Cumulative pregnancy rates after a maximum of nine cycles of modified natural cycle IVF and analysis of patient drop-out: a cohort study

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BACKGROUND: In modified natural cycle IVF (MNV-IVF), treatment is aimed at using the one follicle that spontaneously develops to dominance, using a GnRH antagonist together with gonadotrophins in the late follicular phase only. METHODS: In this single-centre cohort study, nine cycles of MNV-IVF were offered to 268 patients. Cumulative pregnancy rates (CPRs) were calculated and drop-out was analysed. The present study is an extension of earlier studies in which three cycles of MNV-IVF were offered to the same patients. RESULTS: A total of 256 patients completed 1048 cycles (4.1 per patient). Embryo transfer rate was 36.5% per started cycle. Ongoing pregnancy rate was 7.9% per started cycle and 20.7% per embryo transfer. Including treatment-independent pregnancies, the observed CPR after up to nine cycles was 44.4% (95% confidence interval 38.3–50.5) per patient. Pregnancy rates per started cycle did not decline in higher cycle numbers (overall 9.9%). Drop-out rates were high (overall 47.8%). We found that cancellation of oocyte retrieval, fertilization failure and failure to reach embryo transfer are repeating phenomena in subsequent cycles and furthermore that these events predispose for drop-out. CONCLUSIONS: CPR after nine cycles of MNV-IVF in this study was 44.4%. Pregnancy rate per cycle did not decline in higher cycle numbers, possibly due to selective drop-out of poor prognosis patients. Due to the low-risk and patient-friendly nature of the MNC protocol, it seems a feasible treatment option for patients requiring IVF.

Keywords: GnRH antagonist; IVF; minimal stimulation; natural cycle; single embryo transfer

Introduction

In modified natural cycle IVF (MNC-IVF), the one follicle that spontaneously develops to dominance is used for IVF. A GnRH antagonist is administered in the late follicular phase of the natural cycle to prevent unwanted LH-surge and ovulations. Together with the GnRH antagonist, gonadotrophins are used to substitute for an expected fall in estradiol (E₂) levels (Rongiéres-Bertrand et al., 1999).

Rather low pregnancy rates of 0.0–18.3% per started cycle have been found in several studies on MNC-IVF and ICSI (Rongiéres-Bertrand et al., 1999; Ubaldi et al., 2003; Vogel et al., 2003; Kolibianakis et al., 2004; Weghofer et al., 2004; Zhioua et al., 2004; Castelo Branco et al., 2005; Elizur et al., 2005; Pelinck et al., 2006).

However, MNC-IVF offers several advantages. It is a patient-friendly approach due to minimal use of medication, easy oocyte retrieval and negligible risk of ovarian hyper-stimulation syndrome (OHSS). Treatment is easily repeated in consecutive cycles and no resting cycle is necessary in between. Since in most cases only one embryo is available for transfer, the multiple pregnancy rate is low, which is advantageous considering the many problems associated with multiple pregnancies (Fauser et al., 2005). For patients who, for ethical or religious reasons, are opposed to the generation of spare embryos, MNC-IVF forms an attractive alternative to IVF with controlled ovarian stimulation (COS), and legal problems associated with cryopreserved embryos are avoided.

Considering the low-risk and patient-friendly nature of the MNC protocol, it is our opinion that it forms a valuable treatment modality prior to, or as an alternative to, standard IVF with COS. Obviously, a higher number of MNCs will be required to obtain pregnancy rates comparable to those obtained with COS-IVF. In an earlier study, in which patients were interviewed who had undergone either natural cycle IVF or low stimulation IVF with clomiphene citrate, it was found that 66% of patients would opt to undergo six or more cycles before refraining from further treatment (Højgaard et al., 2001). In this study, patients who had undergone COS-IVF...
were also interviewed. Side-effects of hormone treatment as well as stress associated with cancellation of the cycle were perceived as severe or unacceptable by significantly more patients who had undergone COS-IVF as compared with those who had undergone natural cycle or low stimulation IVF (Hoijgaard et al., 2001).

Thus, it seems likely that patients are willing to accept the necessity of a higher number of MNC-IVF cycles in order to obtain acceptable pregnancy rates. Since one treatment cycle of MNC-IVF has a duration of just one menstrual cycle and treatment is easily repeated in consecutive cycles, pregnancy rates per time spent by the patient may be favourable.

So far, little is known about cumulative pregnancy rates (CPRs) after MNC-IVF. We previously found a cumulative ongoing pregnancy rate of 20.8% after three cycles of MNC-IVF (Pelinck et al., 2006).

The additional value of an increase in the number of MNC-IVF cycles to be offered to patients will depend mainly on the willingness of patients to undergo these cycles. In order to determine the optimal number of cycles per patient, it is also important to evaluate whether or not the pregnancy rate per cycle decreases in higher cycle numbers.

In the present cohort study, a maximum of nine cycles of MNC-IVF was offered to patients. We selected nine cycles in order to be able to detect a decline in pregnancy rate, if present, at higher cycle numbers. Available funding also restricted cycle number and we arbitrarily chose nine as the upper limit. Drop-out rates after unsuccessful treatment cycles and pregnancy rates according to cycle number were calculated.

Materials and Methods

Study protocol

The protocol for this study was reviewed and approved by the ethics committee of the University Medical Center Groningen, The Netherlands.

Inclusion criteria for this study were female patient age 18–36 years, first IVF treatment or first IVF treatment after a pregnancy (spontaneously conceived or obtained with COS-IVF), the presence of a regular and proven ovulatory menstrual cycle with a length of 26–35 days and body mass index (BMI: kg/m²) of 18–28. Indications for IVF were tubal pathology, unexplained subfertility, male factor, endometriosis, cervical factor or failed donor inseminations. Patients were not included in the study when an endometriosis cyst was seen on ultrasound. Patients requiring ICSI were not included in this study. Patients with male factor or unexplained subfertility had undergone treatment with intrauterine insemination (IUI) for three to six cycles before starting IVF treatment, as is standard protocol in The Netherlands.

Patients were offered a maximum of nine treatment cycles. Treatments were performed in consecutive menstrual cycles, unless patients requested otherwise. Patients, who decided not to participate in this study, underwent COS-IVF treatment according to standard protocol.

Enrolment of patients to the study took place from March 2001 to August 2004. Treatments were performed between March 2001 and September 2005. All treatments were offered free of charge and prior to starting standard COS-IVF. Until January 2004, medication was fully refunded by insurance companies. In January 2004, refunding policy changed and for some of the patients, medication was no longer refunded.

During the study period, patients who had conceived with MNC-IVF in the past and who returned for treatment after the pregnancy ended, were again offered MNC-IVF but treatments performed upon returning were not included in the cohort studied. Results of the treatments performed in patients after a previous MNC-IVF pregnancy were analysed separately.

End point in the study was pregnancy, defined as either the visualization of at least one intrauterine gestational sac or a proven ectopic pregnancy. Ongoing pregnancy was defined as the presence of an intrauterine gestational sac with fetal heart beat at 12 week gestational age. Live birth was defined as the birth of a living infant after pregnancy duration of at least 24 weeks.

The present study is an extension of earlier studies in which results of the first three cycles of the patients included in this study were described (Pelinck et al., 2005, 2006).

Cycle monitoring

Cycle monitoring was performed as described in detail previously (Pelinck et al., 2005). In short, cycles were monitored by ultrasound and serum E₂ and LH measurements, and cetrotide (GnRH antagonist, 0.25 mg/day: cetrotide®, Serono, The Hague, The Netherlands) together with recombinant FSH (150 IU/day: gonal-F®, Serono Benelux BV, The Netherlands) was started after follicular dominance had developed. Oocyte retrieval was carried out 34 h after ovulation triggering by 10 000 IU of HCG (Pregnyl®, Organon, Oss, The Netherlands) at a follicle size of at least 18 mm and/or plasma E₂ levels of ≥ 1.06 nmol/l.

In cycles where an LH rise was noticed, planning of oocyte retrieval was cancelled according to previously described criteria (Pelinck et al., 2006). For oocyte retrieval, a single lumen aspiration needle was used and no flushing of the follicle was performed. In cases where at the time of planned oocyte retrieval, unexpected ovulation had occurred and tubes were patent, IUI was performed.

Conventional IVF was performed according to standard procedures. No ICSI was done, since patients included in the study had semen quality sufficient for conventional IVF and we expected no higher fertilization rate with ICSI.

Embryo transfer was performed on the third day after oocyte retrieval. For luteal support, HCG 1500 IU was given 5, 8 and 11 days after oocyte retrieval.

Data analysis

Results according to cycle number were calculated per started cycle, per planned, performed and successful oocyte retrieval and are given as percentages. CPRs were calculated as observed CPR per patient, as well as using life table analysis. For life table analysis, two methods were used: the first being traditional life table analysis and the second a corrected estimation, taking into account the number of treatment-independent pregnancies (Kaplan EL, Meier P, 1958). Drop-outs, non-drop-outs and pregnant patients were analysed separately for patient and cycle characteristics. For comparisons, analysis of variance, chi square and 95% confidence intervals (CI) were used where applicable. A separate analysis was performed of results in subsequent cycles of patients who experienced a cancellation of oocyte retrieval, fertilization failure or failure to reach embryo transfer in their first cycle and compared with the results of subsequent cycles of patients where these events did not occur in the first cycle, using 95% CI. A P < 0.05 was considered statistically significant.

Results

Patient characteristics and results of treatment cycles

Patient characteristics are shown in Table 1. Out of 268 included patients, 27 had undergone COS-IVF leading to a pregnancy before entering the study.
Of 268 included patients, 12 withdrew from the study before starting treatment, 5 of these because of the occurrence of a spontaneous pregnancy (1 ectopic, 2 abortions and 2 ongoing). Of the remaining seven, three patients proceeded with COS-IVF and four refrained from all further treatment.

Results according to cycle number are shown in Table 2. Overall, 256 patients started 1048 treatment cycles (4.1 per patient). Median duration of treatment was 19.8 weeks (range 3.7–107.7).

Ninety-four cycles (9.0%) were cancelled before planning of oocyte retrieval. Reasons for cancellation were LH rise or ovulation before or during cetrorelix administration (46 cycles), lack of follicular development or problems with monitoring due to difficult visualization of the ovary (28 cycles), ovarian cysts not spontaneously disappearing (6 cycles), vaginal blood loss during the follicular phase (1 cycle), insufficient semen quality after a febrile episode (1 cycle) and illness or personal reasons (12 cycles).

A further 98 cycles (10.3% per planned oocyte retrieval) were cancelled at the time of planned oocyte retrieval, in one case because of inaccessibility of the ovary and in 97 cases because unexpected ovulation had occurred. Out of 856 oocyte retrievals, 625 were successful (73.0% per attempt). In most cases 1 or 2 oocytes were obtained (576 and 44 cycles, respectively). In five cycles, three or more oocytes were obtained (3, 3, 6, 9 and 20 oocytes, respectively).

In 453 cycles, fertilization occurred (72.5% per successful oocyte retrieval). Due to aberrant fertilization or defective embryo development no embryo transfer was done in 71 of these. In 382 cycles, embryo transfer was done (36.5% per started cycle; 61.1% per successful oocyte retrieval). In 20 cycles, 2 or more embryos were available for transfer and in all of these, double embryo transfer (DET) was done. In all other cycles, one single embryo was transferred (SET).

In 104 cycles, a pregnancy was obtained. One of these occurred spontaneously during a treatment cycle that was cancelled because of an LH surge, 6 occurred after IUI in cases where oocyte retrieval was cancelled because of unexpected ovulation and 97 pregnancies occurred after embryo transfer (91 after SET and 6 after DET). The pregnancy rate was 9.9% (95% CI: 8.1–11.8) per started cycle. Three out of 104 pregnancies were twins (2.9%), of which one occurred after transfer of one single embryo and two occurred after DET. Eighteen pregnancies, including one twin pregnancy, ended in spontaneous abortions, two were ectopic, one was a cervical pregnancy and 83 were ongoing at 12 week gestational age. Ongoing pregnancy rate was 7.9% (95% CI: 6.3–9.6) per

### Table 1: Characteristics of patients undergoing nine cycles of MNV-IVF

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>268</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female patient age (years)*</td>
<td>33.3 (23–36)</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>23.0 (16–34)</td>
</tr>
<tr>
<td>Duration of subfertility (months)*</td>
<td>46.0 (0–121)</td>
</tr>
<tr>
<td>Subfertility (%)</td>
<td>104 (38.8)</td>
</tr>
<tr>
<td>Tubal</td>
<td>82 (30.6)</td>
</tr>
<tr>
<td>Unexplained</td>
<td>106 (39.6)</td>
</tr>
<tr>
<td>Male factor</td>
<td>41 (15.3)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>22 (8.2)</td>
</tr>
<tr>
<td>Cervical factor</td>
<td>8 (3.0)</td>
</tr>
<tr>
<td>Failed AID</td>
<td>9 (3.4)</td>
</tr>
</tbody>
</table>

*aValues are median (range). AID, artificial insemination by donor.

### Table 2: Results according to cycle number of MNV-IVF

<table>
<thead>
<tr>
<th>Cycle number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles started</td>
<td>256</td>
<td>217</td>
<td>181</td>
<td>127</td>
<td>92</td>
<td>69</td>
<td>51</td>
<td>32</td>
<td>23</td>
<td>1048</td>
</tr>
<tr>
<td>OR not planned (%/cycle)</td>
<td>23 (9.0)</td>
<td>18 (9.7)</td>
<td>11 (6.6)</td>
<td>12 (9.4)</td>
<td>5 (5.4)</td>
<td>5 (11.6)</td>
<td>4 (7.8)</td>
<td>–</td>
<td>–</td>
<td>94 (9.0)</td>
</tr>
<tr>
<td>Planned OR cancelled (%/planned OR)</td>
<td>23 (9.9)</td>
<td>18 (9.2)</td>
<td>13 (8.1)</td>
<td>17 (14.8)</td>
<td>10 (11.5)</td>
<td>5 (8.2)</td>
<td>6 (12.8)</td>
<td>4 (12.5)</td>
<td>2 (8.7)</td>
<td>98 (10.3)</td>
</tr>
<tr>
<td>OR performed (%/cycle)</td>
<td>210 (82.0)</td>
<td>178 (82.0)</td>
<td>147 (81.2)</td>
<td>98 (77.2)</td>
<td>77 (83.7)</td>
<td>56 (81.2)</td>
<td>41 (80.4)</td>
<td>28 (87.5)</td>
<td>21 (91.3)</td>
<td>856 (81.7)</td>
</tr>
<tr>
<td>OR successful (%/attempt)</td>
<td>152 (72.4)</td>
<td>134 (75.3)</td>
<td>111 (75.5)</td>
<td>70 (71.4)</td>
<td>56 (72.7)</td>
<td>36 (64.3)</td>
<td>32 (78.0)</td>
<td>18 (64.3)</td>
<td>16 (76.2)</td>
<td>625 (73.0)</td>
</tr>
<tr>
<td>Cycles with fertilization (%/successful OR)</td>
<td>116 (76.3)</td>
<td>93 (69.4)</td>
<td>73 (65.8)</td>
<td>52 (74.3)</td>
<td>42 (75.0)</td>
<td>29 (80.6)</td>
<td>21 (65.6)</td>
<td>13 (72.2)</td>
<td>14 (87.5)</td>
<td>453 (72.5)</td>
</tr>
<tr>
<td>Embryo transfer (%/cycle)</td>
<td>99 (38.7)</td>
<td>76 (35.0)</td>
<td>60 (33.1)</td>
<td>43 (33.9)</td>
<td>37 (40.2)</td>
<td>25 (36.2)</td>
<td>19 (37.7)</td>
<td>11 (34.4)</td>
<td>12 (52.2)</td>
<td>382 (36.5)</td>
</tr>
<tr>
<td>SET</td>
<td>94</td>
<td>73</td>
<td>57</td>
<td>43</td>
<td>35</td>
<td>23</td>
<td>16</td>
<td>9</td>
<td>12</td>
<td>362</td>
</tr>
<tr>
<td>DET</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>–</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>–</td>
<td>20</td>
</tr>
<tr>
<td>Pregnancy rate (%/cycle)</td>
<td>27 (10.5)</td>
<td>20 (9.2)*</td>
<td>19 (10.5)</td>
<td>12 (9.4)*</td>
<td>11 (12.0)*</td>
<td>5 (7.2)*</td>
<td>5 (9.8)*</td>
<td>3 (9.4)*</td>
<td>2 (8.7)</td>
<td>104 (9.9)</td>
</tr>
<tr>
<td>Abortion</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>2*</td>
<td>2</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>Ectopic</td>
<td>–</td>
<td>1*</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1*</td>
<td>–</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>Cervical</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1*</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Ongoing (%/cycle)</td>
<td>25 (9.8)</td>
<td>12 (5.5)</td>
<td>16 (8.8)</td>
<td>11 (8.7)*</td>
<td>10 (10.9)*</td>
<td>5 (7.2)*</td>
<td>3 (5.9)</td>
<td>–</td>
<td>1 (4.3)</td>
<td>83 (7.9)</td>
</tr>
<tr>
<td>Live birth (%/cycle)</td>
<td>24 (9.4)</td>
<td>12 (5.5)</td>
<td>15 (8.3)</td>
<td>11 (8.7)</td>
<td>10 (10.9)</td>
<td>5 (7.2)</td>
<td>3 (5.9)</td>
<td>–</td>
<td>1 (4.3)</td>
<td>81 (7.7)</td>
</tr>
</tbody>
</table>

*aPregnancy after cancelled oocyte retrieval and IUI.
bSpontaneous conception during cycle that was cancelled because of LH surge.

OR, oocyte retrieval.
One pregnancy was interrupted because of severe congenital abnormalities (limb body wall complex). One pregnancy ended in fetal death at 17 week gestation. Live birth was thus 7.7% (95% CI: 6.1–9.4) per cycle.

OHSS did not occur after any of the cycles. Results according to cycle number were not significantly different (95% CI overlapping; not shown). Pregnancy rates per cycle according to subfertility diagnosis were not significantly different (data not shown). CPRs per patient were 43.0% (95% CI: 31.9–54.2), 41.2% (95% CI: 31.4–50.9), 38.5% (95% CI: 22.9–54.0), 35.0% (95% CI: 13.7–56.3), 42.9% (95% CI: 5.4–80.3) and 33.3% (95% CI: 1.9–64.8) for tubal factor, unexplained subfertility, male factor, endometriosis, cervical factor and failed donor inseminations, respectively.

During the study period, 27 patients returned for MNC-IVF after previous MNC-IVF had led to pregnancy (16 spontaneous abortions, 3 ectopic pregnancies and 8 ongoing pregnancies). These patients were again offered nine cycles of MNC-IVF and a further 105 cycles were performed (3.9 per patient). These 105 cycles led to 15 pregnancies, of which 1 miscarried, leading to a pregnancy rate per started cycle of 14.3% (95% CI: 7.5–21.1). All ongoing pregnancies ended in live births. Rate of ongoing pregnancy and live birth was 13.3% (95% CI: 6.7–20.0) per cycle.

Drop-out and CPRs

Drop-out rates and CPRs are specified in Table 3 and Fig. 1.

Out of 268 included patients, 102 (38.1%) left the study before completing 9 cycles because a pregnancy was obtained. Fifteen (5.6%) left the study because of a treatment-independent pregnancy (2 abortions and 13 ongoing pregnancies). Of the remaining 151, 128 (84.8%) dropped out of the study after 0–8 unsuccessful cycles. Of these, 86 (67.2%) proceeded with COS-IVF treatment and 42 (32.8%) stopped treatment altogether. Reasons to stop treatment were related to marital or personal problems in seven cases, illness or operation needed in three cases, problem with sperm donor in one case, problem with semen quality in one case and a problem with the menstrual cycle in one case. One patient moved, and three patients planned to adopt a child. Five patients stated psychological stress or physical burden as the reason to stop further treatment. One patient stated financial problems as the reason to stop treatment. The remaining 19 patients did not state a specific reason for discontinuing treatment.

The drop-out rate (not including those who stopped treatment because of treatment-independent pregnancy) was low after the first and second cycle (3.5 and 6.5%, respectively) and rose sharply thereafter to 13.0–25.5% in further cycles. CPR and ongoing pregnancy rate per patient starting treatment were 40.6% (95% CI: 34.5–46.8) and 32.4% (95% CI: 26.6–38.3). CPR and ongoing pregnancy rate per patient included in the study were 38.8% (95% CI: 32.9–44.8) and 31.0% (95% CI: 25.3–36.6) per patient (Figure 1).
Including treatment-independent pregnancies, CPR and ongoing pregnancy rate per patient included in the study was 44.4% (95% CI: 35.2–53.6) and 35.8% (95% CI: 30.0–41.7) per patient (Figure 1). The cumulative drop-out rate was 47.8% (Figure 1).

CPRs were calculated with life table analysis according to two methods. In the first method, all patients who stopped treatment were censored, leading to a CPR of 59.9% (95% CI: 53.9–65.9). In the second method, patients who stopped treatment because of a spontaneous pregnancy were not censored and considered pregnant in the calculation. All other patients who stopped treatment were censored. CPR according to this method was 63.8% (95% CI: 57.9–69.7).

### Analysis of drop-out

To analyse whether selective drop-out occurred, patients were divided into four groups (patients where a treatment-independent pregnancy occurred excluded): (i) patients dropping out after completing one to four unsuccessful cycles; (ii) patients dropping out after completing five to eight unsuccessful cycles; (iii) patients who completed nine unsuccessful cycles; (iv) patients whose treatment led to pregnancy (cycles in which the pregnancy occurred excluded).

Patient and cycle characteristics of these four groups are presented in Table 4. Age, percentage of primary subfertility and duration of subfertility were not significantly different between groups. The number of oocyte retrievals performed per cycle, as well as fertilization rate and embryo transfer rate were significantly lower in group A compared with groups C and D. When comparing group B with groups C and D, the same trend was seen for number of oocyte retrievals and embryo transfer but differences were not significant. Fertilization rate was significantly lower in group B compared with groups C and D.

In order to analyse whether cancellation of oocyte retrieval, fertilization failure or failure to reach embryo transfer are repeating phenomena in further cycles, results of cycles two to nine of patients where these events occurred were compared with those of patients where they did not.

Results of this analysis are shown in Table 5. The number of performed oocyte retrievals as well as the embryo transfer rate was significantly lower in cycles 2–9 in the group where no oocyte retrieval was performed in the first cycle as compared with the group where an oocyte retrieval was performed.

<table>
<thead>
<tr>
<th>Groupa</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>78</td>
<td>43</td>
<td>21</td>
<td>77</td>
<td>0.20b</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>32.6 (3.2)</td>
<td>33.0 (2.6)</td>
<td>33.4 (2.3)</td>
<td>32.1 (3.0)</td>
<td></td>
</tr>
<tr>
<td>Subfertility primary (%)</td>
<td>50 (64.1)</td>
<td>27 (62.8)</td>
<td>15 (71.4)</td>
<td>47 (61.0)</td>
<td>0.85c</td>
</tr>
<tr>
<td>Duration subfertility (mean ± SD)</td>
<td>51.6 (23.6)</td>
<td>46.8 (19.9)</td>
<td>45.8 (20.8)</td>
<td>43.7 (20.6)</td>
<td>0.16b</td>
</tr>
<tr>
<td>OR performed (%/cycle)</td>
<td>162 (72.6; 66.7–78.6)</td>
<td>211 (77.9; 72.8–82.9)</td>
<td>160 (84.7; 79.4–89.9)</td>
<td>199 (86.5; 82.0–91.0)</td>
<td></td>
</tr>
<tr>
<td>OR successful (%/attempt)</td>
<td>123 (75.9; 69.2–82.6)</td>
<td>148 (70.1; 63.8–76.4)</td>
<td>112 (70.0; 62.8–77.2)</td>
<td>129 (64.8; 58.1–71.6)</td>
<td></td>
</tr>
<tr>
<td>Fertilization (%/successful OR)</td>
<td>64 (52.0; 43.0–61.0)</td>
<td>90 (60.8; 52.8–68.8)</td>
<td>90 (80.4; 72.8–87.9)</td>
<td>101 (78.3; 71.0–85.6)</td>
<td></td>
</tr>
<tr>
<td>ET (%/cycle)</td>
<td>44 (19.7; 14.4–25.1)</td>
<td>72 (26.6; 21.2–31.9)</td>
<td>73 (38.6; 31.5–45.7)</td>
<td>89 (38.7; 32.3–45.1)</td>
<td></td>
</tr>
</tbody>
</table>

*Patients with treatment-independent pregnancies excluded from analysis.

bAnalysis of variance.
cChi square.

A: drop-out after one to four unsuccessful MNCs; B: drop-out after five to eight unsuccessful MNCs; C: nine unsuccessful MNCs completed; D: pregnant (cycle in which pregnancy occurred not included).

Numbers in parentheses are percentages; 95% CI.

<table>
<thead>
<tr>
<th>Results of first cycle</th>
<th>OR not performed</th>
<th>OR performed</th>
<th>Fertilization failure</th>
<th>Fertilization</th>
<th>No ET performed</th>
<th>ET performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>46</td>
<td>210</td>
<td>36</td>
<td>116</td>
<td>157</td>
<td>99</td>
</tr>
<tr>
<td>Number of cycles</td>
<td>138</td>
<td>654</td>
<td>109</td>
<td>333</td>
<td>520</td>
<td>272</td>
</tr>
<tr>
<td>OR performed (%/cycle)</td>
<td>97 (70.3; 62.5–78.1)</td>
<td>549 (83.9; 81.1–86.8)</td>
<td>92 (84.4; 77.5–91.9)</td>
<td>281 (84.0; 80.4–88.4)</td>
<td>418 (80.4; 76.9–83.9)</td>
<td>228 (83.8; 79.4–88.3)</td>
</tr>
<tr>
<td>OR successful (%/attempt)</td>
<td>72 (74.2; 65.3–83.1)</td>
<td>401 (73.0; 69.3–76.8)</td>
<td>67 (72.8; 63.6–82.1)</td>
<td>218 (77.6; 72.6–82.6)</td>
<td>301 (72.0; 67.6–76.4)</td>
<td>172 (75.4; 69.7–81.1)</td>
</tr>
<tr>
<td>Fertilization (%/successful OR)</td>
<td>45 (62.5; 51.1–73.9)</td>
<td>292 (72.8; 68.4–77.3)</td>
<td>31 (46.3; 34.1–58.5)</td>
<td>178 (81.7; 76.4–86.9)</td>
<td>198 (65.8; 60.3–71.3)</td>
<td>139 (80.8; 74.8–86.8)</td>
</tr>
<tr>
<td>ET (%/cycle)</td>
<td>35 (25.4; 18.0–32.8)</td>
<td>248 (37.9; 34.1–41.7)</td>
<td>29 (26.6; 18.1–35.1)</td>
<td>149 (44.7; 39.3–50.2)</td>
<td>161 (31.0; 26.9–35.0)</td>
<td>122 (44.9; 38.8–50.9)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages; 95% CI.
with the group where oocyte retrieval was performed in the first cycle. Patients where fertilization failure occurred in the first cycle showed significantly lower fertilization rate and embryo transfer rate in cycles two to nine as compared with those where fertilization did occur in the first cycle. In patients who failed to reach embryo transfer in the first cycle, fertilization rate and embryo transfer rate were significantly lower in subsequent cycles compared with patients where embryo transfer was done in the first cycle.

Discussion

The present study describes CPRs after nine cycles of MNC-IVF in a cohort of patients. The cohort described was formed by including consecutive patients, and the inclusion rate was almost 100%. Therefore, results of this study can be considered to be obtained in a representative sample of patients under 37 years of age with ovulatory cycles and indication for IVF.

In this study, we calculated observed CPRs as well as an estimation by life table analysis. The difference between the two estimates is rather large and increases in higher cycle numbers. The life table analysis represents an overestimation since it inherently assumes that the chance of pregnancy is the same in drop-outs and those who continue treatment while in our study drop-out was probably selective.

In patients dropping out of the study, age, duration of subfertility and percentage of primary subfertility was not different from those not dropping out. However, the number of cancelled cycles was higher and fertilization rate and embryo transfer rate were lower in the group of drop-outs, showing that cycle cancellation, fertilization failure and failure to reach embryo transfer predispose for drop-out of patients in subsequent cycles. Our analysis also shows that cancellation of the cycle, fertilization failure and failure to reach embryo transfer are repeating phenomena. Surprisingly, in patients with cycle cancellation or failure to reach embryo transfer in the first cycle, the higher rate of cycle cancellation and lower number of embryo transfers in subsequent cycles were not reflected in a lower pregnancy rate. Compared with patients where fertilization did occur in the first cycle, in patients with fertilization failure in the first cycle both fertilization rate and the number of embryo transfers were lower in subsequent cycles, and in this group of patients, a trend towards a lower (not statistically significant) pregnancy rate was found. It is likely that patients dropping out of the study would have had a reduced chance of pregnancy if they had continued treatment. The extent of overestimation of the CPR by the life table approach is of course unknown.

In studies on standard IVF with COS, the life table approach is often used for the analysis of CPRs. In COS-IVF, estimated success rates are also overrated due to selective drop-out of poor prognosis patients. Patients where oocyte yield is low or few embryos are available for transfer, as well as older patients, are often found to be more likely to withdraw from further treatment (De Jong et al., 2002; Stolwijk et al., 2000; Sharma et al., 2002), although in other studies no differences in prognostic factors between drop-outs and those continuing treatment were found (Roest et al., 1998; de Vries et al., 1999). Furthermore, patients considered to have a low chance of pregnancy are often advised to stop treatment (Land et al., 1997; Stolwijk et al., 2000; Olivius et al., 2002). In a recent publication, an overview is given of several studies using life table analysis to calculate estimated success rates after COS-IVF. Estimated success rates from these studies were compared with observed success rates, showing that the extent of overestimation increases with longer follow-up periods and lower observed success rates (Witsenburg et al., 2005).

The observed CPR in our study represents an underestimation of the rate that could be reached in a cohort of patients, since the chance of pregnancy in patients dropping out of the study would not have been zero if they had continued treatment. A realistic estimate of the CPR, corrected for drop-out, will be somewhere between the observed CPR and the life table estimation.

It is rather artificial to correct for drop-outs, since in analysis of IVF results, drop-outs are in most cases not lost to follow-up but rather patients deciding to stop treatment for various reasons. Therefore, drop-out is an inherent part of IVF performance. Corrected estimations can however be used in counseling patients when deciding whether or not to continue treatment.

In the present study, the cumulative drop-out rate was rather high, with 47.8% of the included patients leaving the study before completing nine cycles and without conceiving. Most of these proceeded with COS-IVF (67.2%). Patients refraining from further MNC-IVF may have done so for various reasons. In our study, treatments were offered free of charge and medication was refunded by insurance companies in the majority of treatment cycles and therefore patients' motivation to stop MNC-IVF will in most cases not have been financial in nature. Patients in our study were of course aware of the research setting in which their treatment took place and, being unsure of the chance of success, may have preferred standard COS-IVF. The low multiple pregnancy rate in MNC-IVF is advantageous, but in several studies, it was shown that for many patients, a multiple pregnancy is not considered an adverse outcome (Grobman et al., 2001; Kalra et al., 2003; Child et al., 2004; Murray et al., 2004). The very low risk of OHSS is an advantage of MNC-IVF but since OHSS is also rare in standard COS-IVF, patients may not perceive this as very important. Also, although the MNC protocol appears to be patient-friendly due to minimal use of hormone medication and thus few side-effects, this may be perceived differently by patients. Frequent visits to the clinic and a high number of oocyte retrievals needed to obtain a pregnancy may be burdensome for patients, and disappointments due to cancellation of oocyte retrieval, unsuccessful oocyte retrieval, fertilization failure and failure to reach embryo transfer often occur.

In our study, no active censoring was done, as no patient was denied further treatment after unsuccessful cycles. However, since during the course of this study it became clear that cancellation of oocyte retrieval, fertilization failure and failure to reach embryo transfer are repeating phenomena in further cycles, patients were given information on this, which may have motivated some patients to discontinue MNC-IVF treatment.
The pregnancy rate per started cycle found in this study is low due to considerable loss in every step of the procedure. Further research into adjustments to the protocol, such as changes in timing of ovulation triggering or dose of cetrorelix, is needed to improve its effectiveness, as discussed previously (Pelinck et al., 2006). The application of ICSI may improve fertilization rates and increase the number of embryo transfers. In our study, a rather large number of cycles were cancelled due to lack of follicular development or problems with visualization of the ovary, which could have been prevented by a stricter selection of patients.

Pregnancy rate per cycle and CPR per patient were not different according to indication for IVF. In our study, the proportion of patients with unexplained subfertility is rather high and seems to be higher than in the general population. This may be explained by the fact that only patients with regular and proven ovulatory cycles were included and patients with severe male factor infertility requiring ICSI were not.

In patients returning for treatment after previous unsuccessful MNC-IVF, the pregnancy rate was higher than in our study group (14.3% vs. 9.9% per started cycle, respectively), illustrating that by selecting good prognosis patients results can be considerably improved. Further research is needed to optimize selection of patients who are likely to do well with MNC-IVF. In choosing between MNC-IVF and COS-IVF, benefits and drawbacks of both treatment modalities should be considered, as well as the expected pregnancy rates with either treatment. For instance, poor responders to COS are likely to benefit from MNC-IVF since with COS these patients will have only few oocytes and a low embryo transfer rate. With less time and costs, comparable results could be obtained with MNC-IVF (Tartlatzis et al., 2003; Castelo Branco et al., 2005; Elizur et al., 2005; Ubaldi et al., 2005). In specific situations, such as patients with a history of severe OHSS or those opposing to the creation of supernumery embryos, MNC-IVF may be preferred over COS-IVF. For normal responders to COS, comparative studies are warranted to evaluate the effectiveness of MNC-IVF relative to COS-IVF. Obviously, normal responder patients, a higher number of MNC-IVF cycles will be needed to obtain pregnancy rates comparable to those obtained after stimulated IVF. In this respect, the higher number of oocyte retrievals needed with MNC-IVF and associated risks of infection and bleeding should be taken into account. Any comparative study should not only evaluate effectiveness but also focus on cost-effectiveness and quality of life. Although the drop-out rates in standard COS-IVF are also high, the high drop-out rate in our study suggests that the physical and emotional burden for patients undergoing MNC-IVF is considerable.

MNC-IVF offers several advantages, being associated with a negligible risk of OHSS and a very low multiple pregnancy rate. A treatment cycle has a short duration and treatments are easily repeated in consecutive cycles. Per cycle, MNC-IVF is cheaper than COS-IVF due to minimal use of hormonal medication and in terms of costs per live birth, MNC-IVF may be cost-effective compared with COS-IVF (Groen et al., 2005). In a recent study done in our centre, birthweights of singletons conceived by MNC-IVF and COS-IVF were compared. A significantly higher mean birthweight was found in the MNC-IVF group, whereas patient characteristics in both groups were similar (Keizer et al., 2005). Although this should be confirmed in further studies, it is an important finding since higher birthweights probably reflect better health of newborns. Considering the advantages of MNC-IVF, it is our opinion that it is a valuable treatment option preceding standard IVF with COS, even if pregnancy rates per cycle are lower than those obtained with COS-IVF.

In recent papers, it is often proposed that the best outcome definition of success after IVF is the live birth rate per started treatment consisting of consecutive cycles (Fauser et al., 2002; Vail and Gardener, 2003; Heijnen et al., 2004). When MNC-IVF is applied before COS-IVF, the outcome parameter would be the cumulative live birth rate after sequential treatment with MNC-IVF, if necessary followed by COS-IVF.

Since the CPR after MNC-IVF found in our study is 40.6% and many of those not conceiving with MNC proceeded with COS-IVF, the overall CPR per patient probably will be favourable. In applying sequential treatment with MNC-IVF followed by COS-IVF, those patients conceiving with MNC-IVF will not be exposed to the risks and burden of COS-IVF. For now, implementation of MNC-IVF is hampered by existing reimbursement systems, which are usually based on a maximum number of cycles to be performed, leading to a need to maximize pregnancy rates per cycle rather than per treatment period.

The optimal number of treatment cycles per patient remains unclear. The pregnancy rate per cycle appears to remain constant throughout higher cycle numbers and the decline in steepness of the cumulative pregnancy curve is mainly caused by drop-out of patients during the study, suggesting that patients should be advised to undergo at least nine cycles of MNC-IVF before starting COS-IVF. However, the apparent absence of a decline in pregnancy rates in higher cycle numbers may be caused by selective drop-out of patients with a possible poor prognosis, so nine cycles may not be the suitable number for all patients. Since the occurrence of a cancellation of oocyte retrieval, fertilization failure and failure to reach embryo transfer all seem to be repeating phenomena in further cycles, patient counselling on the number of cycles to be performed should be individualized, taking into account the results of previous cycles.

In conclusion, a CPR of 40.6% was found after nine cycles of MNC-IVF. Including treatment-independent pregnancies, the CPR was 44.4%. Drop-out rates were high, especially in higher cycle numbers. The pregnancy rate per started cycle does not appear to decline in higher cycle numbers, possibly caused by selective drop-out of poor prognosis patients. Considering the advantages of MNC-IVF, the very low multiple pregnancy rate and negligible risk of OHSS in particular, it is our opinion that MNC-IVF offers a valuable treatment modality for patients requiring IVF.

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