Article

Should the practice of double blastocyst transfer be abandoned? A retrospective analysis

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Abstract

As multiple pregnancy is now considered to be an adverse event of an IVF procedure, reducing the multiple pregnancy rates has become the goal of many IVF centres. A large number of double blastocyst transfers in non-selected patients were performed in the authors' institution over recent years, and a retrospective analysis was conducted to investigate if multiple pregnancy rates in such a population are still unacceptably high. In addition, the factors determining the birth of singletons or multiple births following the transfer of two blastocysts was analysed. The live-birth rate per cycle was 35.7%, and the multiple-birth rate was 44.2% of all births. When clinically important variables that performed significantly in univariate analysis were analysed in the multiple regression model, age was negatively correlated with singleton and multiple births, and the transfer of two optimal blastocysts was positively correlated. Based on these results, abandoning the transfer of two blastocysts at least in patients younger than 37 years is recommended, regardless of embryo quality, as even the transfer of two non-optimal blastocysts results in almost 20% multiple births.

Keywords: *blastocyst transfer, embryo transfer, IVF, multiple pregnancy*

Introduction

Multiple pregnancy, a common complication of an IVF/embryo transfer programme, is a great burden to any health system in the world, and presents a serious perinatal risk for both mother and child [European Society for Human Reproduction and Embryology (ESHRE) 2000, 2001; Cohen, 2007]. In the past decade, it has become apparent that transferring two instead of three or more embryos, while maintaining high pregnancy and delivery rates, did not lead to a reduction in the rate of multiple pregnancies, which was the original hope. When transferring two embryos on day 3, multiple pregnancy rates are estimated to be around 10–30% (Schieve *et al.*, 1999; Neubourg *et al.*, 2002; Van Montfoort *et al.*, 2004). Since the introduction of suitable blastocyst media, blastocysts are occasionally transferred to patients, but blastocyst transfer results in an even higher implantation rate (Gardner *et al.*, 1998). Therefore, when two blastocysts are transferred, often more than 50% of the resulting pregnancies are multiple (Gardner *et al.*, 2004; Criniti *et al.*, 2005; Henman *et al.*, 2005; Ryan *et al.*, 2007).

As it is now considered to be an adverse event of an IVF procedure (Martin and Welch, 1998; Senat *et al.*, 1998; European Society for Human Reproduction and Embryology, 2000; Land and Evers, 2003), reducing the multiple pregnancy rate has become the goal of many IVF centres. Single embryo transfer (SET) was introduced (Coetsier and Dhont, 1998; Gerris *et al.*, 1999; Vilska *et al.*, 1999), but although delivery rates per transfer are satisfying (Soderstrom-Anttila and Vilska, 2007), it is clear from the results generated from European registers by ESHRE (Andersen *et al.*, 2007) that most European IVF centres still implement the policy of double embryo transfer (DET). The reasons for this are clear: twin pregnancies are often not regarded as IVF failure by the couple, and DET produces acceptable pregnancy and live-birth rates for both patients and their physicians. Many clinicians are still cautious with the

implementation of SET, because although it has the advantage of reducing the multiple pregnancy rates, it has been shown to generate somewhat lower pregnancy rates compared with DET (De Sutter *et al.*, 2003; Criniti *et al.*, 2005; Van Montfoort *et al.*, 2006).

In the study institution, Maribor University Hospital, culturing embryos to the blastocyst stage is a standard procedure, and both single blastocyst transfer (SBT) and double blastocyst transfer (DBT) are performed. SBT is recommended to patients younger than 35 years for their first two IVF/embryo transfer attempts, and couples are counselled about the risks of DBT, but either one or two blastocysts can be transferred, according to the preference of the couple. Therefore, a large number of DBT in non-selected patients have been performed in the study institution in recent years, and a retrospective analysis was conducted to investigate if multiple pregnancy rates in such a population are still unacceptably high. The studies mentioned previously (Gardner *et al.*, 2004; Criniti *et al.*, 2005; Henman *et al.*, 2005; Ryan *et al.*, 2007), with more than 50% multiple pregnancies when transferring two blastocysts, were prospective, therefore controlling for various factors that may affect the multiple pregnancy rate. Theoretically, this could lead to a higher percentage of multiple pregnancies in a study population than would occur in a non-selected population, a hypothesis which this study aimed to investigate. In addition, the factors determining the birth of singletons or multiple births following the transfer of two blastocysts was analysed.

Materials and methods

This was a retrospective analysis of all patients receiving two blastocysts transferred in a fresh cycle between January 2001 and December 2005 at the Maribor University Hospital, Department of Reproductive Gynecology, in Maribor, Slovenia. Institutional review board approval was not obtained, as this was a retrospective analysis of the institution's database.

Patients were stimulated using a standard long gonadotrophin-releasing hormone (GnRH) agonist/FSH/human menopausal gonadotrophin (HMG) protocol, or a standard GnRH antagonist/FSH/HMG protocol. Ovulation was triggered with the administration of human chorionic gonadotrophin (HCG; Profasi 10,000 IU, Ovitrelle 250 mg, Serono, Italy) when at least two follicles were 17 mm in diameter. Oocytes were retrieved transvaginally under ultrasound guidance 36 h after HCG administration. The oocytes were inseminated either conventionally (IVF) or by intracytoplasmic sperm injection (ICSI) 3–4 h after retrieval.

On the second day of culture, embryo quality was assessed. If there were more than six embryos available on day 2, they were cultured for an additional 48–72 h. Patients gave informed consent allowing extended embryo culture and blastocyst transfer. Before transfer, the blastocyst quality was assessed according to the modified classification system by Kovacic *et al.* (2004), where an optimal blastocyst (B1) is a blastocyst in which the blastocoele is completely expanded. The inner cell mass is round or oval, the trophectoderm is seen as a cohesive epithelium, and there are no excluded blastomeres or cytoplasmic fragments, either in the perivitelline space or in the blastocyst. All other blastocysts (B2–B8) are classified as non-optimal. Embryo transfer was performed on day 5 or, occasionally, on day 6 using a Labotect (Göttingen, Germany) catheter.

It is the institution's policy to transfer no more than two blastocysts. Couples are counselled on an individual basis concerning whether to have one or two blastocysts transferred, but ultimately, it is they who decide whether to have a single or a double blastocyst transfer. Therefore, this is an analysis of patients deciding to have two blastocysts transferred, occasionally against advice.

All patients received luteal phase progestin support with didrogesterone (Dabroston, Belupo) or micronized progesterone (Utrogestan, Laboratoires Besins International) beginning on the day after retrieval. Serum pregnancy tests were performed 12 days after embryo transfer.

Specific outcome measures included transfer rates, implantation rates, pregnancy and ongoing pregnancy rates, live-birth rates, and singleton and twin rates. The transfer rate was calculated by dividing the number of embryo transfers performed by the number of cycles started. The implantation rate was determined by dividing the number of gestational sacs by the number of embryos transferred. The pregnancy rate was calculated by dividing the number of cycles with positive β-HCG by the number of cycles. Live-birth rate was calculated by dividing the number of births by the number of cycles started.

The parameters used in univariate analysis were chosen because they were proven to be statistically significant in previous studies (Bassil *et al.*, 1997; Strandell *et al.*, 2000). Although the number of previous IVF attempts was also proven significant, it could not be used in the analysis, because this information was not included in the database before 2003. Since age was shown to be the most important predictor of multiple birth in an IVF programme, the results are presented by age groups of ≤34, 35–38 and ≥39 years, as these groups are traditionally used in the IVF literature.

Statistical analysis

Statistical analysis involved univariate comparisons among three age groups (≤34, 35–38 and ≥39 years) using Pearson chi-squared test, two-sample case Mann–Whitney U test, and Yates corrected chi-squared test, where appropriate. A number of parameters were compared between pregnancies resulting in a singleton birth and pregnancies resulting in a multiple birth. These factors were included in the univariate logistic regression model with adjustment for method of insemination. Variables proven statistically significant by univariate analysis were then analysed by multivariate logistic regression. A P-value of <0.05 was considered significant.

Results

A total of 1751 cycles was analysed. In **Table 1**, the number of cycles and embryos transferred, pregnancy, implantation and birth rates, and the number of singleton, twin and triplet births relative to patient age is shown. Statistical significances among three age groups are shown. Not included in the table is the number of embryos transferred, as two blastocysts were always transferred per single embryo transfer, accounting for 2800

blastocysts transferred in total. Also not included in the table is the number of cycles without transfer, which was 189 (17.3%), 98 (23.0%), and 64 (27.5%) for the age groups ≤34, 35–38 and ≥39 years, respectively. In total, there were 676 children born in the ≤34, 173 in the 35–38, and 60 in the ≥39 years age groups.

In **Tables 2** and **3**, the parameters used in univariate and multivariate regression analysis are shown, stratified according to patient age. In **Table 2**, significant differences between singleton and multiple pregnancies within and between particular age groups are shown. In **Table 3**, statistical significance for quality of blastocysts transferred between singleton and multiple pregnancies is shown. The differences within the age groups were also analysed with regard to procedure (IVF or ICSI), but there were no significant differences among the age groups (IVF singleton versus IVF multiples and ICSI singleton versus ICSI multiples) for the parameters studied, except for total number of embryos on day 5 for the IVF patients in the ≥39 age group ($P < 0.05$). This can be explained by the small number of patients in this particular subgroup (3 and 5, respectively). In **Table 3**, a significantly higher number of transfers of two optimal blastocysts is shown for multiple pregnancies in IVF and ICSI patients ≤34 years ($P < 0.05$), and for IVF patients aged 35–38 years ($P < 0.05$).

In **Table 4**, significant risk factors from univariate analysis are shown. When all variables that performed significantly in univariate analysis were analysed in the multiple regression model, significance was not observed for any of the parameters (not shown). Therefore, it was decided to include only variables of clinical importance, shown in **Table 5**. In such a model, age showed the highest predictive capacity and was negatively correlated with singleton and multiple births. In addition, the transfer of two optimal blastocysts was positively correlated, in comparison with the transfer of one optimal and one non-optimal blastocyst, or two non-optimal blastocysts.

In **Figure 1**, the distribution of delivery rates and multiple delivery rates per transfer and for different ages is presented. Also, the percentage of multiple deliveries in all births is shown as bars. The results for ages 20, 21, 44 and 45 years are omitted from the chart, although included in the analysis, because these

Table 1. Outcome of IVF/intracytoplasmic sperm injection cycles according to age group.

Variable	*Total*	*≤34 years*	*35–38 years*	*≤39 years*	P-*value*
Cycles	1751	1092	426	233	–
Embryo transfers	1400 (80.0)	903 (82.7)	328 (77.0)	169 (72.5)[a]	0.00044
Positive β-HCG	778 (44.4)	534 (48.9)	179 (42.0)[b]	65 (27.9)[a,c]	<0.0001
Implanted embryos	1132 (40.4)	800 (44.3)	247 (37.7)[b]	85 (25.1)[a,c]	<0.0001
Births	625 (35.7)	453 (41.5)	128 (30.0)[b]	44 (18.9)[a,c]	<0.0001
Singletons	349 (55.8)	237 (52.3)	83 (64.8)[b]	29 (65.9)[a]	0.0158[d]
Twins	268 (42.9)	209 (46.1)	45 (35.2)[b]	14 (31.8)[a,c]	–
Triplets	8 (1.3)	7 (1.5)	0 (0)	1 (2.3)	–

Values are number (percentage). HCG = human chorionic gonadotrophin.
[a]≥39 years versus ≤34 years; [b]35–38 years versus ≤34 years; [c]≥39 years versus 35–38 years; [d]singleton versus multiple birth.

Table 2. Possible predictor variables and birth outcomes according to age group.

Variable	*≤34 years*		*35–38 years*		*≤39 years*	
	SB	*MB*	*SB*	*MB*	*SB*	*MB*
No. of patients	237	216	83	45	29	15
Age (years)	30.2 ± 2.9	29.8 ± 2.9	36.1 ± 1.1	35.9 ± 1.0	40.1 ± 1.2	39.7 ± 1.1
Ampoules	27.6 ± 8.5	27.6 ± 7.7	30.2 ± 7.5[a]	31.8 ± 8.4	31.5 ± 11.2[b]	32.9 ± 7.9
Oocytes retrieved	10.1 ± 4.2	10.1 ± 4.0	9.7 ± 4.1	10.3 ± 4.7	9.1 ± 4.5	10.7 ± 4.6
Oocytes fertilized	6.8 ± 3.1	7.0 ± 2.9	7.0 ± 2.8	6.9 ± 3.1	6.3 ± 2.9	7.7 ± 3.9
Day-2 embryos	6.7 ± 3.0	6.9 ± 2.9	6.8 ± 2.8	6.8 ± 3.1	6.3 ± 2.9	7.7 ± 3.9
Optimal embryos	4.5 ± 2.6	5.0 ± 2.9	4.2 ± 2.7	4.4 ± 2.4	4.0 ± 2.1	5.5 ± 3.4
All blastocysts	4.3 ± 2.1	4.9 ± 2.1[c]	4.5 ± 2.1	5.0 ± 2.2	4.4 ± 2.1	5.0 ± 3.2
Optimal blastocysts	1.3 ± 1.4	2.1 ± 1.8[c]	1.4 ± 1.3	2.0 ± 1.3[c]	1.5 ± 1.2	1.3 ± 1.2
Blastocysts cryopreserved	1.5 ± 1.9	2.3 ± 2.2[c]	1.6 ± 1.9	2.3 ± 2.0[c]	1.6 ± 1.9	2.1 ± 2.7

Values are mean ± SD, unless otherwise stated.
MB = multiple birth; SB = singleton birth.
[a]35–38 years versus ≤34 years; [b]≥39 years versus ≤34 years; [c]within age group.

Table 3. Relationship between quality of blastocysts transferred and birth outcomes according to age group.

Parameter	*≥34 years*		*35–38 years*		*≥39 years*		*Total*		P-*value*
	SB	*MB*	*SB*	*MB*	*SB*		*MB*	*SB*	*MB*
No. of patients	237	216	83	45	29	15	349	276	–
Transfer of two optimal blastocysts	80 (33.8)	124 (57.4)	32 (38.6)	27 (60.0)	14 (48.3)	6 (40.0)	126 (36.1)	157 (56.9)	<0.001
Transfer of one optimal or one non-optimal blastocyst	77 (32.5)	51 (23.6)	25 (30.1)	11 (24.4)	9 (31.0)	4 (26.7)	111 (31.8)	66 (23.9)	0.037
Transfer of two non-optimal blastocysts	80 (33.8)	41 (19.0)	26 (31.3)	7 (15.6)	6 (20.7)	5 (33.3)	112 (32.1)	53 (19.2)	<0.001

Values are numbers (percentage), unless otherwise stated.
MB = multiple birth; SB = singleton birth.

Table 4. Univariate logistic regression of possible predictor variables and birth outcome.

Variable	*SB*	*MB*	P-*value*
No. of patients (%)	349 (55.8)	276 (44.2)	–
Age (years)	32.4 ± 4.2	31.3 ± 4.0	0.0013
No. with tubal infertility (%)	135 (38.7)	99 (35.9)	NS
Ampoules	28.5 ± 8.6	28.6 ± 8.0	NS
Oocytes retrieved	9.9 ± 4.2	10.2 ± 4.2	NS
Oocytes fertilized	6.8 ± 3.0	7.0 ± 3.0	NS
Day 2 embryos	6.7 ± 2.9	7.0 ± 2.9	NS
Optimal embryos	4.4 ± 2.6	4.9 ± 2.8	0.0017
All blastocysts	4.3 ± 2.1	4.9 ± 2.2	0.0017
Optimal blastocysts	1.4 ± 1.4	2.0 ± 1.7	<0.0001
No. of transfer of two optimal blastocysts (%)	126 (36.1)	157 (56.9)	<0.0001
Blastocysts cryopreserved	1.6 ± 1.9	2.3 ± 2.2	<0.0001

Values are mean ± SD unless otherwise stated. NS = not statistically significant.
MB = multiple birth; SB = singleton birth.

Table 5. Coefficient and *P*-value for variables predictive of multiple birth after multivariate logistic regression.

Variable	*Coefficient*	*Standard error*	P-*value*	*Odds ratio (95% confidence interval)*
Age	–0.015	0.005	0.0016	0.99 (0.98–0.99)
No. of optimal blastocysts	0.023	0.021	NS	1.03 (0.98–1.07)
No. of blastocysts cryopreserved	0.018	0.012	NS	1.02 (0.99–1.04)
Transfer of two optimal blastocysts versus transfer of at least one non-optimal blastocyst	0.058	0.058	0.0474	1.12 (1.00–1.26)

NS = not statistically significant.

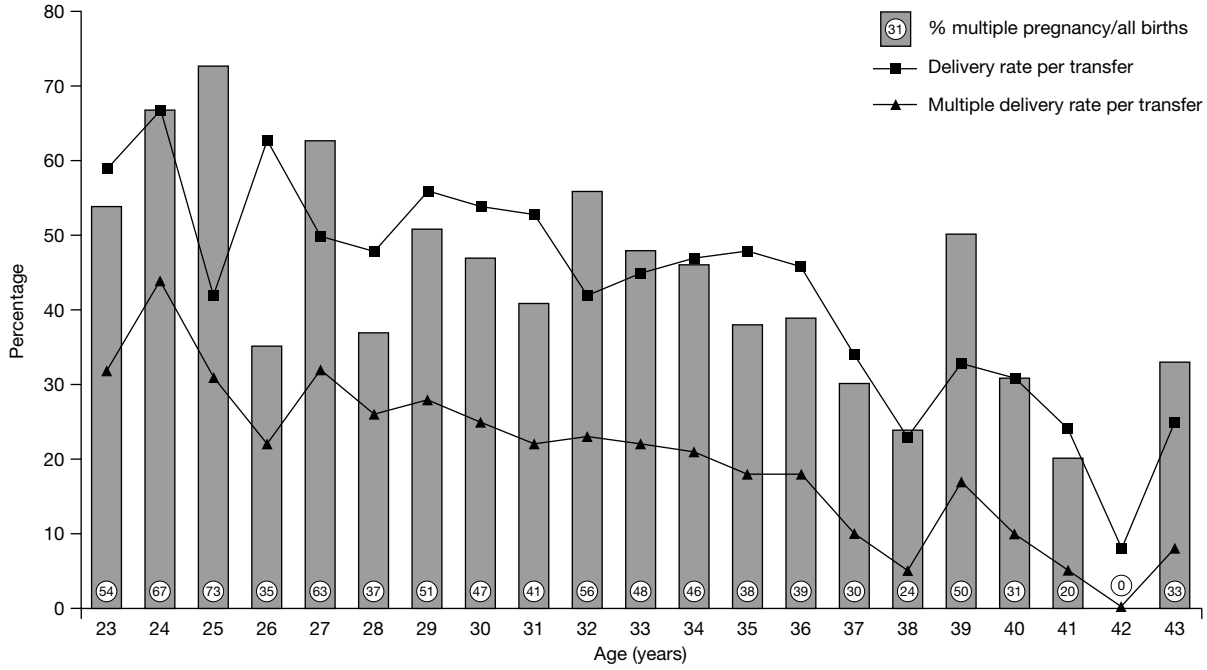

Figure 1. Delivery rates and multiple rates per transfer and according to age.

patients did not have a live birth. The result for age 22 years is also omitted from the chart, because there were no multiple pregnancies in this age group.

Discussion

Prospective studies usually report live-birth rates as live birth per transfer or patient randomized (Lundqvist *et al.*, 2002; Henman *et al.*, 2005), or they report an ongoing pregnancy rate (Coskun *et al.*, 2000; Langley *et al.*, 2001; Van Montfoort *et al.*, 2006), which makes the comparison of live-birth rates difficult. In a study by Strandell *et al.* (2000), which is comparable to the present study in design, and population and sample size (except day-3 embryos were transferred), the live-birth rate per cycle was 29% and the multiple-birth rate was 25.8% of all births, compared with this study's results of 35.7% and 44.2%, respectively. Because this study was a retrospective analysis of a large number of non-selected patients, it is presumed the multiple-birth rates in this study could be regarded as reference rates for DBT.

As anticipated, because of the high blastocyst implantation potential, birth rates per transfer in this report are high, in younger age groups averaging more than 50%, of which almost one-third to one-half are multiple pregnancies. When birth rates per cycle are analysed, transferring two blastocysts in an unselected population results overall in 35.7% births, of which 44.2% are multiple pregnancies. Even in the age group ≥39 years, the multiple pregnancy rate is still over 30%. It is evident from **Figure 1** that the proportion of multiple deliveries in relation to all births is almost constantly one-third to two-thirds, regardless of patient age. This suggests that when transferring two blastocysts, the multiple pregnancy rate depends on the pregnancy chance overall, which is determined by the woman's age. It is clear from previous reports (Henman *et al.*, 2005; Lukassen *et al.*, 2005), and from this one as well, that in women who are 36 years old and younger, where a delivery rate per transfer of more than 30% is expected both in SET and DET, transferring two blastocysts will result in an unacceptably high percentage of multiple pregnancies. This report shows that in women 37 years and older, where delivery rates per transfer begin to drop, transferring two blastocysts still results in a multiple pregnancy rate of more than 20%, which is far higher than the 10% target rate for lowering multiple pregnancy rates (European Society for Human Reproduction and Embryology, 2001). The paradox of declining fertility with advanced maternal age but with increased twinning rate has been described (Beemsterboer, 2006), and this natural phenomenon could have some influence on the high percentage of multiple pregnancies in older age groups.

Patient age, number of embryos available and embryo quality are the most important factors influencing the risk of multiple birth (Bassil *et al.*, 1997; Strandell *et al.*, 2000). Univariate logistic regression in this study has shown that a number of parameters are all significant predictors of multiple birth. In multivariate analysis, where only clinically important parameters were included, age and transfer of two optimal blastocysts were significant, confirming the above mentioned findings. However, these significances are weak, as shown by the loss of significance when all variables that showed significance in univariate analysis were analysed in a model. Veleva *et al.* (2006), who investigated if elective SET is an

acceptable policy for women aged 36–39 years, came to the conclusion that embryo quality rather than chronological age determines the chance of pregnancy. This study's age grouping was slightly different, 35–38 years, and indeed, transferring two optimal blastocysts in this group resulted in 60% multiple births, which is even higher than the 57.4% in the ≤34 group. In women older than 39 years, on the other hand, transferring two optimal blastocysts led to 40% multiple births. Nevertheless, when looking at the age–birth chart, it is evident that neither group was adequately chosen, as a sharp decline in delivery rate per transfer can be observed in women older than 36 years. As suggested by this analysis, many factors determine the chance of a live birth and a multiple birth.

The results presented here also support part of the study of Van Royen *et al.* (1999), who found that in DET the transfer of two top-quality embryos compared with one or none results in a twin rate of 57% compared with 21% and 0%, respectively. In the present study, transferring two optimal blastocysts led to 56.9% multiple births, which decreased to 23.9% and 19.2% when one or no optimal blastocyst was transferred. The persistently high percentage of multiple births when two non-optimal blastocysts were transferred can be explained by the higher implantation potential of blastocysts compared with day 2–3 embryo transfer (Coskun *et al.*, 2000), but this also suggests that transferring two non-optimal blastocysts, even in an unselected population, still results in an unacceptably high multiple pregnancy rate.

Both DBT and SBT have higher pregnancy rates compared with DET and SET (Langley *et al.*, 2001; Henman *et al.*, 2005), but are not widely accepted because blastocyst culture presents a challenge to the embryologist who needs additional education and experience, and because of the higher chance of cycle cancellation which is a great disappointment for any couple. Only approximately 50% of fertilized oocytes reach the blastocyst stage (Gardner *et al.*, 1998; Langley *et al.*, 2001), which leads to cycle cancellation when there is no blastocyst to transfer on day 5. There was cycle cancellation in 20.1% of patients selected for extended embryo culture, which is comparable to some previous reports (Coskun *et al.*, 2000; Lundqvist *et al.*, 2002) but does not confirm the conclusion of Marek *et al.* (1999) who had only a 6.7% cycle cancellation rate when extended embryo culture was applied to non-selected patients. However, this can be partially explained by the higher number of oocytes retrieved in their series, at least in a younger group (~14 in patients <35 years compared with ~10 in patients ≤34 years in ours), because embryo development to the blastocyst stage is increased when a larger number of oocytes are collected (Schoolcraft *et al.*, 1999).

It interesting to note that, in the study population, there were similar numbers of oocytes retrieved and fertilized, embryos on day 2, blastocysts cryopreserved and optimal blastocysts, and similar total numbers of embryos on day 5 across different age groups. This is in contrast to the study by Langley *et al.* (2001), and probably the result of different stimulation protocols used in different age groups. The study centre tend to stimulate patients in older age groups with GnRH antagonists, as this stimulation has been shown to result in a lower number of gonadotrophin ampoules used and more oocytes retrieved (Vlaisavljevic *et al.*, 2003), and fertilization rates, blastulation rates and blastocyst transfer rates are comparable between GnRH agonist and antagonist groups.

Based on the results presented here, and in order to lower the multiple pregnancy rates, abandoning the transfer of two blastocysts at least in patients younger than 37 years is recommended regardless of embryo quality, as even the transfer of two non-optimal blastocysts results in almost 20% multiple births. In the authors' opinion, the right course of action would be to implement compulsory SBT in this age group, as it has been shown that, in women younger than 38 years, cumulative live-birth rates are comparable between patients with elective SBT followed by the transfer of cryostored blastocysts (65.3%) and patients with fresh DBT (64.2%) (Henman *et al.*, 2005). It appears that switching to SBT did not compromise the chance of a live birth in this age group. It is likely that SBT in an unselected population ≥37 years, followed by the transfer of cryostored blastocysts, would not seriously compromise the chance of live birth either. Patients older than 37 years, when blastocysts are available, should be counselled against the transfer of two blastocysts as their chance for multiple pregnancy is also very high. For several more years, because of patient demand and technology limitations, it is not reasonable to assume that this recommendation will be endorsed and SBT will be offered to non-selected patients. However, and as demonstrated by this report, there is already the foundation to expect such a turn of events. A retrospective analysis of SBT is currently being conducted in the study institution, the results of which will be published in a separate manuscript.

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