Minimal Stimulation IVF versus Conventional IVF:  
A Randomized Controlled Trial

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ABSTRACT

BACKGROUND: Minimal stimulation IVF (mini-IVF) is an alternative IVF treatment protocol that may reduce ovarian hyperstimulation syndrome (OHSS), multiple pregnancy rates and cost while retaining high live birth rates.

OBJECTIVE (S): We performed a randomized non-inferiority controlled trial with a pre-specified border of -10% comparing one cycle of mini-IVF with single embryo transfer to one cycle of conventional IVF with double embryo transfer.

STUDY DESIGN: Five hundred sixty four infertile women (age < 39) undergoing their first IVF cycle were randomly allocated to either mini-IVF or conventional IVF. The primary outcome was cumulative live birth rate per woman over a 6-month period. Secondary outcomes included OHSS, multiple pregnancy rates, and gonadotropin use. The primary outcome was cumulative live birth per randomized woman within a time horizon of 6 months.

RESULTS: 564 couples were randomly assigned between February 2009 and August 2013 with 285 allocated to mini-IVF and 279 to conventional IVF. The cumulative live birth rate was 49% (140/285) for mini-IVF and 63% (176/279) for conventional IVF (RR 0.76, 95% CI 0.64-0.89). There were no cases of OHSS after mini-IVF compared to 16 (5.7%) moderate/severe OHSS cases after conventional IVF. The multiple pregnancy rates were 6.4% in mini-IVF compared to 32% in conventional IVF (RR 0.25, 95% CI 0.14-0.46). Gonadotropin consumption was significantly lower with mini-IVF compared to conventional IVF (459 ± 131 versus 2079 ± 389 IU, p<0.0001).

CONCLUSION (S): Compared to conventional IVF with double embryo transfer, mini-IVF with single embryo transfer lowers live birth rate, completely eliminates OHSS, reduces multiple pregnancy rates and reduces gonadotropin consumption.

Keywords: IVF; mini-IVF; clomiphene citrate; OHSS; multiple gestations
Introduction

The current standard of in vitro fertilization (IVF) treatment involves ovarian hyperstimulation with high-doses of gonadotropins in combination with transfer of one or more embryos\(^1\). The major safety issues of conventional IVF are ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies. Women with OHSS are at serious risk of potentially life-threatening conditions involving hospitalization in 1.8% of the cases\(^5\). Multiple pregnancies are associated with high risk of preeclampsia, gestational diabetes, antepartum hemorrhage, anemia, preterm delivery and cesarean section\(^4,6,7-9\). Preterm birth is associated with a high risk of bronchopulmonary dysplasia, necrotizing enterocolitis, and cerebral palsy\(^8,10,11,12\). Additionally, all these complications often lead to expensive hospital admissions\(^13\), which impose a steep burden on societal expenses and health services\(^14\).

One of the most important factors influencing the rate of multiple births is the number of embryos transferred\(^15\). Single embryo transfer (SET) effectively reduces the incidence of multiple pregnancies, but also decreases pregnancy rates per transfer\(^16\). Given this reduction in pregnancy chances, the majority of physicians and patients in the United States are reluctant to practice an SET policy; the percentage of SET in IVF cycles ranged between 8.9-14% among women less than 38 years of age in 2012\(^17\). This resulted in 25-29% multiple pregnancy rates among all pregnancies achieved during the same period of time\(^17\).

Minimal stimulation IVF (mini-IVF) combined with SET has the potential to reduce OHSS and multiple pregnancy rates without significantly lowering live birth rates. Mini-IVF entails the use of clomiphene citrate (CC), which allows an endogenous rise in follicle-stimulating hormone (FSH) to be additive to the ovarian stimulation using low doses of gonadotropins \(^19,20\). In triggering ovulation, mini-IVF
frequently makes use of a gonadotropin-releasing hormone agonist (GnRHa), instead of human chorionic gonadotropin (hCG), to prevent OHSS. A final contentious feature of mini-IVF is a freeze-all-embryo policy to prevent any negative effect of ovarian stimulation on endometrial receptivity\textsuperscript{24}. In observational studies, mini-IVF has been shown to lead to high pregnancy rates, low multiple pregnancy rates, low OHSS rates, and low cost\textsuperscript{21,22}.

The aim of this non-inferiority randomized trial was to compare the effectiveness and safety of the mini-IVF strategy using freeze-all and SET with conventional IVF using fresh double embryo transfer.
Materials and Methods

Study Oversight

This minimal ovarian stimulation trial was designed by investigators at the Academic Medical Center (AMC), Amsterdam and the New Hope Fertility Center (NHFC), New York. All the participants received IVF treatments in a single center (NHFC, New York). The non-inferiority trial was approved by the Institutional Review Board of New York Downtown Hospital (IRB approval reference number: JZ-09-08) and was registered before its start at clinicaltrials.gov: NCT 00799929.

The trial protocol was approved by a protocol review committee and a data and safety monitoring board, both appointed by NHFC and by the institutional review boards of the New York Downtown Hospital and the Biomedical Research Alliance of New York (BRANY). Randomization, monitoring and data analysis were coordinated at the Academic Medical Center in the Netherlands.

Study design and patients

Women were recruited between February 2009 and August 2013 via printed and online media. Participants were responsible to pay for their own medications and they were provided free services (i.e., oocyte retrieval and embryo transfer) as compensation for participation. Inclusion criteria included women aged between 18 and 38, having normal menstrual cycles, requiring a first IVF treatment, and infertility diagnoses of male, unexplained and tubal factors. Exclusion criteria included pre-existing medical conditions (such as diabetes, hypertension, hypothyroidism, and hyperprolactinemia), and BMI <18.5 or >32 kg/m², and day 3 FSH > 12 mIU/mL. Screening tests before initiation of treatment included complete blood count, varicella and rubella titer, pap smear, syphilis, HIV 1 and 2, hepatitis B, hepatitis C, chlamydia,
gonorrhea, prolactin, thyroid-stimulating hormone, cycle day 3 FSH and estradiol ($E_2$). Ultrasound testing was performed at baseline and women with any submucosal or large intramural fibroids requiring surgery were excluded from the study.

Informed consent and randomization

After obtaining written informed consent, women were randomly allocated to mini-IVF or conventional IVF at a 1:1 ratio. The randomization was done at the start of the cycle using sequentially numbered opaque envelopes that had been prepared on basis of a computer-generated list at the Academic Medical Center in the Netherlands and sent to NHFC in New York. Women and medical staff were not blinded for treatment allocation. Outcome data were not disclosed to participants or investigators until completion of the trial.

Mini-IVF

After oral contraceptive pill pre-treatment during 10-14 days, adequate suppression was confirmed with an $E_2$ level of <75 pg/mL. Minimal ovarian stimulation was started with an extended regimen (from day 3 until the day before triggering) of CC (50 mg/day orally) in conjunction with gonadotropin injections (Bravelle and/or Menopur, Ferring, Parsippany, NJ; Follistim, Merck, White House Station, NJ; or Gonal F, EMD Serono, Rockland, MA) starting on cycle day 4-7 with 75-150 IU's daily. No hypothalamic-pituitary suppression was performed and the final maturation of oocytes was induced by a GnRHa nasally (Synarel nasal spray, Pfizer, New York, NY) when the lead follicle reached a diameter of 18 mm or greater (Figure 1). Oocyte retrieval was performed mostly with local anesthesia and follicular flushing was performed as needed. Retrieved oocytes were fertilized by conventional IVF or
ICSI as indicated and subsequently cultured until the blastocyst stage. All blastocysts were vitrified using the CryoTop method (Kitazato Biopharma)\textsuperscript{25}. A single thawed blastocyst was transferred in a subsequent natural or artificially prepared cycle with oral Estrace (Actavis Pharma, Inc, Parsippany, NJ)\textsuperscript{22}.

Conventional IVF

Conventional ovarian stimulation consisted of a long GnRHa protocol using mid-luteal down-regulation (Leuprolide Acetate, Teva, Sellersville, PA) followed by stimulation with daily gonadotropins injections (Bravelle and/or Menopur, Ferring, Parsippany, NJ; Follicst, Merck, White House Station, NJ; or Gonaf, EMD Serono, Rockland, MA) at a dose of 150-300 IU’s daily starting in the early follicular phase (cycle day 3). The final maturation of oocytes was induced with the standard hCG (Novarel, Ferring, Parsippany, NJ; Pregnyl, Merck, White House Station, NJ; or Ovidrel, EMD Serono, Rockland, MA), rather than GnRHa, when at least two follicles reached 18 mm or greater. Oocyte retrieval was performed using mostly general anesthesia because of the presence of a large number of mature follicles. Retrieved oocytes were fertilized by conventional IVF or ICSI according to the same indications as in the mini-IVF protocol and subsequently cultured until the blastocyst stage. Two or one blastocysts– the latter if two embryos were not available or if there was a medical contraindication for DET– were transferred in the fresh cycle. Remaining supernumerary blastocysts were vitrified and two thawed blastocysts or one blastocyst if two were not available were transferred in subsequent naturally or artificially prepared cycles with oral Estrace (Actavis Pharma, Inc, Parsippany, NJ).
Outcomes

The primary outcome was cumulative live birth per randomized woman (including fresh and subsequent frozen embryo transfers) within a time horizon of 6 months. Secondary outcomes were clinical pregnancy rate, OHSS, multiple pregnancy rate, gonadotropin usage, number of retrieved, mature and fertilized oocytes, implantation rate, cancellation rate, and failed fertilization. A clinical pregnancy was defined as at least one intrauterine sac at 6 weeks gestation and live birth was defined as a child born after 22 weeks of gestation or weighing at least 500 grams.

Sample size calculation and statistical analysis

Sample size calculation was based on an expected cumulative live birth rate of 65% in \( \leq 38 \) years old women following conventional IVF based on U.S. SART registry data from 2007 because recruitment started in 2009\(^{17} \). A non-inferiority margin of -10% was considered a clinically significant difference. Thus, 564 women were needed to assure that with a power of 80% the lower limit of a one-sided 95% confidence interval (CI) was within a pre-specified border of -10%.

The effectiveness of mini-IVF versus conventional IVF was expressed as a risk ratio for cumulative live birth, with corresponding 95% CI. The risk difference and 95% CI for live birth as well as the relative risks (RR) and 95% CI for all binary secondary outcomes were calculated. For continuous outcomes, data were expressed as means ± standard deviation (SD) and the difference between both arms was compared using \( t \)-test. Chi-square and Fisher exact tests were used for categorical data as appropriate. The analysis of all outcomes was on an intention to
treat basis. P<0.05 was considered statistically significant. SPSS 22.0 was used to perform all statistical analyses.
Results

A total of 771 women were assessed for eligibility (Figure 2). Of these, 180 women did not fulfill the inclusion criteria (41 had no indication for IVF, 20 had pre-existing medical conditions interfering with IVF treatment, 75 had abnormal screening results, 2 were pregnant at the time of screening, 9 had personal problems, and 33 had multiple reasons) and 27 women did not give informed consent. A total of 564 women were randomly allocated to the mini-IVF strategy or to conventional IVF. Four women in the mini-IVF arm and six women in the conventional IVF arm did not receive the allocated intervention due to withdrawal before starting treatment. Additionally, three women in the mini-IVF arm and six women in the conventional IVF arm dropped-out after starting treatment due to reasons detailed in Figure 2. Cancelled cycles, failed fertilization, and blastocyst developmental failure in each arm are shown in Figure 2. In the mini-IVF arm, of the 281 participants who received the allocated intervention, 6 did not make it to the oocyte retrieval stage (3 had ovarian cyst at the baseline ultrasound, 1 had no follicular development, and 2 prematurely ovulated) and 44 had no embryo transfer for reasons such as failed fertilization, failed blastocyst formation, and spontaneous pregnancy prior to embryo transfer. In the conventional IVF arm, of the 273 participants who received the allocated intervention, 20 did not make it to the oocyte retrieval stage (3 failed to have ovarian suppression and 17 did not have appropriate follicular development) and 22 did not have embryo transfer for similar reasons as those in the mini-IVF arm.

Baseline characteristics did not differ between both arms (Table 1). Most women (68%) were younger than 35 years of age. Primary and secondary outcomes are listed in Table 2. The cumulative live birth rate per randomized woman (including live births from frozen-thawed embryo transfers in both arms of the study) was 49%
in the mini-IVF arm and 63% in the conventional IVF arm, resulting in a RR of 0.78 (95% CI: 0.67 to 0.90). The average absolute difference of mini-IVF versus conventional IVF was -14% (95% CI: -6 to -22%). None of the women in the mini-IVF arm developed OHSS because of the use of GnRHa, while moderate/severe OHSS occurred in 16 (5.7%) women in the conventional IVF arm. Seven of these women were hospitalized and underwent transvaginal paracentesis for symptomatic relief. Multiple pregnancy rate was significantly lower in the mini-IVF arm (6.4% versus 32%, RR= 0.25, 95% CI: 0.14 to 0.46) and they were all monozygotic twins due to the fact that only one embryo was transferred in this arm. Women in the mini-IVF arm required significantly lower total doses of gonadotropins per cycle (459 ± 131 versus 2079 ± 389 IU, p<0.0001).

Laboratory outcome data are summarized in Table 3 and details on the transfer procedures and their outcomes are summarized in Table 4. In total, 340 and 334 fresh and/or frozen embryo transfers procedures were performed in the mini-IVF and conventional IVF arms, respectively. In the mini-IVF arm, strict SET was performed, while in the conventional IVF arm 1.7 embryos on average were transferred in either fresh or subsequent frozen cycles (Table 4). Following the first FET cycle, the implantation rate was significantly higher in the conventional IVF arm (58%) compared to the mini-IVF arm (47%; p=0.02) (Table 4). However, implantation rate declined in subsequent FET cycles in the conventional IVF arm but increased in subsequent FET cycles in the mini-IVF arm to have tendency towards statistical significance in the 3rd FET cycle (30% in the conventional IVF versus 54% in the mini-IVF; p=0.1; Table 4). Additionally, clinical pregnancy rate dropped in subsequent FET cycles in the conventional IVF arm (68% in the first FET cycle then 49%, 40% and 33% in subsequent cycles) while it remained constant in subsequent FET cycles.
in the mini-IVF arm (47% in the first FET cycle then 48%, 54% and 50% in subsequent cycles) (Table 4).
Discussion

This randomized trial compared the mini-IVF strategy including freeze-all and SET to conventional IVF with fresh double embryo transfer in women aged less than 39. The results indicated that the cumulative live birth rate in mini-IVF was 14% lower than that observed in conventional IVF but at the same time mini-IVF completely eliminated OHSS, significantly reduced the risk of multiple pregnancy, and resulted in a 78% reduction in total gonadotropin dose used per cycle.

Our study has several strengths. The design was rigorous and meticulous where only a small number of women withdrew after giving an informed consent and only a small number of women were lost to follow up. The patient population included women of various ethnicities making the results more generalizable. Furthermore, the conventional IVF arm resulted in pregnancy rates, OHSS and multiple pregnancies rates that were comparable to the rates observed in U.S. registries, strengthening our predefined power calculation\(^17\).

There are several limitations to our study. First and foremost, we compared two completely different strategies thus preventing us to disentangle the effects of various components of mini-IVF such as SET and the disengagement of stimulation and transfer. In other words, it is impossible to attribute the observed effects of mini-IVF on live birth rates, OHSS, multiple pregnancy rates and gonadotropin use to the method of ovarian stimulation, to the freeze-all concept or to the single embryo transfer policy as these are all integral parts of mini-IVF. Future randomized controlled trials should focus on strictly evaluating these various components. Second, our study was a single center study, thus hampering generalisability.

Though mini-IVF resulted in significantly lower live birth rates, it is too early to state that it is inferior to conventional IVF. In our power calculation, we used a
reduction of 10% as a clinically relevant reduction in live birth rates following mini-
IVF. The absolute difference observed in our study was -14% with a 95% boundary
of -22% to -6%. Thus, the pre-specified acceptable 10% difference is still within the
95% CI of the observed difference. Furthermore, mini-IVF significantly reduced
OHSS, multiple pregnancy rate and gonadotropin use, thereby increasing safety of
IVF and controlling the cost of unwanted side effects. It is unknown if patients are
willing to trade off these marginally lower live birth rates for increased safety and
reduced costs. It is also unknown whether a lower cost of mini-IVF would motivate
women to undergo more than one mini-IVF cycle for the same price as a single
conventional IVF cycle. In this respect, it would be worthwhile comparing the
cumulative pregnancy and live birth rates of two consecutive mini-IVF cycles with one
conventional IVF cycle. As mini-IVF is also likely to result in less treatment burden
and stress, future trials should consider these additional relevant dimensions into
account. The data provided in this study are the ingredients for shared decision-
making in order to choose between mini-IVF and conventional IVF on a case by case
basis\textsuperscript{39}.

Our mini-IVF protocol was originally developed at Kato Ladies Clinic in Japan
and was successfully adapted and modified by our center\textsuperscript{22}. In contrast to other
treatment protocols where CC was only used for a few days in the early follicular
phase\textsuperscript{27}, CC was administered in our protocol during the whole stimulation phase in
conjunction with a low-dose of gonadotropins. This extended regimen favors the
development of a mild ovarian response but also takes advantage of the ability of CC
to prevent premature LH surge thus reducing the risk of premature ovulation in
addition to decreasing the amount of gonadotropins needed\textsuperscript{19}. We also disengaged
the embryo transfer from the stimulation phase, since it has been demonstrated that
the CC based minimal ovarian stimulation protocol was more efficient when embryos were electively vitrified (preferably at a blastocyst stage) and transferred at a later naturally or artificially prepared frozen embryo transfer cycle\textsuperscript{21}.

Our trial is in line with recent evidence which suggests that segmentation of IVF treatment could overcome the impaired endometrial receptivity frequently occurring in stimulated cycles with gonadotropins\textsuperscript{28}. In this respect, it is of note that the live birth rates per embryo transfer were stable in the mini-IVF arm (39\% in the first frozen embryo transfer cycle then 41\%, 54\% and 50\% in subsequent cycles) while the live birth rates in the conventional IVF arm dropped from 62\% after fresh transfer to 30\% in the fourth frozen embryo transfer cycle. Besides a potential effect on endometrial receptivity, this might indicate an increased embryo quality in the mini-IVF arm as compared to the conventional IVF arm. This hypothesis is yet to be determined.

In conclusion, mini-IVF with SET has a lower live birth rate, eliminates OHSS, reduces gonadotropin consumption, and reduces multiple pregnancy rates compared to conventional IVF with double embryo transfer. How these different dimensions are weighed by couples deciding between mini-IVF or conventional IVF, and whether the lower live birth rate could be offset by a series of “lower cost” mini-IVF cycles, should be the subject of future studies.
References


Table 1. Demographics and baseline characteristics of the participants

<table>
<thead>
<tr>
<th></th>
<th>Mini-IVF</th>
<th>Conventional IVF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized patients</td>
<td>285</td>
<td>279</td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.4±3.6</td>
<td>31.9±4</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>24.7±3.8</td>
<td>24.9±3.8</td>
</tr>
<tr>
<td>Baseline FSH (mIU/mL)</td>
<td>8.6±2.2</td>
<td>8.5±2.3</td>
</tr>
<tr>
<td>Infertility duration (years)</td>
<td>2.4±1.5</td>
<td>2.5±1.5</td>
</tr>
<tr>
<td>Primary infertility</td>
<td>127 (45)</td>
<td>132 (47)</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>207 (73)</td>
<td>207 (74)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>143 (50)</td>
<td>126 (45)</td>
</tr>
<tr>
<td>Black</td>
<td>55 (19)</td>
<td>70 (25)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>42 (15)</td>
<td>46 (16)</td>
</tr>
<tr>
<td>Asian</td>
<td>35 (12)</td>
<td>29 (10)</td>
</tr>
<tr>
<td>Mixed/other</td>
<td>10 (4)</td>
<td>8 (3)</td>
</tr>
<tr>
<td><strong>Infertility diagnoses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubal</td>
<td>78 (27)</td>
<td>101 (36)</td>
</tr>
<tr>
<td>Unknown</td>
<td>69 (24)</td>
<td>66 (24)</td>
</tr>
<tr>
<td>Male</td>
<td>70 (25)</td>
<td>48 (17)</td>
</tr>
<tr>
<td>Mixed male/female</td>
<td>21 (7)</td>
<td>29 (10)</td>
</tr>
<tr>
<td>Other (PCO, DOR, endometriosis, multiple)</td>
<td>47 (16)</td>
<td>35 (12)</td>
</tr>
</tbody>
</table>

Values are expressed as n, mean ± SD or n (%)

P > 0.1 for all comparisons between the mini-IVF and the conventional IVF arms
Table 2. Primary and secondary outcomes in the treatment groups

<table>
<thead>
<tr>
<th>Pregnancy outcome</th>
<th>Mini-IVF</th>
<th>Conventional IVF</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized patients</td>
<td>285</td>
<td>279</td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>161 (57)</td>
<td>211 (76)</td>
<td>0.67 (0.57-0.78)</td>
</tr>
<tr>
<td>Live births</td>
<td>140 (49)</td>
<td>176 (63)</td>
<td>0.78 (0.67-0.90)</td>
</tr>
<tr>
<td>Multiple pregnancy per live births</td>
<td>9 (6.4)</td>
<td>56 (32)</td>
<td>0.25 (0.14-0.46)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stimulation outcome</th>
<th></th>
<th></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total clomiphene dose&lt;sup&gt;c&lt;/sup&gt;</td>
<td>513±101</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Total gonadotropin dose/cycle&lt;sup&gt;c&lt;/sup&gt;</td>
<td>459±131</td>
<td>2079±389</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Days of stimulation&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.7±5.7</td>
<td>10.4±5.7</td>
<td>0.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Peak E&lt;sub&gt;2&lt;/sub&gt; (pg/mL)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1657±1067</td>
<td>3255±2344</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moderate/severe OHSS&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0 (0)</td>
<td>16 (5.7)</td>
<td>&lt;0.0001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as n, n (%) or mean ± SD

<sup>a</sup> t-test  
<sup>b</sup> Chi-square test  
<sup>c</sup> Among those who started ovarian stimulation (n=548)
## Table 3. Laboratory outcome data

<table>
<thead>
<tr>
<th></th>
<th>Mini-IVF</th>
<th>Conventional IVF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized patients</td>
<td>285</td>
<td>279</td>
<td>---</td>
</tr>
<tr>
<td>Oocyte retrievals</td>
<td>275 (97)</td>
<td>253 (91)</td>
<td>0.61&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td># of retrieved oocytes&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.3±3.2</td>
<td>12.8±8</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Inseminated oocytes (IVF or ICSI)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.7±2.8</td>
<td>10±6.7</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fertilized (2PN) oocytes&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.1±2.4</td>
<td>8.3±5.8</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blastocysts (total)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.6±1.9</td>
<td>5.9±4.3</td>
<td>&lt;0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cycles with blastocysts transferred/frozen</td>
<td>234 (82)</td>
<td>235 (84)</td>
<td>0.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as n, mean ± SD or n (%)

<sup>a</sup> *t*-test
<sup>b</sup> Chi-square test
<sup>c</sup> Among those who had oocyte retrieval (n=528)
<sup>d</sup> Among those who reached blastocyst stage (n=469)
Table 4. Outcome of fresh and frozen-thawed embryo transfers in both arms

<table>
<thead>
<tr>
<th></th>
<th>Fresh ET</th>
<th>1st FET</th>
<th>2nd FET</th>
<th>3rd FET</th>
<th>4th FET</th>
<th>5th FET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total # of embryo transfers</td>
<td>---</td>
<td>228</td>
<td>80</td>
<td>26</td>
<td>6</td>
<td>---</td>
</tr>
<tr>
<td>Transferred embryo (s) per cycle</td>
<td>---</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
<td>1±0</td>
<td>---</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>---</td>
<td>106 (47)*</td>
<td>38 (48)</td>
<td>14 (54)</td>
<td>3 (50)</td>
<td>---</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>---</td>
<td>106/228 (47)*</td>
<td>38/80 (48)</td>
<td>14/26 (54)</td>
<td>3/6 (50)</td>
<td>---</td>
</tr>
<tr>
<td>Live birth</td>
<td>---</td>
<td>90 (39)*</td>
<td>33 (41)*</td>
<td>14 (54)</td>
<td>3 (50)*</td>
<td>---</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>---</td>
<td>9 (9.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>---</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>120</th>
<th>111</th>
<th>67</th>
<th>25</th>
<th>9</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total # of embryo transfers</td>
<td>1.7±0.5</td>
<td>1.7±0.5</td>
<td>1.6±0.5</td>
<td>1.5±0.5</td>
<td>1.5±0.5</td>
<td>1.6±0.5</td>
</tr>
<tr>
<td>Transferred embryo (s) per cycle</td>
<td>DET/total # embryo transfers</td>
<td>87/120 (72)</td>
<td>84/111 (75)</td>
<td>37/67 (55)</td>
<td>8/25 (32)</td>
<td>4/9 (44)</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>89 (74)</td>
<td>76 (68)**</td>
<td>33 (49)</td>
<td>10 (40)</td>
<td>3 (33)</td>
<td>0</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>117/207 (56)</td>
<td>113/195 (58)**</td>
<td>42/104 (40)</td>
<td>10/33 (30)</td>
<td>5/13 (38)</td>
<td>0</td>
</tr>
<tr>
<td>Live birth</td>
<td>74 (62)*</td>
<td>67 (60)*</td>
<td>24 (36)*</td>
<td>8 (32)*</td>
<td>3 (33)*</td>
<td>0</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>27 (34)</td>
<td>31 (45)</td>
<td>6 (22)</td>
<td>0</td>
<td>2 (66)</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are expressed as n, mean ± SD, or n (%)

Chi-square test:

- a vs b, p=0.0001, RR= 0.73 (0.62-0.86)
- a vs c, p=0.0003, RR= 0.76 (0.65-0.88)
- d vs e, p=0.61, RR= 1.11 (0.82-1.49)
- f vs g, p=0.16, RR= 1.54 (0.89-2.63)
- h vs i, p=0.62, RR= 1.50 (0.44-5.09)

* vs **, p<0.05
Figure legends

Figure 1: Schematic diagrams of mini-IVF and conventional IVF protocols. In the artificially prepared frozen embryo transfer (part 2) of the mini-IVF protocol, oral estradiol treatment was started on day 3 and given daily then progesterone treatment was added on day 13 onward to the estradiol pills.


Figure 2: Trial profile: screening, eligibility, randomization, and follow-up.
Figure 1

**PART 1**

**Mini IVF**

- Initial Office Visit
- Birth Control Pills (14 d)
- [Randomization]
- 3 - 5
- 21
- Blood & US

**Conventional IVF**

- Initial Office Visit
- Daily Lupron Subcutaneous Injection (28 d)
- [Randomization]
- 3 - 5
- 21
- 3
- Blood & US

**Stimulation**

- Clomid 1 Tab Daily & Low Dose Gonadotropins
- GnRH a Nasal Spray (Ovulation Induction)
- Egg Retrieval & Fertilization IVF/ICSI
- 3
- 8
- 10
- 12
- 14

**PART 2**

**Mini IVF**

- Initial Office Visit
- Birth Control Pills (14 d)
- [Randomization]
- 3 - 5
- 21
- Blood & US

**Conventional IVF**

- Initial Office Visit
- Daily Lupron Subcutaneous Injection (28 d)
- [Randomization]
- 3 - 5
- 21
- 3
- Blood & US

**Stimulation**

- Daily Gonadotropins
- HCG Injection (Ovulation Induction)
- Egg Retrieval & Fertilization IVF/ICSI
- 3
- 8
- 10
- 12
- 14

**Embryo Transfer**

- Daily Estradiol & Progesterone
- Frozen Embryo Transfer
- Pregnancy Test

- 8, 10, 12, 14
- 19
- 26
- Blood & US

- Egg Retrieval & Fertilization IVF/ICSI
- Fresh Embryo Transfer
- Pregnancy Test
- 3 - 5
- 21
- 3
- Blood & US

- Egg Retrieval & Fertilization IVF/ICSI
- Daily Progesterone in Oil Injections
- Fresh Embryo Transfer
- Pregnancy Test
- 3
- 8
- 10
- 12
- 14
- 19
- 26
Figure 2

771 assessed for eligibility

180 Did not meet inclusion criteria
   41 no indication for IVF
   20 pre-existing medical condition preventing/interfering with IVF treatment
   75 abnormal screening results (including BMI, elevated FSH levels, irregular cycles)
   2 pregnant at the time of screening
   9 partnership problems
   33 multiple/other reasons
   27 Declined to participate

564 Randomized

285 Allocated to receive Mini IVF
   3 withdrew consent after randomization
   1 spontaneous pregnancy

281 Received allocated intervention
   6 no egg retrieval
   3 did not start stimulation due to persistent cyst
   2 canceled due to absent follicular development
   2 prematurely ovulated
   44 had no embryo transfer
   2 had no egg retrieved
   9 fertilization failure
   31 had no blastocysts available
   1 spontaneous pregnancy prior to first embryo transfer
   1 withdrawn from treatment
   1 spontaneous pregnancy after failed embryo transfer

279 Allocated to receive conventional IVF
   6 withdrew consent after randomization

273 Received allocated intervention
   20 no egg retrieval
   3 did not start stimulation due to suppression failure
   17 canceled due to insufficient follicular development
   22 had no embryo transfer
   2 fertilization failure
   16 had no blastocysts available
   1 spontaneous pregnancy prior to first embryo transfer
   3 withdrawn from treatment
   2 spontaneous pregnancies after failed embryo transfer
   2 spontaneous abortions (18 and 21 weeks)

3 lost to follow-up

285 Analyzed

4 lost to follow-up

279 Analyzed