Cumulative pregnancy rates after six cycles of modified natural cycle IVF

Pelinck, M. J.; Cantineau, A. E.P.; van Echten-Arends, J.

Published in:
Development of In Vitro Maturation for Human Oocytes

DOI:
10.1007/978-3-319-53454-1_13

Publication date:
2017

Cumulative Pregnancy Rates After Six Cycles of Modified Natural Cycle IVF

13

M.J. Pelinck, MD, PhD, A.E.P. Cantineau, MD, PhD and J. van Echten-Arends, PhD

Introduction

In modified natural cycle (MNC) IVF, treatment is aimed at the use of the one follicle that naturally develops to dominance. In this treatment modality, medication can be used for the prevention of untimely ovulation (GnRH antagonist or indometacin) and for ovulation triggering (hCG). FSH or HMG can be added as add-back for the fall in gonadothrophins caused by the GnRH antagonist, and luteal support may or may not be administered [1]. Medication is explicitly not administered in order to induce multiple follicle development.

Compared to conventional IVF treatment with ovarian stimulation, MNC is a low-risk treatment modality, since the risk of ovarian hyperstimulation syndrome is negligible. Besides this, it offers a patient-friendly modality, since medication is used in low dose and for a few days only, thus causing few side effects. Furthermore, oocyte retrieval is less painful since usually only one follicle is aspirated. A treatment cycle is short and treatments are easily repeated, with no need for a resting cycle in between treatment cycles.

In our centre, a research project on MNC-IVF was started in 2001. Several cohort studies were done, in which patients were offered to undergo MNC-IVF for a maximum of three [2] or nine [3] consecutive cycles, preceding the start of conventional IVF. Treatments were offered for free. Patients requiring ICSI were not included in these studies. Maximum female patient age at inclusion was 36 years.

The clinical protocol used had some small adjustments over time but basically consisted of ultrasound monitoring from cycle day 3 or 8, repeated daily or every other day, according to the size of the leading follicle. When the leading follicle had a diameter of at least 14 mm (measured in three perpendicular planes), daily injections of a GnRH antagonist (0.25 mg) were started combined with 150 IU recombinant FSH. Blood samples were taken to measure LH and E2 levels. Cycles were cancelled when an LH level >20 IU/L was noticed at a follicle size of <15 mm. In cases where an LH level of 20–30 IU/L was noticed at a follicle >16 mm (after medication was started), the oocyte retrieval was planned, since the GnRH antagonist should be capable of

M.J. Pelinck (✉)
Department of Obstetrics and Gynecology, Scheper Ziekenhuis Emmen, Boermarkeweg 60, 7824 AA Emmen, The Netherlands
e-mail: pelinck@hotmail.com

A.E.P. Cantineau · J. van Echten-Arends
Department of Obstetrics and Gynecology, University of Groningen, University Medical Centre Groningen, PO Box 30001, 9700 RB Groningen, The Netherlands
e-mail: a.e.p.cantineau@umcg.nl

J. van Echten-Arends
e-mail: j.van.echten@umcg.nl

R.-C. Chian et al. (eds.), *Development of In Vitro Maturation for Human Oocytes*, DOI 10.1007/978-3-319-53454-1_13

blunting the LH surge enough. In cases where an LH level of >30 IU/L was noticed, the oocyte retrieval was cancelled. Ovulation triggering was done with 10,000 IU hCG when a follicle with a diameter of at least 18 mm was observed. When at the time of the planned oocyte retrieval unexpected ovulation had occurred, tubes were patent and semen of sufficient quality was available, an intra-uterine insemination was performed. Transvaginal ultrasound guided follicle aspiration was performed 34 h after hCG injection. No standard analgesia was given. Fertilization of oocytes was assessed 17–20 h after insemination. Embryo transfer was performed 72–76 h after oocyte retrieval. When additional embryos were available, these were cryopreserved. Luteal support consisted of hCG 1500 IU on day 5, 8 and 11 after oocyte retrieval.

After this research project, MNC was implemented as a standard treatment. MNC was offered to patients for a maximum of 6 cycles to be performed preceding IVF with ovarian stimulation. In the Netherlands, conventional IVF treatments with controlled ovarian hyperstimulation (COH) are refunded for a maximum of three cycles. A contract was made with insurance companies, in which the 6 cycles of MNC substitute the first COH-IVF cycle. A full IVF treatment 'package' thus consisted of up to 6 cycles of MNC, followed by two COH-IVF cycles.

The yearly number of cycles and patients from 2004 onwards are displayed in Fig. 13.1. In the decade that followed, some adjustments have been made to the protocol. Patients requiring ICSI were offered MNC from 2004 onwards. Maximum female age at the start of treatment was changed from 36 to 34 years (December 2007) and embryo transfer day was changed from day 3 to day 2 (January 2009). In cases where more than one embryo was available, double embryo transfer was no longer done, but in all these cases single embryo transfer was performed. Indometacin was added to the protocol in a small number of patients participating in a study [4]. Over time, culture medium and embryo transfer catheters were changed. Cumulative pregnancy rates per patient according to year of start of treatment are shown in Fig. 13.2.

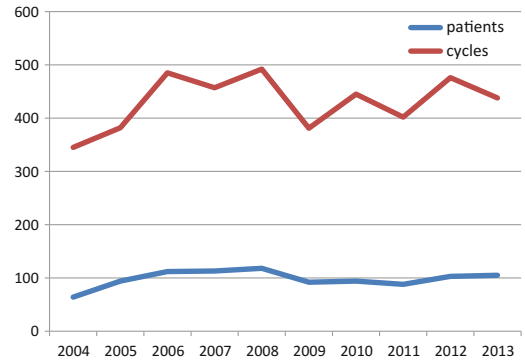

Fig. 13.1 Number of patients undergoing MNC-IVF and number of cycles performed

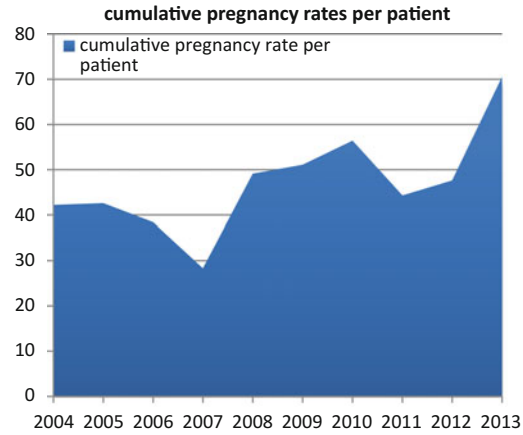

Fig. 13.2 Cumulative pregnancy rates per patient

In this chapter, results of our original studies and cumulative pregnancy rates after 6 cycles of MNC in a larger series of patients are discussed.

What Would Be the Optimal Number of MNC Cycles?

In comparing the results of modified natural cycle IVF to other treatment modalities, it is important to consider that although the pregnancy rate per cycle in general is rather low, duration of treatment is short and treatment can be easily repeated in consecutive cycles. Obviously, compared to standard IVF with ovarian stimulation, a higher number of cycles is necessary to obtain similar pregnancy rates. In our experience, patients are

quite willing to accept the necessity of a higher number of cycles, mainly based on anxiety for hormonal stimulation.

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 order to evaluate the optimal number of MNC-IVF cycles, a study was done in our centre in which a maximum of nine cycles of MNC-IVF was offered to 268 patients. Dropout rates after unsuccessful treatment cycles and pregnancy rates according to cycle number were evaluated [3]. This study is discussed in detail in the following section.

Cumulative Pregnancy Rates After Nine Cycles of MNC-IVF and Analysis of Patient Dropout

For details on methodology, we refer to the original publication [3]. In short, patients aged 18–36 years with an indication for conventional IVF and proven ovulatory cycles were included in this study. Only conventional IVF was performed, and no ICSI was done. All embryo transfers were done on day 3 after oocyte retrieval.

Patients were offered a maximum of nine treatment cycles. Treatments were performed in consecutive menstrual cycles (unless patients requested otherwise) and took place between March 2001 and September 2005. All treatments were offered for free.

End point in this study was pregnancy. Results according to cycle number and actual observed cumulative pregnancy rates per patient were calculated and life table analysis was done.

Patient Characteristics and Results of Treatment Cycles

Patient characteristics are shown in Table 13.1. Of 268 included patients, twelve withdrew from the study before starting treatment, in five of these because of the occurrence of a spontaneous pregnancy. Results according to cycle number are shown in Table 13.2. Overall, 256 patients started 1048 treatment cycles (4.1 per patient). Median duration of treatment was 5.0 months (range 1–24). Ninety-four cycles (9.0%) were cancelled before the 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), or other reasons (28 cycles).

Table 13.1 Patient characteristics

No. of patients	268
Female patient age (years)[a]	33.3 (23–36)
BMI (kg/m^2)[a]	23.0 (16–34)
Duration of subfertility (months)[a]	46.0 (0–121)
Subfertility (%)	
Primary	164 (61.2)
Secondary	104 (38.8)
Indication (%)	
Tubal	82 (30.6)
Unexplained	106 (39.6)
Male factor	41 (15.3)
Endometriosis	22 (8.2)
Cervical factor	8 (3.0)
Failed AID	9 (3.4)

[a]Values are median (range)

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, one or two oocytes were obtained (576 and 44 cycles, respectively). In five cycles, three or more oocytes were obtained (three, three, six, nine and twenty 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

Table 13.2 Results according to cycle number of modified natural cycle IVF

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

OR—oocyte retrieval; *ET*—embryo transfer
[a]Pregnancy after cancelled oocyte retrieval and IUI
[b]Spontaneous conception during cycle that was cancelled because of LH surge

cycles, embryo transfer was done (36.5% per started cycle; 61.1% per successful oocyte retrieval). In 20 cycles, two 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, six 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 six 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 the transfer of one single embryo and two occurred after DET. Ongoing pregnancy rate was 7.9% (95% CI: 6.3–9.6) per started cycle. One pregnancy was interrupted because of severe congenital abnormalities. One pregnancy ended in foetal death at 17 weeks' 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.

Dropout Rates and Cumulative Pregnancy Rates

Dropout rates and cumulative pregnancy rates are specified in Table 13.3 and Fig. 13.3.

Out of 268 included patients, 102 (38.1%) left the study before completing nine cycles because a pregnancy was obtained. Fifteen (5.6%) left the study because of a treatment-independent pregnancy. Of the remaining 151, 128 (84.8%) dropped out of the study after 0-8 unsuccessful cycles. Of these, 86 (67.2%) proceeded with

Table 13.3 Dropout rates and cumulative pregnancy rates

Cycle number	Patients	Pregnancy	CPR[a]	TIP	CPR including TIP[b]	DO	CPR life table[c]	CPR life table[d]
0	268	–	–	5 (1.9)	5 (1.9)	7 (2.6)	–	1.9
1	256	27 (10.5)	27 (10.5)	3 (1.2)	35 (13.1)	9 (3.5)	10.5	13.4
2	217	20 (9.2)	47 (18.4)	2 (0.09)	57 (21.3)	14 (6.5)	18.8	22.1
3	181	19 (10.5)	66 (25.8)	1 (0.06)	77 (28.7)	34 (18.8)	27.3	30.8
4	127	12 (9.4)	78 (30.5)	2 (1.6)	91 (34.0)	21 (16.5)	34.2	38.4
5	92	11 (12.0)	89 (34.8)	–	102 (38.1)	12 (13.0)	42.1	45.8
6	69	5 (7.2)	94 (36.7)	1 (1.4)	108 (40.3)	12 (17.4)	46.3	50.5
7	51	5 (9.8)	99 (38.7)	1 (2.0)	114 (42.5)	13 (25.5)	51.5	56.3
8	32	3 (9.4)	102 (39.8)	–	117 (43.7)	6 (18.8)	56.1	60.4
9	23	2 (8.7)	104 (40.6)	–	119 (44.4)	na	59.9	63.8

Numbers in parentheses are percentages
CPR—cumulative pregnancy rate; *TIP*—treatment-independent pregnancy; *DO*—dropout; *na*—not applicable
[a]CPR calculated over patients starting treatment (n = 256)
[b]CPR calculated over patients included in the study (n = 268)
[c]Life table analysis, treatment-independent pregnancies censored
[d]Life table analysis, treatment-independent pregnancies not censored

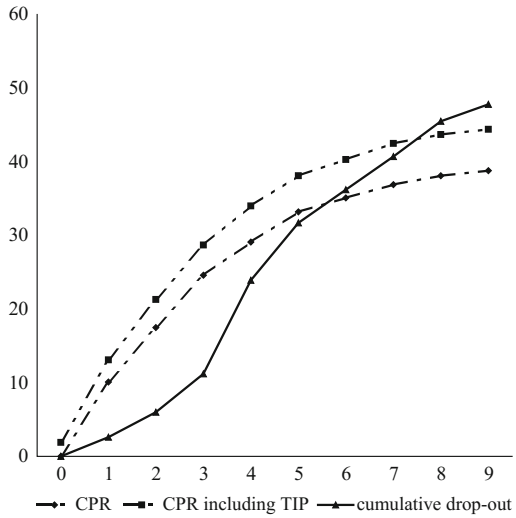

Fig. 13.3 Cumulative pregnancy rates and cumulative dropout rates. *CPR* cumulative pregnancy rate. *TIP*—treatment-independent pregnancy

standard IVF treatment and 42 (32.8%) stopped treatment altogether.

The dropout rate (not including those who stopped treatment because of treatment-independent pregnancy) was low after the first and second cycles (3.5 and 6.5%, respectively) and rose sharply thereafter to 13.0–25.5% in further cycles. Cumulative pregnancy rate per patient starting treatment was 40.6% (95% CI: 34.5–46.8). Including treatment-independent pregnancies, cumulative pregnancy and ongoing pregnancy rate per patient included in the study was 44.4% (95% CI: 35.2–53.6) and 34.7% (95% CI: 28.9–40.5) per patient.

Cumulative pregnancy rates were calculated with life table analysis according to two methods. In the first method, all patients who stopped treatment were censored, leading to a cumulative pregnancy rate 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. Cumulative pregnancy rate according to this method was 63.8% (95% CI: 57.9–69.7).

Analysis of Dropout

To analyse whether selective dropout occurred, patients were divided in four groups (patients where a treatment-independent pregnancy occurred excluded): A. patients dropping out after completing 1–4 unsuccessful cycles; B. patients dropping out after completing 5–8

Table 13.4 Patient and cycle characteristics of dropouts, non-dropouts and pregnant patients

Group[a]	A	B	C	D	*P*
No. of patients	78	43	21	77	
Age (mean ± SD)	32.6 (3.2)	33.0 (2.6)	33.4 (2.3)	32.1 (3.0)	0.20[b]
Subfertility primary (%)	50 (64.1)	27 (62.8)	15 (71.4)	47 (61.0)	0.85[c]
Duration subfertility (mean ± SD)	51.6 (23.6)	46.8 (19.6)	45.8 (20.8)	43.7 (20.6)	0.16[b]
No of cycles	223	271	189	230	
OR performed (%/cycle)	162 (72.6; 66.7–78.6)	211 (77.9; 72.8–82.9)	160 (84.7; 79.4–89.9)	199 (86.5; 82.0–91.0)	
OR successful (%/attempt)	123 (75.9; 69.2–82.6)	148 (70.1; 63.8–76.4)	112 (70.0; 62.8–77.2)	129 (64.8; 58.1–71.6)	
Fertilization (%/successful OR)	64 (52.0; 43.0–61.0)	90 (60.8; 52.8–68.8)	90 (80.4; 72.8–87.9)	101 (78.3; 71.0–85.6)	
ET (%/cycle)	44 (19.7; 14.4–25.1)	72 (26.6; 21.2–31.9)	73 (38.6; 31.5–45.7)	89 (38.7; 32.3–45.1)	

[a]Patients with treatment-independent pregnancies excluded from analysis
A: Dropout after 1–4 unsuccessful modified natural cycles
B: Dropout after 5–8 unsuccessful modified natural cycles
C: 9 unsuccessful modified natural cycles completed
D: Pregnant (cycle in which pregnancy occurred not included)
[b]ANOVA
[c]Chi square
OR—oocyte retrieval; *ET*—embryo transfer

unsuccessful cycles; C. patients who completed nine unsuccessful cycles and D. 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 13.4. Age, percentage of primary subfertility and duration of subfertility were not significantly different between groups. The number of oocyte retrievals performed per cycle was significantly lower in group A compared to groups C and D. Fertilization rate and embryo transfer rate both were significantly lower in group A compared to groups C and D. When comparing group B to groups C and D, the same trend was seen for a number of oocyte retrievals and embryo transfer but differences were not significant. Fertilization rate was significantly lower in group B compared to 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 2–9 of patients where these events occurred were compared to those of patients where they did not. Results of this analysis are shown in Table 13.5. 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 to 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 2–9 as compared to 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 to patients where embryo transfer was done in the first cycle.

Conclusions from This Study

In this study, a cumulative pregnancy rate of 40.6% was found after nine cycles of MNC-IVF. Including treatment-independent pregnancies, the cumulative pregnancy rate was 44.4%.

Table 13.5 Results of subsequent cycles after cancellation of oocyte retrieval, fertilization failure or no embryo transfer in the first cycle

Results of first cycle	OR not performed	OR performed	Fertilization failure	Fertilization	No ET performed	ET performed
No. of patients	46	210	36	116	157	99
Results of cycles 2–9						
No of cycles	138	654	109	333	520	272
OR performed (%/cycle)	97 (70.3; 62.5–78.1)	549 (83.9; 81.1–86.8)	92 (84.4; 77.5–91.4)	281 (84.4; 80.4–88.4)	418 (80.4; 76.9–83.9)	228 (83.8; 79.4–88.3)
OR successful (%/attempt)	72 (74.2; 65.3–83.1)	401 (73.0; 69.3–76.8)	67 (72.8; 63.6–82.1)	218 (77.6; 72.6–82.6)	301 (72.0; 67.6–76.4)	172 (75.4; 69.7–81.1)
Fertilization (%/successful OR)	45 (62.5; 51.1–73.9)	292 (72.8; 68.4–77.3)	31 (46.3; 34.1–58.5)	178 (81.7; 76.4–86.9)	198 (65.8; 60.3–71.3)	139 (80.8; 74.8–86.8)
ET (%/cycle)	35 (25.4; 18.0–32.8)	248 (37.9; 34.1–41.7)	29 (26.6; 18.1–35.1)	149 (44.7; 39.3–50.2)	161 (31.0; 26.9–35.0)	122 (44.9; 38.8–50.9)
Pregnancy (%/cycle)	13 (9.4; 4.4–14.4)	64 (9.8; 7.5–12.1)	7 (6.4; 1.7–11.1)	33 (9.9; 6.6–13.2)	49 (9.4; 6.9–12.0)	28 (10.3; 6.6–14.0)

OR—oocyte retrieval; *ET*—embryo transfer
Numbers in parentheses are percentages; 95% confidence interval

The actual observed cumulative pregnancy rate in our study represents an underestimation of the cumulative pregnancy 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. The cumulative pregnancy rate found with life table analysis represents an overestimation, since it assumes that the chance of pregnancy is the same in dropouts and those who continue treatment while in our study we found that this seems not to be the case. A realistic estimate of the cumulative pregnancy rate, corrected for dropout, will be somewhere in between the actual observed CPR and the life table estimation.

It is rather artificial to correct for dropouts, since in the analysis of IVF results, dropouts are in most cases not lost to follow-up but rather patients deciding to stop treatment for various reasons. Therefore, dropout is an inherent part of IVF performance. Corrected estimations are, however, useful in counselling patients when deciding whether or not to continue treatment. In this study, dropout rates were high, especially in higher cycle numbers.

In patients dropping out of the study, general patient characteristics were not different from those not dropping out. However, we found that cycle cancellation, fertilization failure and failure to reach embryo transfer predispose for dropout of patients in subsequent cycles and also are repeating phenomena in subsequent cycles. We therefore concluded that dropout of patients is probably selective, in the sense that patients with a poorer chance for pregnancy tend to dropout.

Furthermore, we concluded from this study that the optimal number of treatment cycles per patients 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 dropout of patients during the study, suggesting that patients should be advised to undergo at least nine cycles of minimal stimulation before starting standard IVF with ovarian stimulation. However, due to selective dropout of patients with a possible poor prognosis the steady pregnancy rate in all cycle numbers may be only apparent and therefore nine cycles will 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.

Cumulative Pregnancy Rates in a Larger Series of Patients

As mentioned before, after completion of our studies on MNC-IVF, it was introduced as a standard treatment modality in our clinic, to be offered to all suitable new patients for a maximum of 6 cycles. In our original studies, only conventional IVF was performed. After the introduction of MNC as a standard treatment modality, also patients requiring ICSI were offered MNC and over the years, male factor subfertility became the predominant indication for MNC.

In the following section, results of MNC according to age, indication for ART and BMI, are discussed. In our original studies, clinical pregnancy was chosen as end point. In the present analysis, ongoing pregnancy was chosen as end point. So, patients conceiving with MNC but with a not-ongoing pregnancy, who returned for further MNC, were not considered new cases and cycle numbering was continued.

For simplicity's sake, only the three main indications (tubal factor, unexplained subfertility and male factor) are shown. Since the majority of patients in this series were offered a maximum of 6 cycles, only cycles 1–6 were included. For this series, we do not have data on intercurrent spontaneous pregnancies.

Cumulative ongoing pregnancy rates are shown in Fig. 13.4. This graph shows that, also in this larger series, ongoing pregnancy rate does not decline in higher cycles numbers.

Overall, 1744 patients started 7097 cycles and 643 (36.9% per patient) ongoing pregnancies followed. In 49 of cycles, supernumerary embryos were cryopreserved. Out of these, so far, 36 embryo transfers were performed, leading to 6 ongoing pregnancies. Data on cryopreserved embryos are not included in the following analyses.

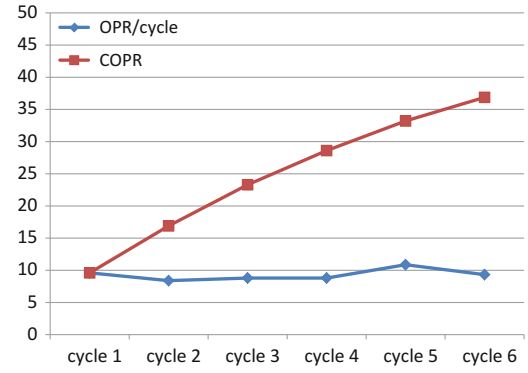

Fig. 13.4 Ongoing pregnancy rate (OPR) and cumulative ongoing pregnancy rate (COPR) according to cycle number

Cumulative Pregnancy Rates According to Patient Age

Results according to female patient age are shown in Table 13.6 and Figs. 13.5 and 13.6. Figure 13.5 shows the number of cycles according to age. The relatively low number of cycles in patients over 34 years is a reflection of the fact that from 2007 onwards, MNC was no longer offered to patients aged over 34 years. Figure 13.6 shows the pregnancy rate and ongoing pregnancy rate according to age. This figure shows that with increasing age there is a gradual decline in pregnancy rates per cycle.

Patients were grouped according to age at first cycle, and results per patient were calculated (Table 13.6). We found no apparent difference in performance in each step of the procedure (number of oocyte retrievals and successful oocyte retrievals, fertilization rate and embryo transfer per cycle all not significantly different), but ongoing implantation rate declines with age, which is of course not surprising.

The difference in pregnancy rates among the groups was not statistically significant. The cumulative ongoing pregnancy rate in patients aged 32–35 and 36–39 was significantly lower than in patients aged 28–31 years. The number of cycles per patient was not different between groups.

Table 13.6 Results according to female patient age

Age	20–23	24–27	28–31	32–35	36–39
No. of patients	43	233	530	792	146
Indication					
Tubal factor	5	40	76	132	33
Unexplained	5	24	79	182	52
Male	33	169	375	478	61
No. of cycles (No/pt)	168 (3.9)	890 (3.8)	2145 (4.0)	3340 (4.2)	554 (3.8)
Oocyte retrieval (%/cycle)	135 (80.4)	778 (87.4)	1880 (87.6)	2879 (86.2)	466 (84.0)
Oocyte retrieval successful (%/attempt)	109 (80.7)	620 (79.7)	1487 (79.1)	2258 (78.4)	357 (76.6)
Fertilization (%/successful oocyte retrieval)	73 (67.0)	408 (65.8)	1024 (68.9)	1529 (67.7)	281 (78.7)
Embryo transfer (%/cycle)	68 (40.5)[a]	374 (42.0)[a]	934 (43.5)[a]	1376 (41.2)[a]	250 (45.1)[a]
Pregnancy (%/cycle)	19 (11.3)[b]	117 (13.2)[c]	269 (12.5)[b,c]	361 (10.8)[c]	56 (10.1)
Ongoing pregnancy (%/cycle) *95% CI*	13 (7.7) *3.6–11.9*	92 (10.3)[c] *8.3–12.4*	223 (10.4)[c] *9.1–11.7*	271 (8.1)[c] *7.2–9.1*	44 (7.9) *5.6–10.2*
Ongoing implantation rate (%/embryo) *95% CI*	18.9 *9.3–27.9*	24.3 *21.8–26.2*	23.6 *20.8–26.4*	19.3 *17.2–21.4*	17.5 *12.7–22.2*
COPR (%/patient) *95% CI*	30.2 *16.2–44.2*	39.5 *33.1–45.9*	42.1 *37.8–42.1*	34.2 *30.8–37.6*	30.1 *22.5–37.7*

[a]In age categories 20–23, 24–27, 28–31, 32–35 and 36–39: 2, 5, 6, 13 and 2 DET
[b]In age categories 24–27 and 28–31: one and two twin pregnancies after DET
[c]In age categories 24–27, 28–31 and 32–35: 1, 3 and 4 pregnancies after IUI or coitus, of which 1, 3 and 3 ongoing

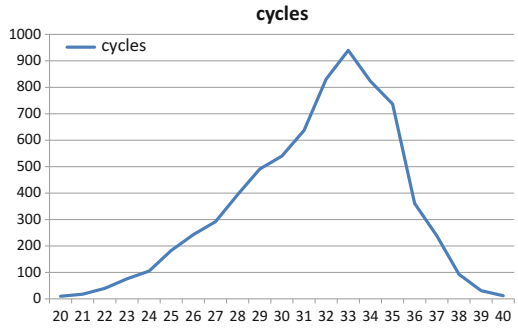

Fig. 13.5 Number of cycles according to female patient age

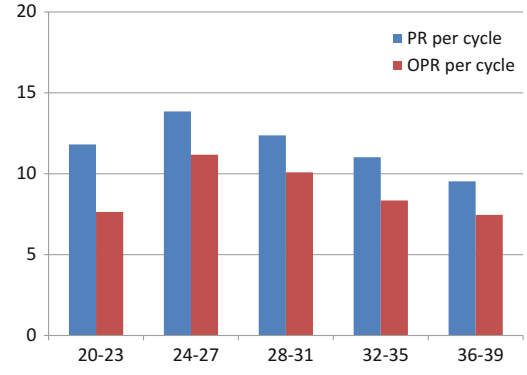

Fig. 13.6 Pregnancy and ongoing pregnancy rates according to female patient age categories

Cumulative Pregnancy Rates According to Indication for ART

Results according to indication for ART are shown in Table 13.7. These data show that the number of oocyte retrievals and the number of successful oocyte retrievals per patient are not different according to indication, but fertilization rate is. Not surprisingly, the lowest fertilization rate was found in male factor and the highest in tubal factor subfertility.

The high fertilization rate in tubal factor subfertility translates into a higher number of embryo transfers per cycle, while in unexplained subfertility, the fertilization rate was higher compared to male factor but the number of embryo transfers was not. Pregnancy rates per

Table 13.7 Results according to indication for ART

Indication for ART	Tubal factor	Unexplained	Male factor
No. of patients	286	342	1116
Mean age (range)	32 (20–38)	32 (20–39)	32 (20–39)
No. of cycles (No/pt)	1143 (4.0)	1329 (3.9)	4625 (4.1)
Oocyte retrieval (%/cycle)	989 (86.5)	1150 (86.5)	3999 (86.5)
Oocyte retrieval successful (%/attempt)	786 (79.5)	891 (77.5)	3154 (78.9)
Fertilization (%/successful oocyte retrieval) *95% CI*	655 (83.3) *80.7–86.0*	625 (70.2) *67.1–73.2*	2035 (64.5) *62.8–66.2*
Embryo transfer (%/cycle) *95% CI*	560 (49.0) *46.0–51.9*	538 (40.5) *37.8–43.2*	1904 (41.2) *39.7–42.6*
Pregnancy (%/cycle)	137 (12.0)	147 (11.1)[a]	538 (11.6)[b]
Ongoing pregnancy (%/cycle) *95% CI*	105 (9.2) *7.5–10.9*	112 (8.4)[a] *6.9–10.0*	426 (9.2)[b] *8.4–10.1*
COPR (%/patient) *95% CI*	36.8 *31.0–42.4*	32.8 *27.7–37.8*	38.2 *35.3–41.1*

[a] Three pregnancies (of which two ongoing) in cycles where oocyte retrieval was cancelled and IUI was done
[b] Five pregnancies (all ongoing) in cycles where oocyte retrieval was cancelled and IUI was done

cycle and cumulative ongoing pregnancy rates per patient were not different between groups. The number of cycles per patient was not different between groups.

Cumulative Pregnancy Rates According to BMI

Results according to BMI are shown in Table 13.8. Unfortunately, we have no data on BMI from patients treated before 2011. The table shows results from patients starting treatment in 2011, 2012, and 2013. Most of the BMIs in this table are self-reported. The low number of patients with BMI $\geq$ 35 is a reflection of the fact that in our centre, these patients are normally not admitted to the IVF program, except in research settings.

The number of (successful) oocyte retrievals, fertilization rate and embryo transfer rate was not different among BMI categories. There is an obvious trend towards a decrease in pregnancy and implantation rate with increasing BMI. The cumulative ongoing pregnancy rate in patients with BMI 30–34 was significantly lower than in patients with BMI 18–24. The number of cycles per patient was not different between groups.

It seems from these results that the lower success rate in obese women is due to a decreased implantation rate and not caused by lower success rate of oocyte retrieval or lower fertilization or embryo transfer rate. This finding is in analogy with what is found in COH-IVF [5].

Performance in First Cycle as a Predictor of Performance in Subsequent Cycles

As discussed before, in our earlier work we found cancellation of oocyte retrieval, fertilization failure and failure to reach embryo transfer to be repeating phenomena in subsequent cycles. In our larger series, we did a similar analysis, results of which are shown in Tables 13.9 and 13.10.

Cancellation of Oocyte Retrieval and Unsuccessful Oocyte Retrieval

Table 13.9 shows the results of subsequent cycles in three groups of patients: oocyte retrieval cancelled [either not planned or planned oocyte retrieval cancelled because of unexpected ovulation] (A), oocyte retrieval unsuccessful in

Table 13.8 Results according to BMI

BMI	18–24	25–29	30–34	35–40
No. of patients	182	100	39	4
Indication				
Tubal factor	25	11	4	–
Unexplained	26	7	2	–
Male	131	82	33	4
No. of cycles (No/pt)	764 (4.2)	417 (4.2)	165 (4.2)	17 (4.3)
Oocyte retrieval (%/cycle)	721 (94.4)	372 (89.2)	146 (88.5)	15 (88.2)
Oocyte retrieval successful (%/attempt)	604 (83.8)	314 (84.4)	123 (84.3)	11 (73.3)
Fertilization (%)	413 (68.4)	186 (59.2)	76 (61.8)	8 (72.7)
Embryo transfer (%/cycle)	382 (50.0)	176 (42.2)	69 (41.8)	8 (47.1)
Pregnancy (%/cycle)	111 (14.5)	54 (13.0)	11 (6.7)	2 (11.8)
Ongoing pregnancy (%/cycle) *95% CI*	91 (11.9) *9.6–14.3*	39 (9.4) *6.5–12.2*	9 (5.5) *1.9–9.0*	1 (5.9) *0–17.3*
Ongoing implantation rate (%/embryo) *95% CI*	91 (23.8) *19.5–28.2*	39 (22.2) *15.9–28.4*	9 (13.0) *4.9–21.2*	1 (12.5) *0–35.9*
COPR (%/patient) *95% CI*	91 (50.0) *42.6–57.4*	39 (39.0) *29.2–48.8*	9 (23.1) *13.5–36.6*	1 (25.0) *0–68.3*

Table 13.9 Results of subsequent cycles after cancellation of oocyte retrieval, unsuccessful oocyte retrieval and successful oocyte retrieval in the first cycle

Results of first cycle	A: OR not performed	B: OR unsuccessful	C: OR successful
No. of patients	242	304	1030
Mean age (range)	31 (20–39)	31 (20–39)	31 (20–39)
Indication			
Tubal factor	46	48	164
Unexplained	40	74	195
Male	156	182	671
Cycles 2-6 of same patients			
No. of cycles (No/pt)	752 (3.1)	1008 (3.3)	3593 (3.5)
Oocyte retrieval (%/cycle) *95% CI*	566 (75.3) *72.1–78.5*	864 (85.7) *83.5–87.9*	3206 (89.2) *88.1–90.3*
Oocyte retrieval successful (%/attempt) *95% CI*	437 (77.2) *73.7–80.7*	629 (72.8) *69.8–75.8*	2567 (80.1) *78.7–81.4*
Fertilization (%/successful oocyte retrieval) *95% CI*	275 (62.9) *58.3–67.6*	427 (67.9) *64.2–71.6*	1763 (68.7) *66.8–70.5*
Embryo transfer (%/cycle) *95% CI*	246 (32.7) *29.3–36.1*	384 (38.1) *35.0–41.2*	1590 (44.3) *42.6–45.9*
Pregnancy (%/cycle) *95% CI*	76 (10.1) *7.9–12.3*	110 (10.9) *9.0–12.9*	417 (11.6) *10.5–12.7*
Ongoing pregnancy (%/cycle) *95% CI*	57 (7.6) *5.6–9.5*	85 (8.4) *6.7–10.2*	333 (9.3) *8.3–10.2*
COPR (%/patient) *95% CI*	23.6 *18.1–29.0*	28.0 *22.8–33.1*	32.3 *29.4–35.2*

[a]First cycles with embryo transfer leading to an ongoing pregnancy excluded

Table 13.10 Results of subsequent cycles after fertilization failure, no embryo transfer and embryo transfer not leading to ongoing pregnancy in the first cycle

Results of first cycle	D: fertilization failure	E: no ET other reason[a]	F: ET, no ongoing pregnancy
No. of patients	331	91	608
Mean age (range)	31 (21–39)	31(22–38)	31 (20–39)
Indication			
Tubal	32	19	113
unexplained	58	27	110
Male	241	45	385
Cycles 2–6 of same patients			
No. of cycles (No/patient)	1234 (3.7)	234 (2.6)	2125 (3.5)
Oocyte retrieval (%/cycle) *95% CI*	1096 (88.8) *87.0–90.6*	211 (90.2) *86.3–94.1*	1899 (89.4) *88.0–90.7*
Oocyte retrieval successful (%/attempt) *95% CI*	892 (81.4) *79.0–83.7*	174 (82.5) *77.2–87.7*	1501 (79.0) *77.2–80.9*
Fertilization (%/successful oocyte retrieval) *95% CI*	532 (59.6) *56.4–62.9*	132 (75.9) *69.4–82.4*	1099 (73.2) *70.9–75.5*
Embryo transfer (%/cycle) *95% CI*	478 (38.7) *35.9–41.5*	108 (46.2) *39.6–52.7*	1004 (47.3) *45.1–49.4*
Pregnancy (%/cycle) *95% CI*	126 (10.2) *8.5–11.9*	30 (12.8) *8.4–17.2*	261 (12.3) *10.9–13.7*
Ongoing pregnancy (%/cycle) *95% CI*	104 (8.4) *6.8–10.0*	23 (9.8) *5.9–13.7*	206 (9.7) *8.4–11.0*
COPR (%/patient) *95% CI*	31.4 *26.3–36.5*	25.3 *16.2–34.4*	33.9 *30.0–37.7*

[a]GV or MI oocyte, >2 pronuclei at fertilization check or >50% fragmentation on day 2 or 3

the first cycle (B), and oocyte retrieval successful in the first cycle (C). This table shows that if oocyte retrieval was cancelled in the first cycle, the number of oocyte retrievals performed in subsequent cycles is lower, as compared to the other two groups of patients. Fertilization rate and embryo transfer rate in this group were lower compared to group C but not B. In group B, the proportion of successful oocyte retrievals was lower than in group C but not A.

Pregnancy rates and ongoing pregnancy rates were not different among groups. The cumulative ongoing pregnancy rate after six cycles was lower in group A compared to group C but not B. The number of cycles per patient was not different between groups. Overall, 480 (30.4%) of patients stopped MNC without completing 6 (unsuccessful) cycles. We do not have data on the number of patients that stopped treatment because of intercurrent spontaneous pregnancies, so the actual cumulative dropout rate is probably a bit lower. The cumulative dropout rates in groups A, B and C, respectively, were 42.8, 34.5 and 26.3%, suggesting that, as we found in our earlier study, dropout is selective in the sense that patients with poor prognosis tend to drop out.

Fertilization and Embryo Transfer

In order to evaluate fertilization failure as a repeating phenomenon, group C from Table 13.9 was subdivided in three groups: fertilization failure (D), no embryo transfer (immature oocyte, >2 pronuclei at fertilization check or >50% fragmentation on day 2 or 3; E) and

embryo transfer performed (first cycles with embryo transfer leading to an ongoing pregnancy excluded; F). Results are shown in Table 13.10. Fertilization rate was lower in group D versus E and F. Embryo transfer rate was lower in group D versus F but not E. Pregnancy and ongoing pregnancy rates as well as cumulative ongoing pregnancy rates were not different between groups. Comparison of results from Tables 13.9 and 13.10 show that embryo transfer rate was significantly lower in group A versus E and F, and cumulative ongoing pregnancy rate was lower in group A than in group F. The number of cycles per patient in group E was lower than in the other groups.

Discussion

In modified natural cycle IVF, treatment is aimed at the use of the one oocyte that is naturally selected. In our opinion, this is a major advantage of the MNC approach, since this one naturally selected oocyte probably represents the best one from a cohort of oocytes. This thought is supported by the fact that a large proportion of embryos arising from MNC are of good quality. Also, the overall implantation rate of embryos from MNC, although unselected, compares favourably to implantation rates of COH embryos [6].

The high implantation rates found in MNC may also be due to better endometrial quality compared to standard IVF. Ovarian stimulation is often reported to be detrimental to endometrial receptivity, especially with high numbers of oocytes and high estradiol levels.

A drawback of MNC-IVF, however, is the considerable loss in every step of the procedure. Cancellation of oocyte retrieval, whether because of lack of follicle development or because of LH rise or unexpected ovulation, unsuccessful oocyte retrieval, fertilization failure and abnormal fertilization frequently occur. In the large series described in this chapter, the embryo transfer rate was 42.3% per started cycle.

We routinely use a GnRH antagonist to prevent untimely ovulation, but still about 10% of planned oocyte retrievals are cancelled because of LH rise or unexpected ovulation. In natural cycle IVF (without the use of a GnRH antagonist), the range in reported cancellation rates is quite wide (3.8–53.1% per started cycle) [7–10]. The lowest cancellation rates are found in studies where quite intensive monitoring was applied, as well as great flexibility regarding planning of oocyte retrieval. Still, one could wonder whether the routine use of a GnRH antagonist in all cycles is justified. It would seem from the data that in a certain amount of cycles, a GnRH antagonist would not have been necessary, but it is unknown which are the patients that do or do not benefit from the GnRH antagonist. Omitting the GnRH antagonist from the protocol will reduce costs related to medication but on the other hand will require more intensive monitoring to obtain reasonable results.

Another way to prevent untimely ovulations is the use of indometacin. Indometacin as a cyclooxygenase inhibitor inhibits the production of prostaglandins, which are needed for follicular rupture and ovulation. Several studies reported on low ovulation rates (0–10%) in cycles where indometacin was added to a (modified) natural cycle protocol [9, 11–13]. In our centre, a randomized study was done in which results were compared in MNC cycles using GnRH antagonist with and without addition of indometacin. The number of patients with at least one ovulation after a maximum of six cycles was not different between groups, and no difference in terms of embryo transfer and pregnancy rate were found. In cycles where an LH surge was observed, the premature ovulation rate was not different between groups. In cycles without LH surge, however, the ovulation rate was significantly lower in the group where indometacin was used [4].

Other adjustments to the MNC protocol, such as changes in timing of ovulation triggering or changes in dosage of GnRH antagonist or gonadotrophins, are imaginable, in order to either raise success rates or reduce costs of medication. In order to improve the effectiveness of the oocyte retrieval procedure, flushing of the follicle is often proposed. So far, no studies comparing flushing to no flushing in (modified) natural cycle

are available, but data from studies on this subject in standard IVF suggest that it is of no benefit [14]. Flushing of the follicle will make the oocyte retrieval procedure more painful and time-consuming. There are no studies available on the necessity of luteal phase support in MNC-IVF. In theory, depletion of granulosa cells from the follicle at oocyte retrieval, as well as the use of GnRH antagonist, gonadotrophins and hCG may influence corpus luteum function. In a small series of cycles where no embryo transfer was performed (n = 24), we found shortened luteal phase and low mid-luteal progesterone levels in about one-third of cases (*unpublished data)*.

Modified natural cycle IVF is a low-risk treatment modality, with a close to zero risk of OHSS and a very low multiple pregnancy rate. Sporadically multiple follicles develop in a modified natural cycle, due to co-dominance of two or three follicles, or due to unintentional ovarian stimulation, when GnRH antagonist and gonadotrophins are started too early in the cycle. In our extended series, in only 5.5% of cycles more than one oocyte was obtained, and in only 9 of these cases (0.13%) $\geq$ 10 oocytes were retrieved. In our original studies and in the extended series described in this chapter, no OHSS occurred.

In 150 out of 7097 cycles (2.1%), 2 or more embryos were available for transfer. When we first started with MNC, double embryo transfer was done in cases where more than one embryo was available, and a twin pregnancy rate of about 2% (monozygous twins after transfer of a single embryo not included) followed. We then changed the transfer policy, and in all cases single embryo transfer was done. The twinning rate is now close to zero, with sporadically occurring monozygous twins. The very low multiple pregnancy rate in MNC is advantageous given the obstetrical risks involved, but in the current era with increasing application of single embryo transfer in COH-IVF, this is becoming a less-relevant argument *pro* MNC in comparison with COH-IVF.

Modified natural cycle IVF is a patient-friendly treatment modality, due to short duration and low dose of hormonal medication, easy oocyte retrieval (analgesia in most cases not required) and easy repeatability in consecutive cycles. On the other hand, frequent visits to the clinic 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. The few studies on patient perceptions on (modified) natural cycle or mild IVF that are available, all report the low dose and short duration of hormonal medication use to be an important positive aspect of these treatments [15–17]. In our cohort studies, we did a small questionnaire study. Patients who had completed a series of MNC-IVF cycles were asked, among other things, whether, looking back on things, they would make the same choice of participating in the study, and 80% of respondents answered positively. Injections of medication and oocyte retrieval were reported burdensome by 5 and 23% of respondents, respectively, whereas cancellation of the cycle (no embryo transfer possible) was reported as burdensome by 83% of respondents (*unpublished data*).

Judging from our data, it does not seem possible to select patients who are likely to do well with MNC-IVF based on patient characteristics such as age, indication for ART and BMI. However, it does seem possible to differentiate patients with relatively poor prognosis for success from those with better chances based on their performance in the first MNC cycle. In particular, cancellation of oocyte retrieval in the first cycle seems to predict poor overall outcome.

In selecting patients for MNC-IVF, it is very important not to consider the expected success rate per se, but to consider what would be the expected success rate after MNC in relation to that of other treatment modalities. For instance, our data show that success rates of MNC decrease with increasing age and higher BMI, but this is due to reduced implantation rates, which would also be expected in these patients when applying COH-IVF.

In specific situations, such as patients with a history of severe OHSS or those opposing to the creation of supernumerary embryos, MNC may

be preferred over COH-IVF. For poor responders to COH, MNC seems an attractive option since with COH these patients will have only few oocytes with low embryo transfer rate. Thanks to the easy repeatability in consecutive months, comparable numbers of oocytes could be obtained with MNC. Whether or not MNC should be the treatment of choice for poor responders to COH remains to be a subject to debate [18–21].

Only few comparative studies on MNC versus COH-IVF are available [22]. Preliminary results from a large randomized controlled trial comparing MNC to COH-IVF in unexplained and mild male factor subfertility suggested equal effectiveness in terms of ongoing pregnancy rates per patient [23]. Cost-effectiveness analysis in this study (only direct costs per pregnancy reported) seems unfavourable for MNC versus COH-IVF [24].

In conclusion, MNC-IVF is a low-risk and patient-friendly treatment modality and in our opinion is a feasible alternative to COH-IVF. The major drawback of MNC is the considerable loss in every step of the procedure, leading to rather low embryo transfer and pregnancy rates per cycle, but thanks to the short duration of a treatment cycle and easy repeatability in consecutive months, results in terms of time to pregnancy are favourable. Results regarding pregnancy rates or cost-effectiveness may be improved by adjustments to the protocol, or by more specific selection of patients.

References

1. Nargund G, Fauser BCJM, Macklon NS, Ombelet W, Nygren K. Frydman R; rotterdam ISMAAR consensus group on terminology for ovarian stimulation for IVF. The ISMAAR proposal on terminology for ovarian stimulation for IVF. Hum Reprod. 2007;22: 2801–4.
2. Pelinck MJ, Vogel NEA, Hoek A, Simons AHM, Arts EGJM, Mochtar MH, Beemsterboer S, Hondelink MN, Heineman MJ. Cumulative pregnancy rates after three cycles of minimal stimulation IVF and results according to subfertility diagnosis: a multicentre cohort study. Hum Reprod. 2006;21: 2375–83.
3. Pelinck MJ, Vogel NEA, Arts EGJM, Simons AHM, Heineman MJ, Hoek A. Cumulative pegnancy rates after a maximum of nine cycles of modified natural cycle IVF and analysis of patient drop-out: a cohort study. Hum Reprod. 2007;22:2463–70.
4. Rijken-Zijlstra TM, Haadsma ML, Hammer C, Burgerhof JGM, Pelinck MJ, Simons AHM, van Echten-Arends J, Land JA, Groen H, Hoek A. Effectiveness of indometacin to prevent ovulation in modified natural cycle IVF: a randomized controlled trial. Reprod Biomed Online; 27: 297–304.
5. Sobaleva S, El-Toukhy T. The impact of raised BMI on the outcome of assisted reproduction: current concepts. J Obstet Gynaecol. 2011;31:561–5.
6. Gordon JD, DiMattina M, Reh A, Botes A, Celia G, Payson M. Utilization and success rates of unstimulated in vitro fertilization in the United States: an analysis of the society for assisted reproductive technology database. Fertil Steril. 2013;100:392–5.
7. Omland AK1, Fedorcsák P, Storeng R, Dale PO, Abyholm T, Tanbo T. Natural cycle IVF in unexplained, endometriosis-associated and tubal factor infertility. Hum Reprod 2001, 12: 2587–2592.
8. Pelinck MJ, Hoek A, Simons AH, Heineman MJ. Efficacy of natural cycle IVF: a review of the literature. Hum Reprod Update. 2002;8:129–39.
9. Lenton EA. Natural cycle IVF with and without terminal hCG: learning from failed cycles. Reprod Biomed Online. 2007;15:149–55.
10. Von Wolff M, Nitzschke M, Stute P, Bitterlich N, Rohner S. Low-dosage clomiphene reduces premature ovulation rates and increases transfer rates in natural-cycle IVF. Reprod Biomed Online. 2014;29: 209–15.
11. Nargund G, Waterstone J, Bland J, Philips Z, Parsons J, Campbell S. Cumulative conception and live birth rates in natural (unstimulated) IVF cycles. Hum Reprod. 2001;16:259–62.
12. Kadoch IJ, Al-Khaduri M, Phillips SJ, Lapensée L, Couturier B, Hemmings R, Bissonnette F. Spontaneous ovulation rate before oocyte retrieval in modified natural cycle IVF with and without indometacin. Reprod Biomed Online. 2008;16:245–9.
13. Kawachiya S1, Matsumoto T, Bodri D, Kato K, Takehara Y, Kato O. Short-term, low-dose, non-steroidal anti-inflammatory drug application diminishes premature ovulation in natural-cycle IVF. Reprod Biomed Online 2012, 24: 308–313.
14. Wongtra-Ngan S1, Vutyavanich T, Brown J. Follicular flushing during oocyte retrieval in assisted reproductive techniques. Cochrane Database Syst Rev; 2010, 9: CD004634.
15. Højgaard A1, Ingerslev HJ, Dinesen J. Friendly IVF: patient opinions. Hum Reprod 2001, 16: 1391–1396.
16. Pistorius EN, Adang EM, Stalmeier PF, Braat DD, Kremer JA. Prospective patient and physician preferences for stimulation or no stimulation in IVF. Hum Fertil (Camb). 2006;9:209–16.
17. Garel M, Blondel B, Karpel L, Blanchet V, Breart G, Frydman R, Olivennes F. Women's views on

friendly IVF: a qualitative preliminary study. J Psychosom Obstet Gynaecol. 2009;30:101–4.
18. Schimberni M, Morgia F, Colabianchi J, Giallonardo A, Piscitelli C, Giannini P, Montigiani M, Sbracia M. Natural-cycle in vitro fertilization in poor responder patients: a survey of 500 consecutive cycles. Fertil Steril. 2009;92:1297–301.
19. Kadoch IJ, Phillips SJ, Bissonnette F. Modified natural-cycle in vitro fertilization should be considered as the first approach in young poor responders. Fertil Steril. 2011;96:1066–8.
20. Polyzos NP, Blockeel C, Verpoest W, De Vos M, Stoop D, Vloeberghs V, Camus M, Devroey P, Tournaye H. Live birth rates following natural cycle IVF in women with poor ovarian response according to the Bologna criteria. Hum Reprod. 2012;27:3481–6.
21. Kedem A, Tsur A, Haas J, Yerushalmi GM, Hourvitz A, Machtinger R, Orvieto R. Is the modified natural in vitro fertilization cycle justified in patients with "genuine" poor response to controlled ovarian hyperstimulation? Fertil Steril. 2014;101:1624–8.
22. Allersma T, Farquhar C, Cantineau AEP. Natural cycle in vitro fertilisation (IVF) for subfertile couples. Cochrane Database Syst Rev. 2013;8: CD010550.
23. Bensdorp AJ, Slappendel E, Koks C, Oosterhuis J, Hoek A, Hompes P, et al. The INeS study: prevention of multiple pregnancies: a randomised controlled trial comparing IUI COH versus IVF eSET versus MNC IVF in couples with unexplained or mild male subfertility. BMC Women's Health. 2009;9:35.
24. Tjon-Kon-Fat RI, Bensdorp AJ, Maas J, Oosterhuis GJE et al. An economic analysis comparing IVF with a single embryo transfer and IVF with a modified natural cycle to IUI with hyperstimulation (the INeS trial). Hum Reprod. 2013;28 S1: Abstract 0–171.