the day of egg collection.
Immature oocytes are cultured in IVM medium for 24h.
In vitro matured oocytes are inseminated following IVM.
Start to give Estradiol from the day of egg collection.
Start to give Progesterone from the day ICSI.

3 days

Embryos produced from mature oocytes are on day 3
Transfer embryos produced from mature and immature oocytes
Embryos produced from immature oocytes are on day 2
The percentages of completed treatment cycles distributed by natural cycle IVF/M, IVM or COH. Total of 378 patients started 417 treatment cycles.
The percentages of completed treatment cycles based on age groups distributed by natural cycle IVF/M, IVM or COH.
<table>
<thead>
<tr>
<th>Variable</th>
<th>IVF/M</th>
<th>IVM</th>
<th>COH</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles started (patients)</td>
<td>153 (140)</td>
<td>63 (56)</td>
<td>201 (182)</td>
</tr>
<tr>
<td>No. of cycles completed (patients)</td>
<td>151 (138)</td>
<td>63 (56)</td>
<td>196 (177)</td>
</tr>
<tr>
<td>Age (y), mean ± SD</td>
<td>31.4 ± 3.7</td>
<td>31.4 ± 4.0</td>
<td>34.4 ± 4.8</td>
</tr>
<tr>
<td>No. of oocytes retrieved (mean ± SD)</td>
<td>1,826 (12.1 ± 5.6)</td>
<td>1,017 (16.1 ± 10.3)</td>
<td>2,079 (10.6 ± 6.6)</td>
</tr>
<tr>
<td>Oocytes matured</td>
<td>1,216 (66.6)</td>
<td>658 (64.7)</td>
<td>1,875 (90.2)</td>
</tr>
<tr>
<td>Oocytes fertilized</td>
<td>996 (81.9)</td>
<td>519 (78.9)</td>
<td>1,485 (79.2)</td>
</tr>
<tr>
<td>Zygotes cleaved</td>
<td>928 (93.2)</td>
<td>469 (90.4)</td>
<td>1,429 (96.2)</td>
</tr>
<tr>
<td>No. of embryos transferred (mean ± SD)</td>
<td>462 (3.1 ± 0.8)</td>
<td>210 (3.3 ± 0.6)</td>
<td>513 (2.5 ± 0.4)</td>
</tr>
<tr>
<td>Clinical pregnancies obtained</td>
<td>61 (40.4)</td>
<td>26 (41.3)</td>
<td>74 (37.8)</td>
</tr>
<tr>
<td>Embryos implanted</td>
<td>82 (17.8)</td>
<td>35 (16.7)</td>
<td>103 (20.1)</td>
</tr>
<tr>
<td>Live births</td>
<td>21 (34.4)</td>
<td>8 (30.8)</td>
<td>35 (47.3)</td>
</tr>
<tr>
<td>Singleton</td>
<td>18</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Twin</td>
<td>3</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Ongoing pregnancies</td>
<td>23 (37.7)</td>
<td>8 (30.8)</td>
<td>21 (28.4)</td>
</tr>
<tr>
<td>Miscarriages</td>
<td>17 (27.9)</td>
<td>10 (38.5)</td>
<td>18 (24.3)</td>
</tr>
</tbody>
</table>

Note: Values are number (percentage) unless otherwise noted.
* Significantly different compared with the other two groups (P < .05).
Interpretation

• Natural cycle IVF/M together with IVM-alone treatments can offer more than 50% of infertile women acceptable pregnancy and implantation rates.
Comparison of pregnancy outcomes in natural cycle IVF/M treatment with or without mature oocytes retrieved at time of egg collection

Seong-Ho Yang¹, Pasquale Patrizio², San-Hyun Yoon¹, Jin-Ho Lim¹, and Ri-Cheng Chian³*

¹Maria Fertility Hospital, Seoul, Korea, ²Yale Fertility Center, Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University School of Medicine, New Haven, CT, USA, ³Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada
Table 2. Comparison of pregnancy and live birth rates of natural cycle IVF/M treatment in women with or without mature oocytes collected at the time of egg retrieval.

<table>
<thead>
<tr>
<th>Groups</th>
<th>A</th>
<th>B</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (cycles)</td>
<td>283 (314)</td>
<td>53 (55)</td>
<td>–</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>31.2 ± 3.6</td>
<td>30.4 ± 3.3</td>
<td>NS</td>
</tr>
<tr>
<td>No. of mature oocytes retrieved (mean ± SD)</td>
<td>739 (2.4 ± 1.6)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>No. of immature oocytes retrieved (mean ± SD)</td>
<td>2,780 (8.9 ± 5.0)</td>
<td>515 (9.4 ± 4.9)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of oocytes matured <em>in vitro</em> (%)</td>
<td>1,826 (65.7)</td>
<td>363 (70.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Total numbers of oocytes matured (mean ± SD)</td>
<td>2,565 (8.2 ± 3.5)</td>
<td>363 (6.6 ± 3.5)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of oocytes fertilized (%)</td>
<td>2,101 (81.9)</td>
<td>294 (81.0)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of <em>in vivo</em> matured oocytes fertilized (mean ± SD)</td>
<td>644 (2.1 ± 1.4)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>No. of <em>in vivo</em> matured oocytes cleaved (mean ± SD)</td>
<td>610 (1.9 ± 1.4)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>No. of <em>in vitro</em> matured oocytes fertilized (mean ± SD)</td>
<td>1,457 (5.1 ± 1.4)</td>
<td>294 (4.9 ± 2.2)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of <em>in vitro</em> matured oocytes cleaved (mean ± SD)</td>
<td>1,324 (4.2 ± 2.6)</td>
<td>262 (4.8 ± 2.7)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of zygotes cleaved (%)</td>
<td>1,934 (92.1)</td>
<td>262 (89.1)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of embryos transferred (mean ± SD)</td>
<td>859 (2.7 ± 0.4)</td>
<td>147 (2.7 ± 0.5)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of clinical pregnancies (%)</td>
<td>126 (40.1)</td>
<td>19 (34.5)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of embryos implanted (%)</td>
<td>139 (16.2)</td>
<td>22 (15.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Live births per cycle (%)*</td>
<td>93 (29.6)</td>
<td>9 (16.4)</td>
<td>0.043</td>
</tr>
<tr>
<td>Singleton</td>
<td>74</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td>Twins</td>
<td>18</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Triplets</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Miscarriage rate per clinical pregnancies (%)*</td>
<td>33 (26.2)</td>
<td>10 (52.6)</td>
<td>0.019</td>
</tr>
</tbody>
</table>

Group A: The patients with mature oocytes retrieved at egg retrieval.
Group B: The patients without mature oocytes retrieved at egg retrieval.
* Significantly different between groups.
• Although the clinical pregnancy rates are not different regardless of whether the mature oocytes retrieved or not at the time of egg retrieval, the live birth rate is higher (P <0.05) when the mature oocytes are obtained at the time of the egg retrieval.
Mild stimulation cycle IVF/M

- Clomiphene Citrate (CC) 50-100 mg/day
- HMG 75-150 IU/day
- HCG
- OPU
- IVM
- Day 3 or Day 5 cryopreservation
- 36 h
- IVF for M-II oocytes and ICSI for IVM oocytes
- Culture embryos from in vivo and in vitro matured (6-48 h) oocytes
- E2, P4
- Day 3 or Day 5 for ET
- HCG check

Day 0 3 5 14
Conclusions

• Mild stimulation cycle IVF combined with IVM is an efficient treatment for women with infertility, especially for women under 35 yrs of age;

• Mild stimulation IVF/M prove to be not just alternatives to standard treatment, but potentially first-line treatment choices.
An ideal reference for infertility specialists and reproductive endocrinologists alike, this comprehensive text discusses current natural and mild approaches to in vitro fertilization (IVF) and the development of in vitro maturation (IVM) as a clinical treatment. It is divided thematically into four sections. Part I presents the basic science of ovarian endocrinology and the mechanism of oocyte maturation, including follicular development and the importance of mitochondrial changes. The differences between natural cycle IVF and stimulated IVF are presented in part II, including the standard ovarian stimulation protocols and their outcomes and the etiology, prevention and management of ovarian hyperstimulation syndrome (OHSS). Minimal and mild stimulation for IVF is discussed in part III, including mild stimulation protocols for oncology patients and the new minimally invasive INVO procedure. In vitro maturation as a clinical treatment is the subject of part IV, discussing the methodology of immature oocyte retrieval as well as other laboratory aspects and congenital and obstetrical outcomes of babies born from IVM.

Combining expertise from thought leaders around the world, Development of In Vitro Maturation for Human Oocytes will be a go-to resource for infertility researchers and practitioners and reproductive endocrinologists working with infertile couples looking for safer and more cost-effective options for treatment.
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- Natural IVF & IVM
- Biological efficiency of oocytes
- Optimising health outcomes in IVF
- Perinatal outcomes after ART
- Access to IVF & public funding, Add ons in IVF
- Factors affecting Implantation
- New technology and Revolution in hysteroscopic surgery
- Debates by renowned experts

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ISMAAR has submitted this programme to the European Accreditation Council for Continuing Medical Education (EACCME).
Thank you!

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