Human Reproduction Vol.21, No.9 pp. 2375–2383, 2006 doi:10.1093/humrep/del192
Advance Access publication June 3, 2006.

Cumulative pregnancy rates after three cycles of minimal stimulation IVF and results according to subfertility diagnosis: a multicentre cohort study

M.J.Pelinck[1,5], N.E.A.Vogel[1], A.Hoek[1], A.H.M.Simons[1], E.G.J.M.Arts[1], M.H.Mochtar[2], S.Beemsterboer[3], M.N.Hondelink[4] and M.J.Heineman[1]

[1]Section of Reproductive Medicine, Department of Obstetrics and Gynaecology, University Medical Center Groningen, Groningen, [2]Center for Reproductive Medicine, Department of Obstetrics and Gynaecology, Academic Medical Center, [3]Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Vrije Universiteit Medical Center, Amsterdam and [4]Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Isala Clinics, Location Sophia, Zwolle, The Netherlands

[5]To whom correspondence should be addressed at: Section of Reproductive Medicine, Department of Obstetrics and Gynaecology, University Medical Center Groningen, Hanzeplein 1, Groningen 9700 RB, The Netherlands. E-mail: pelinck@hotmail.com

BACKGROUND: In minimal stimulation IVF, treatment is aimed at using the single oocyte that spontaneously develops to dominance. To prevent untimely ovulation, a GnRH antagonist is administered in the late follicular phase of the natural cycle together with recombinant FSH for substitution. Owing to the lack of ovarian stimulation, minimal stimulation IVF is a low-risk and patient-friendly treatment. In this study, effectiveness of minimal stimulation IVF was studied. METHODS: In this prospective multicentre cohort study, minimal stimulation IVF was offered to 350 patients. All indications for conventional IVF were included. Main outcome measures were pregnancy rates per cycle and cumulative pregnancy rates after three cycles. RESULTS: A total of 336 patients completed 844 cycles (2.5 per patient). The overall ongoing pregnancy rate per started cycle was 8.3% [95% confidence interval (CI) 6.4–10.2%]. The cumulative ongoing pregnancy rate after up to three cycles was 20.8% (95% CI 16.4–25.3%) per patient. No differences were found according to indication for IVF. CONCLUSIONS: Minimal stimulation IVF seems suitable for all indications studied. Pregnancy rates are encouraging. Owing to the low-risk and patient-friendly nature of this protocol, it seems a feasible treatment option for patients requiring IVF.

Key words: GnRH antagonist/IVF/minimal stimulation/natural cycle/single-embryo transfer

Introduction

In minimal stimulation IVF, treatment is aimed at using the one dominant follicle that spontaneously develops in a natural cycle. Because of the minimum use of medication, minimal stimulation IVF offers several advantages. A GnRH antagonist is only used in the late follicular phase, to prevent untimely LH surges and the consequent cancellation of oocyte retrieval, as is the administration of gonadotrophins to substitute for the expected fall in estradiol (E_2) (Paulson *et al*., 1994; Rongières-Bertrand *et al*., 1999).

Because gonadotrophins are administered in a low dose and only one or few follicles develop, the risk of the ovarian hyperstimulation syndrome (OHSS) is negligible. Minimal stimulation IVF is also a patient-friendly treatment as medication is administered for a few days only, causing few side effects, and the duration of a treatment cycle is considerably shorter than standard IVF with controlled ovarian stimulation (COS). As usually only one follicle is aspirated, oocyte retrieval is easy and short lasting and can be performed without analgesia (Ramsewak *et al*., 1990). As opposed to COS-IVF, in minimal stimulation IVF, no resting cycle is necessary after a failed treatment cycle, and treatments are easily repeated in consecutive cycles. Because usually no spare embryos are generated, minimal stimulation IVF is an attractive treatment option for patients who, for ethical or religious reasons, are opposed to the generation of spare embryos (Biggers and Summers, 2004).

Data on efficacy of minimal stimulation IVF according to cause of subfertility are scarce. So far, only small studies describing minimal stimulation IVF have been published with pregnancy rates per started cycle varying between 0.0 and 18.3% (Rongières-Bertrand *et al*., 1999; Kadoch *et al*., 2003; Ubaldi *et al*., 2003; Vogel *et al*., 2003; Kolibianakis *et al*., 2004; Weghofer *et al*., 2004; Zhioua *et al*., 2004; Elizur *et al*., 2005; Pelinck *et al*., 2005). In most of these studies, ICSI was performed in all cycles, either electively because of the expected small number of oocytes or because of severe male factor infertility (Rongières-Bertrand *et al*., 1999; Ubaldi *et al*., 2003; Vogel *et al*., 2003; Weghofer *et al*., 2004; Zhioua *et al*.,

2004). In a pilot study performed in the University Medical Center Groningen, Groningen, where cumulative pregnancy rates after a maximum of three cycles of minimal stimulation IVF were assessed, only conventional IVF was performed. In this study, ongoing pregnancy rates per started cycle were 14.0, 14.3 and 10.7% for tubal factor, unexplained and male factor subfertility, respectively, indicating an advantage for tubal factor and unexplained subfertility (Pelinck *et al*., 2005).

It is unclear how efficacy of minimal stimulation IVF compares to COS-IVF. In two recent studies, similar pregnancy rates per cycle were found in minimal stimulation IVF and COS-IVF (Weghofer *et al*., 2004; Elizur *et al*., 2005). However, these studies included only women of ≥40 years of age or poor-responder patients, so these results are not applicable to the general IVF population (Weghofer *et al*., 2004; Elizur *et al*., 2005).

To evaluate effectiveness of minimal stimulation IVF however, cumulative pregnancy rates are more relevant than pregnancy rates per started cycle. Owing to the patient-friendly nature of the minimal stimulation protocol, it is possible that dropout rates will be relatively low. Moreover, as treatments are easily repeated in consecutive cycles, pregnancy rates per time spent by the patient may be favourable. In the pilot study performed in our centre (University Medical Center Groningen), we found a cumulative ongoing pregnancy rate of 34.0% after three cycles of minimal stimulation IVF (Pelinck *et al*., 2005).

The purpose of the present multicentre cohort study was 2-fold. First, we wished to evaluate effectiveness of minimal stimulation IVF according to cause of subfertility, and secondly, cumulative pregnancy rates after three cycles were calculated. So far, this is the largest series of minimal stimulation IVF.

Materials and methods

Study protocol

In this multicentre cohort study, the University Medical Center Groningen (centre A), the Academic Medical Center Amsterdam (centre B), the Vrije Universiteit Medical Center Amsterdam (centre C) and the Isala Clinics Zwolle (centre D) participated.

The study protocol was reviewed and approved by the local ethical committees of the participating centres. Inclusion criteria for this study were female patient age 18–36 years, first IVF treatment ever or first IVF treatment after a pregnancy, the presence of a regular and proven ovulatory menstrual cycle with a length of 26–35 days and BMI (kg/m^2) of 18–28. Indications for IVF were tubal pathology, unexplained subfertility, male factor, endometriosis, cervical factor or failed artificial inseminations with donor semen (AID). Patients were not included in the study in case 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 three free treatment cycles. In a subgroup of patients in centre A, nine free cycles were offered. For this study, the first three cycles were analysed. Treatments were performed in three consecutive menstrual cycles, unless patients requested otherwise. Patients who decided not to participate in this study underwent COS-IVF treatment according to standard protocol.

Inclusion of the patients took place from January 2001 to June 2004. Treatments were performed between January 2001 and January 2005. This study is an extension of a pilot study performed in centre A, in which 50 patients were studied (Pelinck *et al*., 2005).

Ultrasound monitoring was started on cycle day 3 or 8 and repeated daily or every other day, according to the size of the lead follicle. Follicle diameter was measured in three perpendicular planes, and the mean value was taken. When a lead follicle with a mean diameter of at least 14 mm was observed, daily injections of 0.25 mg of the GnRH antagonist cetrorelix (Cetrotide®, Serono, the Hague, the Netherlands) together with 150 IU recombinant FSH (r-FSH, Gonal-F®, Serono Benelux BV, the Netherlands) were started. Cetrorelix was continued up to and including the day of ovulation triggering, and r-FSH was continued up to the day of ovulation triggering. Patients were instructed to have their injections in the evening and at the same time daily, to ensure a 24 h interval between injections.

Blood was taken for assessment of serum concentrations of LH and E_2 on the days ultrasound was performed. In centres A and B, LH levels were determined the same day and were taken into account for planning of oocyte retrieval and ultrasound examinations. In centres C and D, LH levels were not taken into account for planning of oocyte retrieval and ultrasound examinations, because results usually were not yet available at the time of planning. In centres A, B and C, E_2 levels were available at the time of planning, whereas in centre D they were not.

Blood samples were taken in the morning, so serum concentrations reflected levels 12–16 h after administration of the medication.

Ovulation triggering was achieved by subcutaneous injection of 10 000 IU of HCG (Pregnyl®, Organon, Oss, the Netherlands) when a follicle with a diameter of at least 18 mm was observed and/or plasma E_2 levels were ≥0.8 nmol/l.

Cycles were cancelled when an LH level of ≥20.0 IU/l was noticed at a follicle size of <15 mm (before medication was started). In cases where an LH level of 10.0–30.0 IU/l was noticed at a follicle size of ≥15 mm (after medication start), the cycle was not cancelled because we hypothesized that cetrorelix should be capable of blunting the LH surge enough to allow for planned oocyte retrieval. In cases where an LH level of ≥30.0 IU/l was noticed, planning of oocyte retrieval was cancelled.

Transvaginal ultrasound-guided follicle aspiration was performed 34 h after ovulation triggering. A single lumen aspiration needle was used. No flushing of the follicle was performed. Analgesia was only given on patient request. Only large (dominant) follicles were aspirated. 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 local standard procedures. Embryo transfer was performed on the third day after oocyte retrieval. For luteal support, HCG 1500 IU (Pregnyl®, Organon) was given 5, 8 and 11 days after oocyte retrieval.

Pregnancy was defined as the ultrasound visualization of an 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 amenorrhoea.

Data analysis

Patient characteristics according to participating centre and indication for IVF were compared using Kruskal–Wallis and Chi-square tests where applicable. Results (per started cycle, per oocyte retrieval, per embryo transfer and per patient, according to indication for IVF and cycle number) are given as percentages with 95% confidence intervals (CI). A separate analysis was performed of results in second and third

cycles of patients who experienced a cancellation of oocyte retrieval, an unsuccessful oocyte retrieval or fertilization failure in their first cycle and compared with the results of second and third cycles of patients where these events did not occur in the first cycle.

Results

Patient characteristics

In centres A, B, C and D, 303, 21, 16 and 10 patients were included, respectively. Patient characteristics according to participating centre were not significantly different (Table I).

Patient characteristics according to indication for IVF are summarized in Table II. The median age of the patients and the median duration of subfertility were significantly different between indications ($P = 0.02$ and $P < 0.001$, respectively). The median BMI was not different between indications ($P = 0.92$). Subfertility was primary in 56.0% of patients and secondary in 44.0% and significantly different between indications, secondary subfertility being most frequent in patients with tubal factor ($P = 0.001$).

Overall results

Results according to indication for IVF and cycle number are summarized in Tables III and IV. Results according to participating centre showed no significant differences (data not shown).

Figures 1 and 2 are summaries of both Tables III and IV. Overall, 57 patients dropped out of the study, in 14 cases before start of any treatment (Figure 1). In five of these, the reason for dropout was the occurrence of a spontaneous pregnancy. Forty-three patients dropped out of the study after one or two unsuccessful treatment cycles, in seven of these because of the occurrence of a spontaneous pregnancy.

A summary of all started cycles is shown in Figure 2. A total of 844 cycles were started in 336 patients. Of 844 started cycles, 55 (6.5%) were cancelled before medication was started because of lack of follicular development (16 cases), premature LH rise or ovulation (25 cases) or other reasons (14 cases). A further 25 cycles where cancelled after cetrorelix and r-FSH administration was started, because of an LH surge or ovulation (15 cases), stop in follicular growth (5 cases) or other reasons (5 cases).

Of 844 started cycles, 764 oocyte retrievals were planned (90.5%). Of these, 69 (9.0%) were cancelled, in one case because of inaccessibility of the ovary during oocyte retrieval and in 68 cases because of premature ovulation, where despite correct administration of medication, no follicle was present at the time of planned oocyte retrieval. Of the cycles where oocyte retrieval was planned, LH levels on the day medication was started and on the day ovulation was triggered were known in 730 and 735 cases, respectively. In 158 cases (21.6%), LH level was ≥10.0 IU/l at the time medication was started. Of these, 30 (19.0%) were cancelled at the time of oocyte

Table I. Patient characteristics according to participating centre

	Centre A	Centre B	Centre C	Centre D	Total	*P*
Number of patients (*n*)	303	21	16	10	350	
Female patient age (years)	33.0 (22–37)	34.0 (25–36)	32.0 (29–36)	32.0 (23–36)	33.0 (22–37)	0.94[a]
BMI (kg/m^2)	23.0 (16–34)	24.0 (18–29)	21.5 (20–26)	23.5 (21–33)	23.0 (16–34)	0.35[a]
Subfertility [*n* (%)]						
Primary	172 (56.8)	11 (52.4)	11 (68.8)	2 (20.0)	196 (56.0)	0.09[b]
Secondary	131 (43.2)	10 (47.6)	5 (31.3)	8 (80.0)	154 (44.0)	
Duration of subfertility (months)	46.0 (0–121)	47.0 (8–111)	52.0 (17–90)	42.0 (8–77)	46.0 (0–121)	0.31[a]
Indication [*n* (%)]						
Tubal	95 (31.4)	7 (33.3)	5 (31.3)	2 (20.0)	109 (31.1)	0.22[b]
Unexplained	117 (38.6)	4 (19.0)	8 (50.0)	3 (30.0)	132 (37.7)	
Male factor	47 (15.5)	7 (33.3)	1 (6.3)	4 (40.0)	59 (16.9)	
Endometriosis	25 (8.3)	1 (4.8)	2 (12.5)	—	28 (8.0)	
Cervical factor	10 (3.3)	—	—	1 (10.0)	11 (3.1)	
Failed AID	9 (3.0)	2 (9.5)	—	—	11 (3.1)	

AID, artificial insemination by donor. Values are expressed as median (range) where applicable.
[a]Kruskal–Wallis test.
[b]Chi-square test.

Table II. Patients' characteristics according to indication for IVF

	Tubal	Unexplained	Male factor	Endometriosis	Cervical factor	Failed AID	Total	*P*
Number of patients (*n*)	109	132	59	28	11	11	350	
Female patient age (years)	33.0 (22–36)	32.0 (23–36)	33.0 (23–37)	31.5 (26–36)	35.0 (30–36)	35.0 (30–36)	33.0 (22–37)	0.02[a]
BMI (kg/m^2)	22.0 (17–34)	23.0 (16–30)	23.0 (18–33)	22.0 (19–30)	23.0 (18–26)	21.0 (18–34)	23.0 (16–34)	0.92[a]
Subfertility [*n* (%)]								
Primary	45 (41.3)	82 (62.1)	33 (55.9)	22 (78.6)	5 (45.5)	9 (81.8)	196 (56.0)	0.001[b]
Secondary	64 (58.7)	50 (37.9)	26 (44.1)	6 (21.4)	6 (54.5)	2 (18.2)	154 (44.0)	
Duration of subfertility (months)	34.0 (0–98)	51.0 (0–121)	47.0 (3–111)	47.5 (8–98)	50.5 (3–105)	64.5 (31–107)	46.0 (0–121)	≤0.001[a]

AID, artificial insemination by donor. Values are expressed as median (range) where applicable.
[a]Kruskal–Wallis test.
[b]Chi-square test.

Table III. Results according to indication for IVF

Indication	Tubal	Unexplained	Male factor	Endometriosis	Cervical factor	Failed AID	Total
Number of patients included	109	132	59	28	11	11	350
Number of patients started	106	128	55	26	10	11	336
Cycles started (number per patient)	263 (2.5)	323 (2.5)	136 (2.5)	62 (2.4)	29 (2.9)	31 (2.8)	844 (2.5)
Cycles cancelled before medication started	16	21	7	9	2	—	55
Percentage per cycle (95% CI)	6.1 (3.1–9.0)	6.5 (3.8–9.2)	5.1 (1.4–8.9)	14.5 (5.6–23.5)	6.9 (0.0–16.3)	0.0	6.5 (4.8–8.2)
Cetrorelix administration (days), median (range)	3.0 (1–12)	3.0 (1–11)	3.0 (1–9)	3.0 (1–6)	3.0 (1–5)	3.0 (1–9)	3.0 (1–12)
Cycles cancelled before HCG	5	9	7	4	—	—	25
Percentage per cycle (95% CI)	1.9 (0.2–3.6)	2.8 (1.0–4.6)	5.1 (1.4–8.9)	6.5 (0.2–12.7)	0.0	0.0	3.0 (1.8–4.1)
OR planned	242	293	122	49	27	31	764
Percentage per cycle (95% CI)	92.0 (88.7–95.4)	90.7 (87.5–93.9)	89.7 (84.5–94.9)	79.0 (68.7–89.4)	93.1 (83.7–100.0)	100.0	90.5 (88.5–92.5)
Planned OR cancelled	22	26	7	6	3	5	69
Percentage per planned OR (95% CI)	9.1 (5.4–12.8)	8.9 (5.6–12.2)	5.7 (1.5–9.9)	12.2 (2.9–21.6)	11.1 (0.0–23.2)	16.1 (2.9–29.3)	9.0 (7.0–11.1)
OR performed	220	267	115	43	24	26	695
Percentage per cycle (95% CI)	83.7 (79.1–88.2)	82.7 (78.4–86.9)	84.6 (78.4–90.8)	69.4 (57.6–81.1)	82.8 (68.7–96.8)	83.9 (70.7–97.1)	82.3 (79.7–85.0)
OR successful	170	201	86	35	14	17	523
Percentage per attempt (95% CI)	77.3 (71.6–82.9)	75.3 (70.0–80.6)	74.8 (66.7–82.9)	81.4 (69.5–93.3)	58.3 (38.2–78.5)	65.4 (46.7–84.0)	75.3 (72.0–78.5)
TMSC (million), median (range)	89.0 (1.0–560.0)	58.0 (1.0–760.0)	18.0 (0.807–120.0)	130.0 (30.8–750.0)	30.0 (4.3–260.0)	3.65 (0.300–8.3)	54.0 (0.300–760.0)
Cycles with fertilization	140	136	46	32	10	12	376
Percentage per successful OR (95% CI)	82.4 (76.5–88.2)	67.7 (61.1–74.3)	53.5 (42.7–64.2)	91.4 (82.0–100.0)	71.4 (47.3–95.6)	70.6 (48.5–92.7)	71.9 (68.0–75.8)
Embryo transfer	120	112	41	26	10	8	317
Percentage per cycle (95% CI)	45.6 (39.5–51.8)	34.7 (29.4–40.0)	30.1 (22.3–38.0)	41.9 (29.4–54.5)	34.5 (16.8–52.1)	25.8 (10.1–41.5)	37.6 (34.2–40.9)
Single-embryo transfer	114	106	39	24	8	8	299
Double-embryo transfer	6	6	2	2	2	—	18
Pregnancy	28	30[a]	15	8	2	2	85[a]
Percentage per cycle (95% CI)	10.6 (6.8–14.5)	9.3 (6.1–12.5)	11.0 (5.7–16.4)	12.9 (4.4–21.4)	6.9 (0.0–16.3)	6.5 (0.0–15.3)	10.1 (8.0–12.1)
Percentage per embryo transfer (95% CI)	23.3 (15.6–31.1)	25.9 (17.6–34.2)[a]	36.6 (21.5–51.6)	30.8 (12.7–48.9)	20.0 (5.3–45.3)	25.0 (0.0–55.6)	26.5 (21.5–31.5)[a]
Abortion	3	6	3	—	1	—	13
Ectopic	—	1	—	—	—	—	1
Cervical	—	—	—	1	—	—	1
Ongoing	25	23	12	7	1	2	70
Percentage per cycle (95% CI)	9.5 (5.9–13.1)	7.1 (4.3–10.0)	8.8 (4.0–13.7)	11.3 (3.3–19.3)	3.4 (0.0–10.2)	6.5 (0.0–15.3)	8.3 (6.4–10.2)
Percentage per embryo transfer (95% CI)	20.8 (13.4–28.2)	20.5 (12.9–28.2)	29.3 (15.1–43.5)	26.9 (9.5–44.3)	10.0 (0.0–29.0)	25.0 (0.0–55.6)	22.1 (17.4–26.7)
Cumulative ongoing pregnancy rate	25	23	12	7	1	2	70
Percentage per patient (95% CI)	23.6 (15.3–31.8)	18.0 (11.2–24.8)	21.8 (10.7–32.9)	26.9 (9.5–44.3)	10.0 (0.0–29.0)	18.2 (0.0–41.4)	20.8 (16.4–25.3)
Live birth rate	24	21[b]	12	7	1	2	67[b]
Percentage per patient (95% CI)	22.6 (14.5–30.8)	16.5 (9.9–23.1)[b]	21.8 (10.7–32.9)	26.9 (9.5–44.3)	10.0 (0.0–29.0)	18.2 (0.0–41.4)	19.9 (15.6–24.3)[b]

AID, artificial insemination by donor; CI, confidence interval; OR, oocyte retrieval; TMSC, total motile sperm count.
[a]One ectopic pregnancy after cancelled oocyte retrieval and intrauterine insemination.
[b]One pregnancy: outcome unknown; not included in calculation.

Table IV. Results according to cycle number

Cycle number	Cycle 1	Cycle 2	Cycle 3	Total
Cycles started	336	277	231	844
Cycles cancelled before medication started	17	20	18	55
Percentage per cycle (95% CI)	5.1 (2.7–7.5)	7.2 (4.1–10.3)	7.8 (4.3–11.3)	6.5 (4.8–8.2)
Cetrorelix administration (days), median (range)	3.0 (1–12)	3.0 (1–11)	3.0 (1–8)	3.0 (1–12)
Cycles cancelled before HCG	9	9	7	25
Percentage per cycle (95% CI)	2.7 (0.9–4.4)	3.2 (1.1–5.4)	3.0 (0.8–2.3)	3.0 (1.8–4.1)
OR planned	310	248	206	764
Percentage per cycle (95% CI)	92.3 (89.3–95.2)	89.5 (85.9–93.2)	89.2 (85.1–93.3)	90.5 (88.5–92.5)
Planned OR cancelled	30	22	17	69
Percentage per planned OR (95% CI)	9.7 (6.3–13.0)	8.9 (5.3–12.5)	8.3 (4.4–12.1)	9.0 (7.0–11.1)
OR performed	280	226	189	695
Percentage per cycle (95% CI)	83.3 (79.3–87.4)	81.6 (76.9–86.2)	81.8 (76.7–86.9)	82.3 (79.7–85.0)
OR successful	210	167	146	523
Percentage per attempt (95% CI)	75.0 (69.8–80.2)	73.9 (68.1–79.7)	77.2 (71.1–83.3)	75.3 (72.0–78.5)
TMSC (million), median (range)	68.0 (1.0–760.0)	54.0 (0.807–710.0)	51.0 (0.911–510.0)	56.0 (0.807–760.0)[a]
Cycles with fertilization	158	118	100	376
Percentage per successful OR (95% CI)	75.2 (69.3–81.2)	70.7 (63.6–77.7)	68.5 (60.8–76.2)	71.9 (68.0–75.8)
Embryo transfer	136	99	82	317
Percentage per cycle (95% CI)	40.5 (35.1–45.8)	35.7 (30.0–41.5)	35.5 (29.2–41.8)	37.6 (34.2–40.9)
Single-embryo transfer	127	95	77	299
Double-embryo transfer	9	4	5	18
Pregnancy	38	24[b]	23	85[b]
Percentage per cycle (95% CI)	11.3 (7.9–14.8)	8.7 (5.3–12.0)	10.0 (6.0–13.9)	10.1 (8.0–12.1)
Percentage per embryo transfer (95% CI)	27.9 (20.2–35.6)	23.2 (14.7–31.7)[b]	28.0 (18.1–38.0)	26.5 (21.5–31.5)[b]
Abortion	2	8	3	13
Ectopic	—	1	—	1
Cervical	—	—	1	1
Ongoing	36	15	19	70
Percentage per cycle (95% CI)	10.7 (7.3–14.1)	5.4 (2.7–8.1)	8.2 (4.6–11.8)	8.3 (6.4–10.2)
Percentage per embryo transfer (95% CI)	26.5 (18.9–34.0)	15.2 (7.9–22.4)	23.2 (13.9–32.5)	22.1 (17.4–26.7)

CI, confidence interval; OR, oocyte retrieval; TMSC, total motile sperm count.
[a]Cryopreserved semen not included in calculation.
[b]One ectopic pregnancy after cancelled oocyte retrieval and intrauterine insemination.

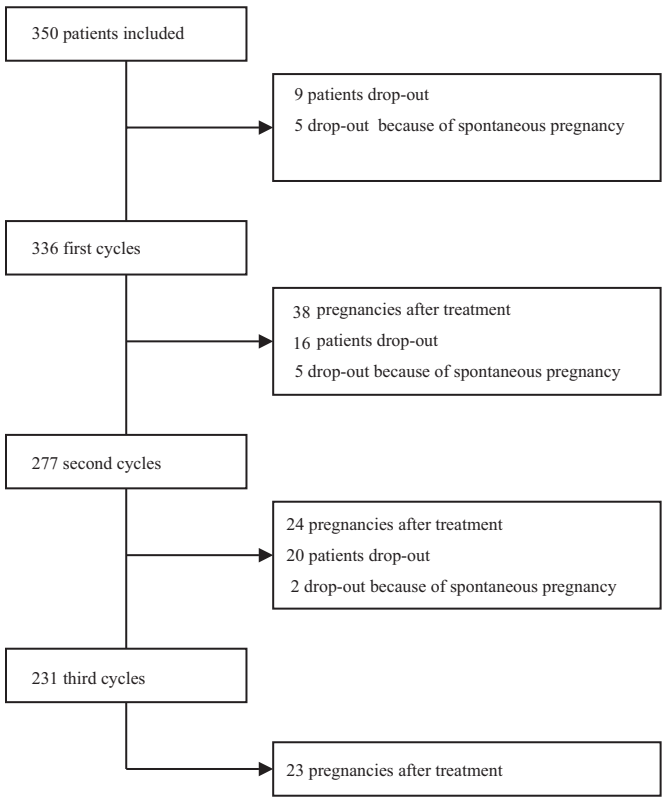

Figure 1. Number of dropouts and pregnancies according to cycle number.

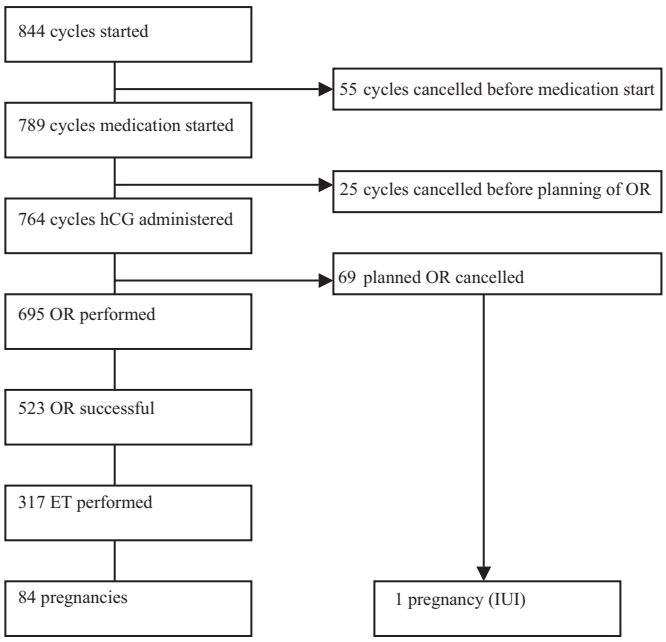

Figure 2. Summary of overall results.

retrieval. On the day ovulation was triggered, LH level was ≥10.0 IU/l in 111 cases (15.1%). Of these, 38 (34.2%) were cancelled due to ovulation at the time of planned oocyte retrieval.

Of 695 oocyte retrievals, 523 were successful (75.3%). In 40 of these, two or more oocytes were obtained (two oocytes in 35 cases, three oocytes in two cases and four, nine and 20 oocytes in the remaining three cases). In most of the cases where two or more oocytes were obtained, one single follicle or two co-dominant follicles were aspirated. In two and four cases, respectively, three and four follicles were aspirated. In the remaining three cases, 5, 6 and 11 follicles were aspirated.

In 376 of a total of 523 successful oocyte retrievals, fertilization occurred (71.9%). No transfer was carried out in 59 of these due to aberrant fertilization or defective embryo development [three pronuclei (PN), absence of cleavage or excessive fragmentation].

Overall, 317 embryo transfers were performed (37.6% per started cycle). In 299 of these, one single embryo was available for transfer. In 16 cases, two embryos were available. Three and four embryos were available in one case each. In all these cases, two embryos were transferred.

A total of 299 single-embryo transfers led to 78 pregnancies. In all centres except centre C, embryos were screened for the presence of multinucleated blastomeres (MNB). Of 280 single-embryo transfers where presence of MNB, amount of fragmentation (0%, ≤10%, 10–40% or >40%) and number of blastomeres on day 2 and day 3 were noted, 84 (30.0%) were of excellent quality, that is, no MNB, four or five blastomeres on day 2 and at least 7 on day 3 and ≤10% fragmentation (Van Royen *et al.*, 1999). Of these, 34 implanted (40.5%). Of 37 embryos showing MNB at any stage, five ongoing implantations occurred (13.5%).

Eighty-five pregnancies followed. One pregnancy occurred after cancelled oocyte retrieval and IUI, 78 occurred after transfer of one embryo and six after transfer of two embryos. Thirteen pregnancies ended in miscarriages, one was ectopic and one was a cervical pregnancy. Seventy pregnancies were ongoing. Four twin pregnancies occurred, two after transfer of one embryo and two after transfer of two embryos. Of the twin pregnancies, one miscarried and three were ongoing. Pregnancy rate per started cycle was 10.1%, of which 4.7% were twins. The ongoing pregnancy rate per started cycle was 8.3%, of which 4.3% were twins. The pregnancy rate and ongoing pregnancy rate per embryo transfer were 26.5 and 22.1%. The cumulative ongoing pregnancy rate after three cycles was 20.8% per patient.

One pregnancy was interrupted because of severe congenital abnormalities (limb–body wall complex). One pregnancy ended in fetal death at 17 week gestation. Outcome was unknown for one ongoing pregnancy. Live birth rate, not including the pregnancy lost to follow-up, was thus 19.9% per patient.

Of 208 patients completing three unsuccessful cycles, 127 continued with minimal stimulation IVF, 46 started COS-IVF and 35 refrained from further treatment.

Results according to indication for IVF

Results according to indication for IVF are summarized in Table III. For the indications tubal factor, unexplained, male factor, endometriosis, cervical factor and failed AID, respectively, 263, 323, 136, 62, 29 and 31 cycles were started. There were no significant differences in the number of cancelled cycles.

In failed AID, cryopreserved semen was used for IVF. Median total motile sperm count (TMSC) of the used semen in this group was 3.7×10^6. For the other indications, median TMSC ranged from 18.0×10^6 (male factor) to 130.0×10^6 (endometriosis). Fertilization per successful oocyte retrieval was significantly lower in male factor and unexplained subfertility as compared with tubal factor and endometriosis (Table III). The number of embryo transfers per started cycle was significantly lower for male factor as compared with tubal subfertility (Table III).

Pregnancy rates and live birth rates were not significantly different between indications (Table III).

Results according to cycle number

Results according to cycle number are summarized in Table IV and Figure 1. Fifty-nine patients completed one cycle, 46 patients completed two cycles and 231 patients completed three cycles, for a total of 844 cycles.

Between cycle numbers, the differences in cancellation rates, oocyte retrieval rate, fertilization rate, embryo transfer rate and pregnancy rates were not significant (Table IV and Figure 1)

The results of second and third cycles of patients who experienced a cancellation of oocyte retrieval, an unsuccessful oocyte retrieval or fertilization failure in their first cycle and the results of second and third cycles of patients where these events did not occur in the first cycle are summarized in Table V.

In 56 patients, oocyte retrieval was not performed in the first cycle. In 36 of these, this was due to LH rise or ovulation during cetrorelix administration or because of unexpected ovulation at the time of planned oocyte retrieval. These 36 patients completed a further 62 cycles (34 second and 28 third cycles), of which 13 were cancelled again due to LH rise or ovulation [21.0% (95% CI 10.6–31.3)] and nine were cancelled for other reasons (14.5%), and 40 oocyte retrievals [64.5% (95% CI 52.4–76.7)] were performed. For comparison, in 300 patients, oocyte retrieval was not cancelled in the first cycle or was cancelled for reasons other than LH rise or ovulation. These patients completed a further 446 cycles (243 second and 203 third cycles), of which 35 were cancelled due to LH rise or ovulation [7.8% (95% CI 5.3–10.4)], significantly less than in the group where the first cycle was cancelled. Thirty-six second and third cycles were cancelled for reasons other than LH rise or ovulation (8.1%). The number of oocyte retrievals performed in second and third cycles was 375 [84.1% (95% CI 80.6–87.5)], significantly higher than in the group where the first cycle was cancelled.

For 70 patients, oocyte retrieval was not successful in the first cycle. These 70 patients completed a further 118 cycles (64 second and 54 third cycles), of which 14 were cancelled and 104 oocyte retrievals were performed (88.1%), of which 65 were successful [62.5% per attempt (95% CI 53.0–72.0)]. For comparison, oocyte retrieval was successful in the first cycle in 210 patients. These patients completed a further 298 cycles (162 second and 136 third cycles), of which 50 were

Table V. Results in second and third cycles according to performance in first cycle

Results of first cycle	First cycle cancelled (LH rise/ovulation)	First cycle not cancelled (or cancel other reason)	First cycle OR unsuccessful	First cycle OR successful	First cycle no fertilization	First cycle fertilization
Cycle 1 (*n*)	36	300	70	210	52	158
Cycles 2 and 3 of same patients (*n*)	62	446	118	298	92	206
Cancel (LH rise/ovulation)	13	35				
Percentage per cycle (95% CI)	21.0 (10.6–31.3)[a]	7.8 (5.3–10.4)[a]				
Cancel (other reason)	9	36				
Percentage per cycle (95% CI)	14.5 (5.6–23.5)	8.1 (5.5–10.7)				
Cancel total	22	71	14	50	15	35
Percentage per cycle (95% CI)	35.5 (23.3–47.6)[b]	15.9 (12.5–19.4)[b]	11.9 (5.9–17.8)	16.8 (12.4–21.1)	16.3 (8.6–24.0)	17.0 (11.8–22.2)
OR performed	40	375	104	248	77	171
Percentage per cycle (95% CI)	64.5 (52.4–76.7)[c]	84.1 (80.6–87.5)[c]	88.1 (82.2–94.1)	83.2 (78.9–87.6)	83.7 (76.0–91.4)	83.0 (77.8–88.2)
OR successful			65	200	63	137
Percentage per attempt (95% CI)			62.5 (53.0–72.0)[d]	80.6 (75.6–85.7)[d]	81.8 (73.0–90.6)	80.1 (74.0–86.2)
TMSC (million), median (range)					30.0 (1.30–410.0)	59.0 (0.81–710.0)
Cycles with fertilization					30	115
Percentage per successful OR (95% CI)					47.6 (35.0–60.2)[e]	83.9 (77.7–90.2)[e]
Embryo transfer	19	162	35	123	27	96
Percentage per cycle (95% CI)	30.6 (18.9–42.4)	36.3 (31.8–40.9)	29.7 (21.3–38.1)	41.3 (35.6–47.0)	29.3 (19.9–38.8)[f]	46.6 (39.7–53.6)[f]
Pregnancy	4	43[g]	11[g]	29	7	22
Percentage per cycle (95% CI)	6.5 (0.21–12.7)	9.6 (6.8–12.4)	9.3 (4.0–14.7)	9.7 (6.3–13.2)	7.6 (2.1–13.1)	10.7 (6.4–15.0)

CI, confidence interval; OR, oocyte retrieval; TMSC, total motile sperm count.
[a,b,c,d,e,f]Same letters indicate significant differences.
[g]One pregnancy after cancelled oocyte retrieval and intrauterine insemination.

cancelled and 248 oocyte retrievals were performed (83.2%). The number of successful oocyte retrievals per attempt was significantly higher compared with the group where oocyte retrieval was not successful in the first cycle [200 oocyte retrievals successful: 80.6% per attempt (95% CI 75.6–85.7)].

For 52 patients, no fertilization occurred after successful oocyte retrieval in the first cycle. Indications for IVF were tubal factor, unexplained subfertility, male factor, endometriosis, failed AID and cervical factor in 14, 23, 10, 1, 2 and 2 cases, respectively. These 52 patients completed a further 92 cycles (48 second and 44 third cycles), of which 15 were cancelled, and 77 oocyte retrievals were performed (83.7%), of which 63 were successful (81.8% per attempt). Median TMSC in these cycles was 30.0×10^6 (range 1.30–410.0). Fertilization occurred in 30 cases [47.6% (95% CI 35.0–60.2)]. Twenty-seven embryo transfers were performed [29.3% (95% CI 19.9–38.8)], leading to seven pregnancies (7.6%). For comparison, in 158 patients, fertilization did occur after successful oocyte retrieval in the first cycle. Indications for IVF were tubal factor, unexplained subfertility, male factor, endometriosis, failed AID and cervical factor in 52, 61, 23, 16, 3 and 3 cases, respectively. These patients completed a further 206 cycles (114 second and 92 third cycles), of which 35 were cancelled and 171 oocyte retrievals were performed (83.0%), of which 137 were successful (80.1% per attempt). Median TMSC in these cycles was 59.0×10^6 (range 0.81–710.0). Fertilization rate and number of embryo transfers were significantly higher as compared with the group where fertilization failure occurred in the first cycle [fertilization in 115 cases: 83.9% (95% CI 77.7–90.2); embryo transfer in 96 cases: 46.6% (95% CI 39.7–53.6)]. Twenty-two pregnancies occurred (10.7%).

Discussion

This study describes the largest series of minimal stimulation IVF available so far. The overall ongoing pregnancy rate per started cycle was 8.3%, with a cumulative ongoing pregnancy rate after up to three cycles of 20.8%.

Pregnancy rates according to indication for IVF showed no significant differences. The results of this study suggest therefore that minimal stimulation IVF is applicable for all indications for conventional IVF. Although for male factor infertility compared with tubal factor, both fertilization and embryo transfer rates were significantly lower, these differences were not reflected in a lower pregnancy rate. Fertilization rate in male factor was lower than in endometriosis, but the embryo transfer rate was not. This lower fertilization rate in male factor infertility is not surprising because these patients have diminished semen quality. However, when fertilization did occur, implantation rates found in our study were very good. For unexplained infertility, the fertilization rate was significantly lower than for tubal factor and endometriosis, but the embryo transfer rate and pregnancy rates were not. This lower fertilization rate in unexplained infertility is not surprising either, because fertilization failure is a common finding in these patients (Takeuchi *et al*., 2000; Hershlag *et al*., 2002; Jaroudi *et al*., 2003; Bungum *et al*., 2004). For this category of patients also, once fertilization did occur, we found good implantation rates. For cervical factor infertility, results seemed rather poor, but because the number of patients was small, no firm conclusions can be drawn.

The number of cancelled oocyte retrievals was rather high (17.7%). Of 844 started cycles, 80 (9.5%) were cancelled before planning of oocyte retrieval. An additional 69 cycles were cancelled at the time of planned oocyte retrieval. This

cancellation rate seems to be in accordance with cancellation rates reported in literature. In earlier studies on minimal stimulation IVF, in 101 of a total of 531 described cycles, oocyte retrieval was cancelled (19.0%) (Meldrum *et al*., 1994; Paulson *et al*., 1994; Rongières-Bertrand *et al*., 1999; Kadoch *et al*., 2003; Vogel *et al*., 2003; Kolibianakis *et al*., 2004; Weghofer *et al*., 2004; Zhioua *et al*., 2004; Elizur *et al*., 2005).

Of 149 cancellations, 108 (12.8% per started cycle) were related to a rise in LH or ovulation. In natural cycle IVF without the use of a GnRH antagonist, of a total of 1572 described cycles, 314 were cancelled because of an LH rise or ovulation (20.0%) (Omland *et al*., 2001; Ballesteros *et al*., 2002; Bauman *et al*., 2002; Pelinck *et al*., 2002; Lukassen *et al*., 2003). This raises the question whether the apparently small decrease in cancellation rate in the minimal stimulation protocol justifies the inconvenience and costs of treatment with GnRH antagonist and gonadotrophins. Because no studies comparing natural cycle IVF to minimal stimulation IVF are available, no conclusions can be drawn on this issue. A study comparing minimal stimulation IVF with natural cycle IVF, including a cost-effectiveness analysis, seems warranted.

On the other hand, changes in the minimal stimulation protocol may reduce the number of LH rises and premature ovulations, thus raising effectiveness. A higher dose or more frequent administration of cetrorelix, ovulation triggering at a smaller follicle size or a smaller interval between HCG administration and oocyte retrieval could all be helpful in this respect. Another approach to the reduction of the number of premature ovulations is the use of indomethacin to prevent follicular rupture (Nargund *et al*., 2001).

In this study, the number of successful oocyte retrievals seems rather low (75.1% per attempt). In all oocyte retrievals, a single lumen aspiration needle was used, and no flushing of the follicle was done. Flushing of the follicle may raise effectiveness of the oocyte retrieval but also will make the procedure more painful and time-consuming (Tan *et al*., 1992; Daya *et al*., 1995).

In this study, the high number of cancelled and unsuccessful oocyte retrievals led to a low number of embryo transfers per started cycle (37.3%), but due to a good implantation rate (25.7% per transferred embryo), the pregnancy rate was acceptable. Thirty percentage of single embryos transferred were of excellent quality and showed an implantation rate of 40.5% per embryo. The overall implantation rate found in this study seems similar to implantation rates of embryos obtained after COS-IVF (Andersen *et al*., 2005), which is surprising since in most cases, only one embryo was available for transfer and unlike the case for COS-IVF, selection of the best-quality embryo was not possible. An explanation for this could be that from a cohort of oocytes, the one that naturally develops to dominance represents the best-quality oocyte. An alternative explanation could be that the implantation environment in minimal stimulation IVF is better than in COS-IVF. The supraphysiological E_2 levels after ovarian stimulation are suggested to be correlated with disturbed endometrial receptivity (Devroey *et al*., 2004). Therefore, the physiological hormone levels present in minimal stimulation IVF may be associated with a better endometrial receptivity as compared with COS.

In 40 oocyte retrievals, two or more oocytes were obtained (4.7% per cycle; 5.8% per oocyte retrieval). In most of these cases, two co-dominant follicles were present when medication was started and both continued to grow and were aspirated, or two oocytes were obtained from one dominant follicle. However, in nine of these cases (1.1% per cycle; 1.3% per oocyte retrieval), three or more large follicles were aspirated, although only one dominant follicle (≥14 mm) had been present when medication was started. Apparently, in rare cases, the administration of r-FSH leads to ovarian stimulation, even when it is started after presumed follicular dominance at a follicle size of 14 mm. An alternative explanation is that an ovarian cyst was mistaken for a dominant follicle, and follicular dominance had not yet developed in these cases.

In this study, the multiple pregnancy rate was very low with 4.7%, which is advantageous considering the many problems associated with multiple pregnancies (Fauser *et al*., 2005). Although two twin pregnancies occurred after transfer of one single embryo, the application of elective single-embryo transfer in those cases where more than one is available could lead to a further reduction in multiple pregnancies after minimal stimulation IVF (Gerris, 2005).

It is unclear what the optimal number of cycles per patient would be. Overall, results were not significantly different according to cycle number. However, the occurrence of a cancellation of oocyte retrieval, unsuccessful oocyte retrieval and fertilization failure all seem to be repeating phenomena in further cycles. Patient counselling on the number of cycles to be performed should therefore be individualized, taking into account the performance in the first cycle.

So far, no cost-effectiveness analyses concerning minimal stimulation IVF are available. Per cycle, minimal stimulation will be far cheaper than COS-IVF due to less medication use. On the other hand, more cycles of minimal stimulation compared with COS-IVF will be needed to obtain a comparable number of pregnancies per patient. Future research should clarify how costs per obtained pregnancy or live birth after minimal stimulation IVF compare with those after COS-IVF.

Minimal stimulation IVF offers a low-risk and patient-friendly protocol, being associated with a very low risk of OHSS and little hormonal medication use, short duration of a treatment cycle and easy oocyte retrieval. No resting cycle is necessary after a failed cycle, and treatments can be performed in consecutive cycles. Although effectiveness per started cycle is rather low, cumulative pregnancy rates after up to three cycles are reasonable and probably comparable with those after one treatment cycle of COS-IVF, which takes a comparable time span to be performed (Andersen *et al*., 2005).

Based on the advantages of minimal stimulation IVF, it is our opinion that it is a feasible treatment for all patient categories studied. There are some groups of patients for whom minimal stimulation IVF forms a particularly valuable alternative to COS-IVF. These include patients with a history of OHSS, who will benefit from the lack of ovarian stimulation and patients who are opposed to the generation of supernumerary embryos, who will appreciate the fact that in most cases only one oocyte is obtained. Also in poor responders to COS-IVF, it seems logical to apply minimal stimulation IVF, as 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 minimal stimulation. In one study comparing minimal stimulation IVF with COS-IVF in poor responders, similar pregnancy rates were found (Elizur *et al*., 2005).

In conclusion, minimal stimulation seems suitable for all indications for conventional IVF. Owing to considerable loss in every step of the procedure, the embryo transfer rate is low, but this is compensated by a favourable implantation rate. Pregnancy rates found in this study are encouraging. Because of the low-risk and patient-friendly nature of this protocol, it is our opinion that minimal stimulation is a feasible treatment option for patients requiring IVF.

Acknowledgements

This study was supported by a grant from the University Medical Center Groningen, Groningen, the Netherlands and from ZonMw, the Netherlands (grant number 945-02-002).

Part of these data were presented at the 18th Annual Meeting of ESHRE, 1–3 July 2002, Vienna, Austria, at the Annual Meeting of ASRM, 15 October 2002, Seattle, USA, and at the 20th Annual Meeting of ESHRE, 27–30 June 2004, Berlin, Germany. Part of these data have been published before (Pelinck *et al*., 2005).

References

Andersen AN, Gianaroli L, Felberbaum R, de Mouzon J, Nygren KG, The European IVF-monitoring programme (EIM) and European Society of Human Reproduction and Embryology (ESHRE) (2005) Assisted reproductive technology in Europe, 2001. Results generated from European registers by ESHRE. Hum Reprod 20,1158–1176.

Ballesteros A, Landeras J, Sagastegui CG, Molla M, Pérez-Cano I and Remohi J (2002) Natural cycle in poor responders. Fertil Steril 78(Suppl 1),P-108.

Bauman R, Mihaljević D, Kupešić S and Kurjak A (2002) In vitro fertilization in spontaneous cycles – our experience. Eur J Obstet Gynecol Reprod Biol 102,184–187.

Biggers JD and Summers MC (2004) When to avoid creating surplus human embryos. Hum Reprod 19,2457–2459.

Bungum L, Bungum M, Humaidan P and Andersen CY (2004) A strategy for treatment of couples with unexplained infertility who failed to conceive after intrauterine insemination. Reprod Biomed Online 8(5),584–589.

Daya S, Gunby J, Hughes EG, Collins JA, Sagle MA and YoungLai EV (1995) Natural cycles for in-vitro fertilization: cost-effectiveness analysis and factors influencing outcome. Hum Reprod 10,1719–1724.

Devroey P, Bourgain C, Macklon NS and Fauser BCJM (2004) Reproductive biology and IVF: ovarian stimulation and endometrial receptivity. Trends Endocrinol Metab 15,84–90.

Elizur SE, Aslan D, Shulman A, Weisz B, Bider D and Dor J (2005) Modified natural cycle using GnRH antagonist can be an optional treatment in poor responders undergoing IVF. J Assist Reprod Genet 22,75–79.

Fauser BCJM, Devroey P and Macklon NS (2005) Multiple birth resulting from ovarian stimulation for subfertility treatment. Lancet 365,1807–1816.

Gerris JM (2005) Single embryo transfer and IVF/ICSI outcome: a balanced appraisal. Hum Reprod Update 11,105–121.

Hershlag A, Paine T, Kvapil G, Feng H and Napolitano B (2002) In vitro fertilization-intracytoplasmic sperm injection split: an insemination method to prevent fertilization failure. Fertil Steril 77(2),229–232.

Jaroudi K, Al-Hassan S, Al-Sufayan H, Al-Mayman H, Qeba M and Coskun S (2003) Intracytoplasmic sperm injection and conventional in vitro fertilization are complementary techniques in management of unexplained infertility. J Assist Reprod Genet 20(9),377–381.

Kadoch JJK, Frydman NNF, Castelo Branco AAC, Lédéé Bataille Franchin RRF and Frydman RRF (2003) IVF-ET using naturally-selected dominant follicles for poor prognosis patients: a hypothesis. Fertil Steril 80(Suppl 3),P-199.

Kolibianakis E, Zikopoulos K, Camus M, Tournaye H, Van Steirteghem A and Devroey P (2004) Modified natural cycle for IVF does not offer a realistic chance of parenthood in poor responders with high day 3 FSH levels, as a last resort prior to oocyte donation. Hum Reprod 19,2545–2549.

Lukassen HGM, Kremer JAM, Lindeman EJM, Braat DDM and Wetzels AMM (2003) A pilot study of the efficacy of intracytoplasmic sperm injection in a natural cycle. Fertil Steril 79,231–232.

Meldrum DR, Rivier J, Garzo G, Wisot A, Stubbs C and Hamilton F (1994) Successful pregnancies with unstimulated cycle oocyte donation using an antagonist of gonadotropin-releasing hormone. Fertil Steril 61,556–557.

Nargund G, Waterstone J, Bland JM, Philips Z, Parsons J and Campbell S (2001) Cumulative conception and live birth rates in natural (unstimulated) IVF cycles. Hum Reprod 16,259–262.

Omland AK, Fedorcsák P, Storeng R, Dale PO, Åbyholm T and Tanbo T (2001) Natural cycle IVF in unexplained, endometriosis-associated and tubal factor infertility. Hum Reprod 16,2587–2592.

Paulson RJ, Sauer MV and Lobo RA (1994) Addition of a gonadotropin releasing hormone (GnRH) antagonist and exogenous gonadotropins to unstimulated in vitro fertilization (IVF) cycles: physiologic observations and preliminary experience. J Assist Reprod Genet 11,28–32.

Pelinck MJ, Hoek A, Simons AHM and Heineman MJ (2002) Efficacy of natural cycle IVF: a review of the literature. Hum Reprod Update 8,129–139.

Pelinck MJ, Vogel NEA, Hoek A, Arts EGJM, Simons AHM and Heineman MJ (2005) Minimal stimulation IVF with late follicular phase administration of the GnRH antagonist cetrorelix and concomitant substitution with recombinant FSH: a pilot study. Hum Reprod 20,642–648.

Ramsewak SS, Kumar A, Welsby R, Mowforth A, Lenton EA and Cooke ID (1990) Is analgesia required for transvaginal single-follicle aspiration in in vitro fertilization? A double-blind study. J In Vitro Fert Embryo Transf 7,103–106.

Rongières-Bertrand C, Olivennes F, Righini C, Fanchin R, Taïeb J, Hamamah S, Bouchard P and Frydman R (1999) Revival of the natural cycles in in-vitro fertilization with the use of a new gonadotrophin-releasing hormone antagonist (Cetrorelix): a pilot study with minimal stimulation. Hum Reprod 14,683–688.

Takeuchi S, Minoura H, Shibahara T, Shen X, Futamura N and Toyoda N (2000) In vitro fertilization and intracytoplasmic sperm injection for couples with unexplained infertility after failed direct intraperitoneal insemination. J Assist Reprod Genet 17(9),515–520.

Tan SL, Waterstone J, Wren M and Parsons J (1992) A prospective randomized study comparing aspiration only with aspiration and flushing for transvaginal ultrasound-directed oocyte recovery. Fertil Steril 58,356–360.

Ubaldi F, Rienzi L, Baroni E, Ferrero S, Iacobelli M, Minasi M, Martinez F and Greco E (2003) GnRH antagonist and natural ICSI cycle in poor responder patients. Hum Reprod 18(Suppl 1),P-327.

Van Royen E, Mangelschots K, De Neuborg D, Valkenburg M, Van de Meerssche M, Ryckaert G, Eestermans W and Gerris J (1999) Characterisation of a top quality embryo, a step towards single-embryo transfer. Hum Reprod 14,2345–2349.

Vogel NEA, Pelinck MJ, Arts EGJM, Hoek A, Simons AHM and Heineman MJ (2003) Effectiveness of the modified natural cycle ICSI: results of a pilot study. Fertil Steril 80 (Suppl 3),P-7.

Weghofer A, Margreiter M, Bassim S, Sevelda U, Beilhack E and Feichtinger W (2004) Minimal stimulation using recombinant follicle-stimulating hormone and a gonadotropin-releasing hormone antagonist in women of advanced age. Fertil Steril 81,1002–1006.

Zhioua F, Zhioua A, Chaker A, M'Solly S and Meriah S (2004) Efficacy of intracytoplasmic sperm injection in a natural cycle with GnRH antagonists. Hum Reprod 19(Suppl 1),P-299.

Submitted on March 6, 2006; resubmitted on April 13, 2006; accepted on April 24, 2006