

Should we advise patients undergoing IVF to start a cycle leading to a day 3 or a day 5 transfer?

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BACKGROUND: The aim of this study was to compare ongoing pregnancy rates per started cycle between patients randomized at consultation to have embryo transfer either on day 3 or on day 5 of in-vitro culture. **METHODS:** All patients <43 years of age for whom IVF was indicated were allowed to participate in the study (day 3 group, 234 patients; day 5 group, 226 patients). Ovarian stimulation was performed either using GnRH antagonists/recombinant FSH (rFSH) (day 3, 70.1% of patients; day 5, 72.6% of patients) or using the long GnRH agonist protocol/urinary gonadotropins (day 3, 29.9% of patients; day 5 27.4% of patients). **RESULTS:** The random decision to initiate a cycle leading to day 5 as compared with a day 3 transfer was associated with a significantly lower chance of embryo cryopreservation (day 3, 61.5%; day 5, 50.4%; $P < 0.02$). Ongoing pregnancy rate per started cycle did not differ between the two groups compared [day 3, 32.1%, 95% confidence interval (CI) 26.4–38.2%; day 5, 33.2%, 95% CI 27.3–39.5%]. **CONCLUSIONS:** Advising patients at consultation to initiate an IVF cycle leading to a day 5 as compared with a day 3 transfer does not appear to increase the probability of ongoing pregnancy, and is associated with a significantly lower probability of obtaining cryopreserved embryos.

Key words: blastocyst transfer/day 3 transfer/GnRH antagonists/ongoing pregnancy rate/recombinant FSH

Introduction

A shift from day 3 to day 5 embryo transfer has occurred in recent years as several retrospective studies suggested that replacement of blastocysts enhances the reproductive outcome of IVF (for review see Kolibianakis and Devroey, 2002).

Randomized controlled trials (RCTs) comparing the two methods, however, showed that the probability of pregnancy is not dependent on the type of embryo transferred (Blake *et al.*, 2004). Moreover, similar implantation rates were achieved by replacing equal numbers of embryos either on day 3 or on day 5 of in-vitro culture (Kolibianakis and Devroey, 2002).

Randomized comparisons between day 3 and day 5 transfer using sequential media have so far been performed by applying randomization after initiation of stimulation (Gardner *et al.*, 1998), at oocyte retrieval (Utsunomiya *et al.*, 2002) or at fertilization (Coskun *et al.*, 2000; Karaki *et al.*, 2002; Levron *et al.*, 2002; Rienzi *et al.*, 2002). The patients included in the above studies satisfied specific criteria regarding the number of follicles present on a certain day of stimulation, the achievement of oocyte retrieval or the presence of a predefined number of 2PN oocytes. Counseling patients on the mode of embryo transfer based on the results of these studies is, of course, conditional. The couple will only know

the type of embryo transfer after a certain stimulation day is reached, after oocyte retrieval or after insemination of the oocytes retrieved.

To date, no information about the probability of ongoing pregnancy in patients randomized to day 3 or day 5 transfer is available at the moment of consultation, before the outcome of stimulation or fertilization is known.

The aim of this study was to compare ongoing pregnancy rates per started cycle between patients randomized, prior to initiation of stimulation, to have embryo transfer either on day 3 or on day 5 of in-vitro culture.

Materials and methods

Patient population

Four hundred and sixty patients treated by IVF at the Centre for Reproductive Medicine of the Dutch-Speaking Brussels Free University from January 2001 until December 2003 were included in the study. Patients could enter the study only once. Randomisation was performed by the attending physician according to a computer-generated list. The sequence of randomization was not concealed. Inclusion criteria were age <43 years and presence of indication for IVF. Exclusion criteria were preimplantation genetic screening (for aneuploidy or for a specific disease) and azoospermia. The current study was approved by our Institutional Review Board.

Ovarian stimulation

Two ovarian stimulation protocols were used in the present study. Initially, the long GnRH agonist protocol using Buserelin (Suprefact; Hoechst, Frankfurt, Germany) combined with HMGs (Menopur; Ferring Pharmaceuticals A/S) was applied (Van de Velde *et al.*, 1998). A combination of GnRH antagonist and recombinant gonadotropins was introduced in turn, and gradually replaced the long agonist protocol. GnRH antagonist 0.25 mg/day (Orgalutran; NV Organon, Oss, The Netherlands) was used to inhibit premature LH surge and was always started on the morning of day 6 of stimulation while recombinant FSH (rFSH) (Puregon; NV Organon) was started on day 2 of the cycle (Kolibianakis *et al.*, 2004).

The starting dose of gonadotrophins was determined according to the patient's age and/or previous response to ovarian stimulation (range 75–450 IU). The dose of gonadotrophins remained constant during the first 5 days of stimulation, and could be adjusted if necessary.

Final oocyte maturation was achieved by 10 000 IU of HCG (Pregnyl; NV Organon) when at least three follicles of ≥ 17 mm were present on ultrasound.

Oocyte retrieval and IVF procedure

Oocyte retrieval was carried out 36 h after HCG administration by transvaginal ultrasound-guided aspiration of follicles. Conventional IVF was performed in 120 couples, ICSI in 312 couples and both conventional IVF and ICSI in 28 couples. The ICSI and IVF procedures have been described in detail previously (Devroey *et al.*, 1995; Devroey and Van Steirteghem, 2004). Embryos were cultured in sequential media (Vitrolife, Gothenburg, Sweden). As a matter of principle, one to two embryos were transferred on day 3 or day 5 after oocyte retrieval. Embryos were classified as top quality (score 3), medium quality (score 2) or low quality (score 1), as described previously (De Vos *et al.*, 1999; Gardner and Schoolcraft, 1999). Supernumerary embryos were frozen at the blastocyst stage in both groups, as has been described previously (Zikopoulos *et al.*, 2004a).

Luteal supplementation

The luteal phase was supplemented with daily vaginal administration of 600 mg natural micronized progesterone in three separate doses (Utrogestan; Besins, Brussels, Belgium), starting 1 day after oocyte retrieval and continued until 7 weeks of gestation if pregnancy was achieved.

Hormonal measurements

Hormonal assessment was performed at initiation of stimulation, on day six of stimulation and additional blood samples were taken as required between day 6 of stimulation and HCG administration. Serum LH, FSH, HCG, estradiol (E_2) and progesterone levels were measured by means of the automated Elecsys immunoanalyser (Roche Diagnostics, Mannheim, Germany). Intra-assay and inter-assay coefficients of variation were $<3\%$ and $<6\%$ for FSH, $<5\%$ and $<10\%$ for E_2 and $<3\%$ and $<5\%$ for progesterone, respectively.

Outcome measures

Primary outcome measure was detection of ongoing pregnancy defined as pregnancy progressing beyond the 12th week of gestation. Secondary outcome measures were achievement of embryo transfer and achievement of cryopreservation of at least one embryo.

Statistical analysis

Power analysis

A power analysis showed that in order to detect a difference of 5% in ongoing pregnancy rates between the two groups compared assuming a baseline ongoing pregnancy of 30% at an α level of 0.05 and β of 0.2, 1416 patients should be included in each group. This is hardly a realistic task for one centre, and would be very difficult to achieve even in the context of a multicentre trial. Therefore, the aim of the current study was to provide an estimate of the difference in ongoing pregnancy rate per started cycle between day 3 and day 5 on a relatively large patient population. To the best of our knowledge, this is the largest randomized comparison between day 3 and day 5 transfer using sequential media.

Statistical tests

Fisher's exact test was used to analyse nominal variables in the form of frequency tables. Normally distributed (Kolmogorov–Smirnov test with Lilliefors correction) metric variables were tested with the *t*-test for independent samples, while non-normally distributed metric variables were analysed using the Mann–Whitney *U*-test. All tests were two-tailed with a confidence level of 95% ($P < 0.05$). Values are expressed as mean \pm standard error of the mean, unless otherwise stated.

Results

Similar indications for treatment were present between patients who started a cycle leading to a day 3 or a day 5 transfer. Andrological infertility was present in 65.8% and 64.6% of couples, idiopathic infertility in 15.4% and 17.3%, tubal factor infertility in 9.4% and 10.2%, endometriosis in 5.6% and 4.4% and anovulation in 3.8% and 3.5% of couples, respectively. Additional patient characteristics and stimulation data in the day 3 and day 5 groups are shown in Table I. Randomization resulted in two comparable patient populations both before and after initiation of stimulation.

A significantly lower proportion of patients achieved an embryo transfer in the day 5 as compared with the day 3 group (Table II). In seven (3.0%) and 11 (4.9%) patients in the day 3 and day 5 group, respectively, no oocyte retrieval was performed because of poor ovarian response. In four (1.8%) and five (2.3%) patients with oocytes retrieved, in the day 3 and day 5 group, respectively, no fertilization occurred. In patients with fertilized oocytes, poor embryo quality/arrest in culture was responsible for no transfer in four patients (1.8%) in the day 3 group and 19 patients (9.1%) in the day 5 group ($P < 0.001$). Blastulation rate in the day 5 group calculated as the number of blastocysts developed divided by the number of 2PN oocytes available was $50.7 \pm 2.4\%$. Where embryo transfer was performed, similar numbers of embryos were replaced in both groups (Table I).

Similar ongoing pregnancy rates per started cycle were obtained in patients randomized to initiate a cycle leading to a day 3 or a day 5 transfer (Table II). Moreover, similar implantation rates in patients who had an embryo transfer and similar multiple pregnancy rates were present in the day 3 and day 5 group (Table II).

A significantly lower chance of not having embryos available for cryopreservation was present in the day 5 compared

Table I. Patient characteristics and stimulation data in the day 3 and in the day 5 group

	Day 3 (<i>n</i> = 234)	Day 5 (<i>n</i> = 226)	<i>P</i>
Female age (years)	31.3 (0.3)	31.5 (0.2)	NS
Previous IVF/ICSI trials	0.7 (0.1)	0.8 (0.1)	NS
FSH at initiation of stimulation (IU/l)	6.9 (0.3)	6.7 (0.2)	NS
Starting dose of FSH (IU)	184.7 (3.7)	184.4 (2.8)	NS
Type of stimulation (% of patients)			NS
GnRH agonists/urinary gonadotrophins	29.9	27.4	
GnRH antagonists/rFSH	70.1	72.6	
Duration of FSH stimulation (days)	10.4 (0.2)	10.4 (0.2)	NS
Total units of FSH (IU)	2011.1 (53.5)	1999.9 (50.4)	NS
Number of follicles \geq 17 mm on the day of HCG	5.1 (0.2)	4.9 (0.2)	NS
E ₂ on the day of HCG administration (pg/ml)	2034.5 (83.2)	1999.5 (88.8)	NS
Progesterone on the day of HCG administration (ng/ml)	1.2 (0.5)	1.2 (0.5)	NS
Number of cumulus–oocyte complexes ^a	12.1 (0.5)	12.0 (0.5)	NS
2PN oocytes	7.0 (0.3)	7.4 (0.4)	NS
Fertilization rate (%)	61.3 (1.7)	63.4 (1.7)	NS
Number of embryos transferred ^b	1.9 (0.1)	1.8(0.1)	NS
Number of embryos cryopreserved ^c	4.2 (0.3)	3.6 (0.3)	NS
Proportion of patients with cryopreserved embryos (%) ^d	61.5 (144/234)	50.4 (114/226)	0.02
Quality score of transferred embryos	2.7 (0.15)	2.5 (0.1)	NS

Data are given as mean (standard error).

^aFor patients in whom oocyte retrieval was performed.

^bFor patients in whom embryo transfer was performed.

^cFor patients in whom cryopreservation was feasible.

^dConsidering all patients who started a cycle.

NS = not significant.

with the day 3 group, and a significantly lower number of embryos was cryopreserved in cases where cryopreservation was feasible (Table I).

Discussion

This study has shown that advising patients at consultation to initiate an IVF cycle leading to a day 5 transfer does not appear to increase the probability of ongoing pregnancy compared with day 3 transfer. Moreover, it is associated with a significantly higher chance of not performing embryo transfer and a significantly lower probability of cryopreservation.

To the best of our knowledge, this is the first study to estimate the probability of ongoing pregnancy in patients randomized to perform a day 3 or day 5 transfer at consultation, and the largest randomized trial comparing day 3 and day 5 transfer using sequential media.

Although two different protocols were used for ovarian stimulation in the current study, randomization resulted in a similar proportion of patients in the day 3 and day 5 group assigned in the two stimulation schemes.

The results of the current study are in support of the existing evidence from RCTs that show no difference in the pregnancy rate between day 3 and day 5 groups despite variations regarding the time of randomization (Kolibanakis and Devroey, 2002; Blake *et al.*, 2004). In addition, the current study confirms that an increase in embryo transfer cancellation rate and a decreased chance for cryopreservation is to be expected when initiating a day 5 compared with a day 3 transfer (Blake *et al.*, 2004).

The results of the present study suggest that infertile patients should not be advised to initiate a cycle leading to a day 5 transfer unless it is considered necessary (e.g. if prim-

Table II. Pregnancy outcome in the day 3 and in the day 5 group

	Day 3	Day 5
Patients who initiated an IVF cycle (<i>n</i>)	234	226
Patients who reached oocyte retrieval [<i>n</i> (%)]	227 (97.0)	215 (95.1)
Patients with embryo transfer ^a [<i>n</i> (%)]	218 (93.2) ^a	190 (84.1) ^b
Positive HCG [<i>n</i> (%)]	96 (41.0)	94 (41.6)
Biochemical pregnancies [<i>n</i> (%)]	11 (11.5)	12 (12.8)
First trimester miscarriage [<i>n</i> (%)]	10 (10.4)	6 (6.4)
Extrauterine pregnancy [<i>n</i> (%)]	0 (0)	1 (1.1)
Ongoing pregnancies (<i>n</i>)	75	75
Ongoing pregnancy rate per started cycle [% (95% CI)]	32.1 (26.4–38.2)	33.2 (27.3–39.5)
Ongoing pregnancy rate per oocyte retrieval [% (95% CI)]	33.1 (27.2–39.4)	33.2 (27.3–39.5)
Ongoing pregnancy rate per embryo transfer [% (95% CI)]	34.4 (28.4–40.9)	39.5 (32.8–46.5)
Ongoing implantation rate (% \pm SEM)	24.5 \pm 2.5	26.6 \pm 2.7
Multiple pregnancy rate [<i>n</i> (%)]	20 (26.7)	15 (20.0)

^aConsidering all patients who started a cycle.

^b*P* < 0.001.

SEM = standard error of the mean.

plantation genetic diagnosis is required). Initiating a cycle leading to a day 5 transfer will result in a significantly higher proportion of patients without embryos to transfer on day 5 and significantly more patients with no embryos available for cryopreservation, compared with initiating a cycle leading to day 3 transfer. On the other hand, the probability of pregnancy appears to be similar between the two groups, regardless of the type of transfer performed. At the same time, blastocyst transfer is associated with an increased workload, cost and resources, which are considered to be its greatest disadvantage among embryologists (Hartshorne and Lilford, 2002).

It is interesting to note that regardless of the time of patient randomization, no difference in pregnancy rates is present in currently published RCTs comparing day 3 and day 5 transfer. In addition, similar implantation rates have been achieved where similar numbers of embryos were transferred between the two groups compared (Kolibianakis and Devroey, 2002).

This apparent similarity in implantation rates has occurred despite the fact that blastocysts have a greater implantation potential compared with a cleavage-stage embryo, as it is characterized by an activated embryonic genome (Braude *et al.*, 1988) and is by definition at a more advanced stage of development. It should not be forgotten, however, that the comparison between day 3 and day 5 transfer in RCTs concerns pregnancy achievement following the transfer of embryos developed in in-vitro culture for 3 or 5 days. The possibility cannot be excluded that although the sequential media currently used for blastocyst culture allow embryos to develop to the blastocyst stage, they might compromise their implantation potential compared with blastocysts developed *in vivo* (Alikani *et al.*, 2000; Racowsky *et al.*, 2000).

Alternatively, the abnormal endometrium at oocyte retrieval, which was shown to be present in 100% of cases in agonist (Ubaldi *et al.*, 1997) and antagonist cycles (Kolibianakis *et al.*, 2002), might obscure a superior implantation potential of blastocysts developed *in vitro*. The above hypothesis could be assessed by a randomized trial in which embryos from donors are transferred in recipients either in day 3 or on day 5 of in-vitro culture.

On the other hand, the presence of abnormal endometrium at oocyte retrieval probably does not justify the assumption that blastocyst transfer leads to a better synchronization with endometrium compared with day 3 transfer.

No cases of monozygotic twinning were observed in the current study. Although monozygotic twinning has also been reported to occur in day 3 transfers (Sills *et al.*, 2000; Schachter *et al.*, 2001), a higher incidence of monozygotic twinning in day 5 compared with day 3 transfer has been suggested by several authors (Racowsky *et al.*, 2000; da Costa *et al.*, 2001; Tarlatzis *et al.*, 2002). A case of quintuplet pregnancies following transfer of two blastocysts has recently been reported (Unger *et al.*, 2004; Zikopoulos *et al.*, 2004b). This issue may require further investigation.

In conclusion, advising patients at consultation to initiate an IVF cycle leading to a day 5 transfer does not appear to increase the probability of ongoing pregnancy compared with initiating an IVF cycle leading to day 3 transfer, and it is

associated with a significantly lower probability of achieving cryopreservation.

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