Chapter | *4*

Prevention of multiple pregnancies after IVF in women 38 and older: a randomised study

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Introduction

Multiple pregnancy rates after in vitro fertilization (IVF) treatment are substantial. In the Netherlands approximately 25% of the ongoing pregnancies after IVF is a multiple pregnancy and this is in line with rates observed in other European countries (148). Almost half of the children after IVF is part of a multiplet.

Multiple pregnancies are accompanied by a higher mortality and morbidity rate due to premature birth and low birth weight. Prematurity occurs in 5-10% of singleton pregnancies and in 40-60% of twin pregnancies after IVF (14). The same appears to be true for the risk of low birthweight which occurs in 5-10% and 50-71% respectively (149,150,151, 152,153,154,55). Perinatal mortality is 5 times higher in twin pregnancies and the chance of neurological morbidity is 8 times higher compared with singleton pregnancies (14). All this implies that in twin pregnancies the chance of having one or two children that either have suffered perinatal death or have become severely neurological damaged may approach 8% versus 0.5% in singletons. Twin pregnancies also imply a higher risk for the mother such as preterm labour, gestation induced hypertension, diabetes and vaginal blood loss (153). Costs of an IVF treatment do not only contain the medical treatment costs but also the costs of obstetrical and neonatal care. Such costs are considerably higher in twin pregnancies (155,55). Clinicians and patients have become increasingly aware that multiple pregnancies should not be viewed as an undisputable success and should be avoided if possible.

Retrospective research has suggested that by transfer of 2 embryos instead of 3 in women under 35 years of age the pregnancy rate is not significantly different whereas the multiple pregnancy rate is significantly reduced in the group where 2 embryos were transferred (156). This finding has led to a major decrease in the rate of transfer of 3 embryos, at least in most European centers.

Recent studies showed that in patients younger than 38 years, in whom at least 3 good quality embryos were available, single embryo transfer (SET) compared to dual embryo transfer yields reduced ongoing pregnancy rates (36,40,41,157,43,44). However, this reduced rate has to be set against the advantage of the elimination of multiple pregnancies (43,44). The reduced success rates may be compensated by performing an additional treatment cycle or by applying a high-quality frozen-thawed embryo program (43,44).

No randomised controlled trials in this research field have been performed in women above 38 years. Because implantation will considerably decrease with age (158) pregnancy rates are decreased by a factor 2 and ongoing pregnancy rates are only one third of those in the younger age class (159). Therefore most clinicians agree that SET is not advisable in women of 38 years and older (51). Little is known on the feasibility of transferring 2 in stead of 3 embryos in women of this age in order to decrease the incidence of

multiple gestations. The present study aims to answer the question whether dual instead of triple embryo transfer during IVF treatment in patients over 38 years will substantially reduce the number of multiple pregnancies while the chance of a term (>37 weeks gestational age) live birth per started treatment still remains acceptable. The outcome parameter term live birth per treatment instead of per cycle is used because the goal of an IVF treatment is having a healthy baby after completion of an IVF treatment consisting of a series of IVF cycles and subsequent replacement of frozen embryos.

Materials and Methods

Study design

A two center controlled randomised study was performed. Randomisation was carried out using sealed envelops opened by the study coordinator on the phone. Study approval was obtained by the local ethics committee of the University Medical Centre Utrecht and the Rijnstate Hospital Arnhem, the Netherlands.

Patients

Patients on the waiting list for IVF or IVF/ICSI were recruited for the study. Recruitment took place in 2 hospital centers for reproductive medicine in the period October 2001 through December 2003. Patients were eligible for inclusion in the study if they were 38 years and older and had an indication for an IVF or IVF/ICSI treatment either for the first time or after a previous IVF or IVF/ICSI childbirth. No other inclusion criteria were applied. Patients were informed about the study by word of mouth by a doctor and in writing by a patient information leaflet. Randomisation was performed during the IVF or IVF/ICSI intake after checking for inclusion and exclusion criteria. Written informed consent was obtained from all patients.

Treatment groups

All participants were randomised into one of two embryo transfer strategy groups. The first group was intended to undergo a transfer of a maximum of 2 embryos in the first 3 cycles (dual embryo transfer strategy: DET-group). In order to compensate for a possible reduction in pregnancy rate in this group, patients were offered a fourth reimbursed cycle in which the choice for the transfer of 2 or 3 embryos was left to the couple. The second group was intended to have the transfer of a maximum of 3 embryos in the first 3 treatment cycles (three embryo transfer strategy: TET-group). Randomisation for the whole treatment period was performed before information about embryo quality was available because we wanted to investigate a general policy applicable in clinical practice based on age without pre-selection on embryo quality.

Ovarian Stimulation Protocol

All cycles were performed by a long agonist suppression protocol (leuprolide, Lucrin: Abbott B.V., Amstelveen, The Netherlands; 0,2 mg/day, sc, or triptorelin, Decapeptyl: Ferring B.V. Hoofddorp, The Netherlands; 0,1 mg/day, sc). After downregulation was established recombinant FSH (recFSH) (Gonal-F; Serono Benelux B.V., Amsterdam, The Netherlands, or Puregon; N.V. Organon, Oss, The Netherlands), in a sc dose of 150 IU daily was started (stimulation day 1). Dose adjustments during the first cycle or in subsequent cycles were performed on an individual basis. Human chorionic gonadotropin (hCG) (Profasi, 10.000 IU, sc; Serono Benelux B.V., Amsterdam, The Netherlands, or Pregnyl, 5000-10.000 IU, sc; N.V. Organon, Oss, The Netherlands, or Ovitrelle, 250 microg, sc; Serono Benelux B.V., Amsterdam, The Netherlands) was administered for final oocyte maturation when the largest follicle had reached a diameter of at least 18 mm and at least 1 additional follicle > 14 mm was observed. 36 hours later oocyt retrieval was performed and embryos were transferred 3 or 4 days after oocyte pick up. Luteal phase support was started on the day of oocyte pick up.

Methods of analysis

Little information is available on cumulative term live birth rates in subsequent cycles in this age group. Moreover we did not know whether patients were willing to remain in the randomised group if they did not get pregnant in the first one or two cycles. Therefore, we decided to perform a pilot study first in which we aimed to include approximately 50 patients. Depending on the results a decision on the continuation of the trial was to be taken or suggestions for further research would be given. The two treatment groups were compared using the t-test and the ^{2}-test. A $p<0.05$ was considered statistically significant. The mean number of cycles, oocyte pick ups en embryo transfers were compared using a Mann Whitney U test.

The primary outcome measure was the cumulative term (>37 weeks gestational age) live birth rate. Additionally, we provided information about the live births. To calculate the primary endpoints we first performed an intention to treat analysis (ITT-analysis) and constructed a Kaplan Meier survival curve, in which non-pregnant patients who did not proceed to a subsequent cycle were censored. This method assumes that these patients would have had the same chance of getting pregnant as the patients who did continue treatment (non-informative censoring). However, it is well possible that the cumulative rate will be too optimistic if patients with poorer prognosis drop out selectively (160,161,127). Therefore, an adapted Kaplan Meier curve was calculated, in which we assumed that the patients who did not continue treatment had no chance of getting pregnant (162). The first curve represents an optimistic chance, the second curve a pessimistic chance and we assume that the true cumulative term live birth rate is somewhere in between. Second a per-protocol analysis (PPA) was performed to account for couples

who switched from the DET strategy to the TET strategy being not pregnant after the first or second cycle. The cumulative term live birth rate for 4 cycles in the DET-group and 3 cycles in the TET-group was compared using the confidence interval of the difference between the 2 groups and a z-test.

Statistics Package for Social Sciences for Windows, version 11.5 (SPSS Inc., USA) was used for data analysis.

Results

Fourty five patients were included in the study. A total of 112 cycles were performed, 66 in the DET-group and 46 in the TET-group. The flowchart of the study according to CONSORT guidelines is shown in Figure 1.

The two groups were comparable regarding patient characteristics, cycle characteristics and treatment characteristics except for number of cycles, oocyte pick up and embryo transfers due to the treatment strategy (Table 1).

In the dual embryo transfer group, 23 first cycles, 20 second cycles, 15 third cycles en 8 fourth cycles were conducted. In the triple embryo transfer group, 22 first cycles, 15 second cycles and 9 third cycles were carried out. In the DET-group 3 patients had 3 embryos transferred in the fourth cycle. The optimistic cumulative term live birth rate (assuming that drop outs have the same chances as patients who continued) in the DET-group after 4 cycles was 47.3% and in the TET-group after 3 cycles was 40.5% (Figure 2). The difference between the DET and TET-group was 6.8% in the favour of the DET-group (95% CI -25;38) (p=0.7). The pessimistic cumulative term live birth rate in the DET and TET-group did not differ statistically (Table 3). In the DET-group 4 patients (17.4%) switched to another embryo transfer policy whereas 0 patients switched in the TET-group. Two patients in the DET-group (8.6%) conceived spontaneously. When excluding this patients in the analysis (per-protocol analysis) the optimistic and pessimistic cumulative term live birth rate in the DET and TET-group did not differ statistically (Table 2). The cumulative singleton live birth after 4 cycles in the DET-strategy and 3 cycles in the TET-strategy was 47.3% versus 37.0%.

The percentage of patients with at least one top quality embryo (Day 3: ≥8 cells, <10% fragmentation; Day 4: Morula, complete compaction, <10% fragmentation) in the DET-group was 54% and in the TET-group 67%. This difference was not statistically significant (p=0.3).

Transferring the required number of embryos per strategy was not always possible. In the DET-group in 20% of the started cycles embryo transfer of 2 embryo's was not possible because there were less than 2 embryos available. In the TET-group in 28.2% of the started cycles embryo transfer of three embryos was not possible because there

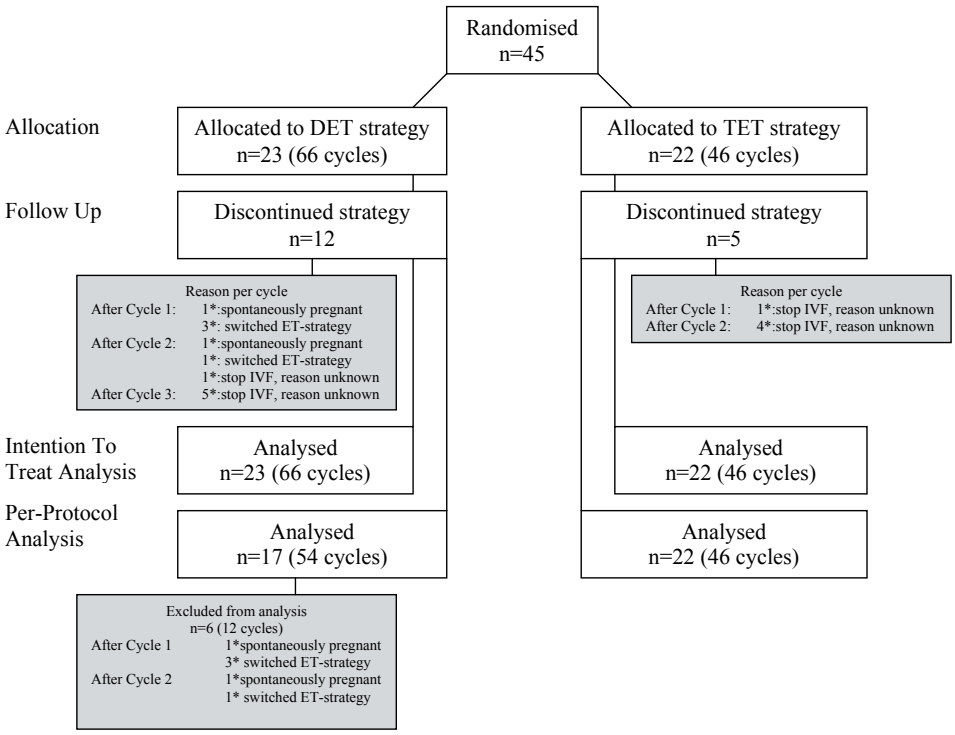

Figure 1. Flow chart according to CONSORT guidelines

were less than 3 embryos available. Cryopreservation in the DET-group was possible in 6 cycles and in the TET-group in 1 cycle. Transfer of cryopreserved embryos did not lead to an ongoing pregnancy. The ongoing (>12 weeks) implantation rate was 7.5% (95% CI 3.5;13.8) in the DET-group and 11.6% (95% CI 6.3;19) in the TET-group. De difference between the 2 groups was not significant (p=0.3).

In the DET-group there were no multiple pregnancies 0% (95% CI 0;24). In the TET-group there were 3 twin pregnancies 30% (95% CI 7;65). The difference in twin rate was marginally significant (p=0.05).

The mean gestational age in the DET-group was 39.8 weeks (range 38.1 – 42.3 weeks) and in the TET-group 39.5 weeks (range 35.4 - 42.1 weeks) (p=0.8). The mean birth weight in the DET-group was 3729.8 grams (range 2020 - 5030 grams) and in the TET-group 3298,3 grams (range 2000 – 4240 grams) (p=0.3). One child in a singleton pregnancy from the DET-group suffered intra uterine death after 31.5 weeks of gestation.

Table 1. Characteristics of patients, cycles and treatments in the DET-group and TET-group. All characteristics are based on the initial randomisation.

		DET-group	TET-group	p
Characteristics per patient		***23 patients***	***22 patients***	
Age (years)		40.8 (±1.7)	41.1 (±2.5)	NS
Dur inf (years) (range)		3.7 (±2.5)	3.2 (±2.4)	NS
Primary Infertility (%)		57	41	NS
Cause of inf (%)	***Cervical***	4.3	0	NS
	Anovulation	0	4.5	NS
	Tubal	21.7	22.7	NS
	Male	39.1	22.7	NS
	Unexpl	34.8	50	NS
No of Cycles (NC)		2.9 (±1.1)	2.1 (±0.9)	0.01*
NC with Oocyte Pick Up		2.7 (±1)	2 (±0.8)	0.01*
NC with Embryo Transfer		2.6 (±1)	1.9 (±0.8)	0.02*
Characteristics per cycle		***66 cycles***	***46 cycles***	
No cancelled cycles[a]		4 (6)	3 (6.1)	NS
No oocytes[b]		7.7 (2-21)	7.6 (2-19)	NS
No embryos[b]		4.4 (1-13)	5.1 (1-14)	NS
≥3 embryos available[a]		40 (60.6)	33 (71.8)	NS
No embryos transferred[b]		2.0 (1-3)	2.7 (1-3)	<0.001*
No cryopreserved embryos[b]		0.3 (0-5)	0.07 (0-3)	0.14
	Cycle no	**1 2 3 4**	**1 2 3**	
No of Started Cycles		23 20 15 8	22 15 9	NS
No Clin Pregn		7 7 2 2	6 3 2	NS
No Ong Pregn		3 4 2 2	6 2 2	NS
No Singlet Preg		3 4 2 2	4 2 1	NS
No Multi Preg		0 0 0 0	2 0 1	0.05**
No Live Birth		3 4 2 1	6 2 2	NS
No Term Live Birth		3 4 2 1	5 2 1	NS

Values are mean (±standard deviation) or [a]number (percentage), [b]mean (range) per embryo transfer,
*Mann Whitney U test, **Pearson χ^2 test

Up till the date of November 1, 2005 in the DET-group all patients had continued treatment after 1 completed cycle, 1 patient did not continue treatment after 2 cycles and 5 patients did not continue treatment after 3 cycles. The total rate of couples not completing the treatment strategy for other reasons than getting pregnant was 26%. In the TET-group 1 patient did not continue treatment after 1 cycle and 4 patients after the second cycle. The total rate of couples not completing the treatment strategy was 23% in this group. There was no significant difference in patient or cycle characteristics between the drop outs and patients who finished the full treatment strategy.

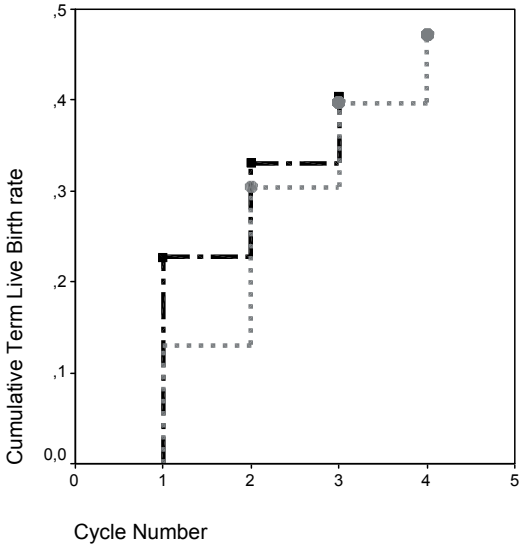

Figure 2. Cumulative Optimistic Term Live Birth rate (%) in DET and TET-group for intention to treat analysis.

Discussion

This study is the first randomised controlled trial comparing cumulative ongoing pregnancy rates after dual and triple embryo transfer in women of 38 years and older. It suggests that by applying dual instead of triple embryo transfer in subsequent cycles as standard strategy in patients of 38 years and older it is possible to reduce multiple pregnancy rates. Since the study was set up as a feasibility study, the numbers are too small to justify firm conclusions. The difference in the number of multiplets is obvious but the confidence intervals are wide and statistical significance on the edge.

Table 2. Cumulative Optimistic (Opt) en Pessimistisc (Pess) Term Live Birth rate (%) in DET and TET group for intention to treat and per protocol analysis

	Intention to Treat Analysis				**Per Protocol Analysis**			
	Opt DET	**Opt TET**	**Pess DET**	**Pess TET**	**Opt DET**	**Opt TET**	**Pess DET**	**Pess TET**
1 cycle	**13**	**22.7**	13	22.7	8.7	22.7	8.7	22.7
2 cycles	**30.4**	**33**	30.4	31.8	29	33	26.1	31.8
3 cycles	**39.7**[1b]	**40.5**[1ab]	39.1[2b]	36.4[2ab]	41.9[3b]	40.5[3ab]	34.8[4b]	36.4[4ab]
4 cycles	**47.3**[1a]		43.5[2a]		41.9[3a]		34.8[4a]	

[1a] difference 6.8% (95% CI -25;38) p=0,7;[1b] difference -0.8% (95% CI -31;29) p=0.96 ;[2a] difference 7.1% (95% CI -21;36) p=0.6;[2b] difference 2.7% (95% CI -26;31) p=0.9;[3ab] difference 1.4% (95% CI -31;34) p=0.9 ; [4ab] difference -1.6 (95% CI -30;26) p=0.9

In our experience it was quite difficult to recruit couples from those who were considered eligible, possibly due to the fact that couples in this age group anticipate an advantage of replacing a high number of embryos. To ensure that a difference in pregnancy rates is indeed smaller than 10% it would take 600 couples, based on the present findings. Such a study would imply a multi center set up in more than one country, an almost impossible endeavour.

Large but retrospective studies did not find differences in pregnancy rates per cycle performing DET compared to TET (52,53). Obviously such studies lacks the insight into the accumulation of pregnancies in subsequent cycles. Our findings show a trend in reduction of the per cycle chance of pregnancy when the number of embryos transferred is reduced. However, from the data shown it appears that application of a two embryo transfer strategy in women over 38 years will not change the final perspective of obtaining the desired healthy child. Furthermore the multiple pregnancy rate was significantly lower in the DET-group.

To accept the DET approach in daily practice it is important that, instead of looking at success in IVF treatment in terms of ongoing pregnancy rate per cycle, physicians and patients learn to look at success in terms of term live birth per whole IVF treatment or per treatment period (163). When using milder, more patient friendly, stimulation protocols the term live birth per whole IVF treatment or per treatment period could become higher because the drop out rate may possibly be decreased and more IVF cycles can be conducted in the same period of time (164).

By taking live birth per whole IVF treatment as endpoint the discussion will arise whether a twin counts as 1 or 2 live births. A patient who delivers 2 babies will be less inclined towards starting a next IVF treatment for a second child. Especially in women 38 years and older having 2 babies from one serie of IVF attempts may be the only way to obtain a family with two children. To date it is not clear how to incorporate this item in the process of deciding on embryo transfer strategy, where health of the offspring is balanced against the desire for a completed family.

In the light of the ongoing discussion on single and dual embryo transfer in women younger than 38 years the issue of the use of DET or TET in women above 38 years is very much comparable. By replacing less embryos the live birth rate per cycle seems to drop but by conducting an extra treatment cycle the cumulative term live birth rate after more cycles will be equal in the DET and the TET-group. In our study, transfer of cryopreserved embryos did not result in additional pregnancies. In larger groups this could possible prove to be different, although it is reasonable to assume that cryopreservation and transfer of cryopreserved embryos is less frequent in women above 38 years (165).

The study of cumulative cycles in this trial delivered methodological problems in the course of the subsequent treatment cycles. First, there is the problem of drop outs. The overall drop out rates in the course of four and three treatment cycles were not different

from those reported in the literature (160,32,166). Drop outs hamper the simple calculation of cumulative term live birth rates. To deal with this problem it was decided to calculate socalled optimistic and pessimistic scenarios (167,168).

A second problem within this study are the patients who switched from a DET to TET strategy in the course of the study period. For this reason we also conducted a per protocol analysis. Four patients in the DET-group switched from their allocated number of 2 embryos to transfer into 3 embryos and as such can be considered protocol violators. Therefore they were not included in the per-protocol analysis. Moreover, two patients in the DET-group became spontaneously pregnant between treatment cycles. These patients were also not included in the per-protocol analysis since the spontaneous pregnancies are not a direct result of the treatment given. Despite a considerable number of switchers and spontaneous pregnancies in the DET-group the per protocol analysis did not show a significant difference between both strategies. This finding proves that the almost identical cumulative term live birth rates between both strategies in the intention to treat analysis were not caused by switchers or spontaneous pregnancies.

A third methodological issue that emerged in the course of the study was the number of embryos that became actually available for transfer. Older women are expected to have less follicles, less oocytes and therefore less embryos available for transfer (169,170). In our study women had a relatively high number of oocytes at oocyte pick up. Except for the inclusion criteria mentioned in the materials and methods no other inclusion criteria were used to include patients. All patients between 38 and 45 years with an indication for IVF had the possibility to embark the study protocol. In our study in both treatment strategies about 20% could not receive the allocated number of embryos because there were simply not enough embryos.

Introducing dual embryo transfer in women above 38 years may require big efforts from both the clinician and the couple. The couple should be made aware of the balance between their short term desire for offspring and their long term appreciation of raising healthy children. If structured, written information about risks and complications of multiple pregnancies and the consequences of the transfer of less embryos is provided, patients will probably become more inclined to the transfer of 2 embryos rather than 3. Introducing the dual embryo transfer as a standard policy, from which deviation is not allowed as a principle, patients may not easily put pressure on the physician to obtain consent for a 3 embryos transfer. However, if patients have to pay for the IVF cycle by themselves, choosing for dual embryo transfer when being well informed about the lower pregnancy rate will be a difficult choice. If a country has an adequate reimbursement system there is a main task for the politicians to create the legislation in such a manner that dual embryo transfer in women of 38 years and older is mandatory (171,48).

In summary, this study suggests that in women of 38 years and over a dual embryo transfer strategy after IVF may result in similar cumulative pregnancy rates compared

with a triple embryo transfer strategy, while reducing multiple pregnancy rates. This seems to be at the expense of an increase in the number of cycles needed to obtain these results, an expense that seems nicely balanced against the great advantages of multiple pregnancy prevention.