Reproductive BioMedicine Online (2010) **20**, 836–842

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ARTICLE

Cumulative live-birth delivery after IVF/ICSI since the progressive introduction of single-embryo transfer

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Abstract The only way to decrease the incidence of multiple pregnancies in the IVF/intracytoplasmic sperm injection (ICSI) population is to introduce single-embryo transfer (SET). This study investigated the impact of the progressive introduction of SET for the whole IVF/ICSI population from the patients' point of view by calculating the cumulative live-birth delivery rate. During a 5-year period (2001–2005), the outcome of 2164 cycles with oocyte aspiration in 1047 patients was analysed. A subanalysis was made to calculate the additional effect of frozen–thawed cycles. Survival analysis was performed with the Kaplan–Meier method and the endpoint was live-birth delivery. In this 5-year period, the cumulative live-birth delivery rate per patient was 51% after three IVF/ICSI cycles and 58% after six cycles. With a more permissive method of survival analysis, these results were 64% and 85%, respectively. The additional effect of the frozen–thawed cycles since reimbursement was only 5%. SET was progressively introduced in this period leading to a twin live-birth delivery rate of only 6.7%. It is concluded that a favourable outcome was observed for the cumulative live-birth delivery rate since the introduction of SET but with a disappointing additional effect of the frozen–thawed cycles.

KEYWORDS: cumulative live-birth delivery, IVF/ICSI outcome, single-embryo transfer, survival analysis

1472-6483/$ - see front matter
doi:10.1016/j.rbmo.2010.02.013

Introduction

Single-embryo transfer (SET) is the only option to prevent a high incidence of multiple pregnancies in IVF/intracytoplasmic sperm injection (ICSI). However, in randomized controlled trials (Bergh, 2005; Gerris, 2005), it was shown that this transfer policy can decrease the ongoing pregnancy and/or delivery rate particularly when more embryos with high implantation potential are available. Observational studies have shown that pregnancy/delivery rates are acceptable and unaffected when compared with the whole IVF/ICSI population. Most randomized studies have shown proof of concept and have validated embryo selection criteria while cohort studies have appreciated the impact of a progressive introduction of SET where the maintenance of the current pregnancy rate for the whole programme was put forward as an acceptable endpoint.

Daya (2005) discussed that success in IVF/ICSI by pregnancy/delivery rates per cycle is easy to calculate but does not take into account the number of cycles for each patient nor the time frame of the treatment. It is widely used and may be a good instrument to illustrate the performance of an IVF centre. However, it is of little value for counselling the patient entering an IVF programme. These patients are anxious to know whether this tedious and expensive treatment is offering any prospect of success, the birth of a healthy child.

In order to counsel patients correctly on their chance of a live-birth delivery when starting an IVF/ICSI treatment, this study investigated the cumulative live-birth delivery rate for all patients starting IVF/ICSI treatment from 2001 to 2005 by means of survival analysis. During this 5-year period, SET was progressively introduced. Since the prospective randomized study conducted in 1998 (Gerris et al., 1999), patients were encouraged to undergo elective single-embryo transfer particularly in first and second cycles. This embryo transfer policy was part of a study in 2001, investigating the impact of the patients' choice for SET in first IVF/ICSI cycles (De Neubourg et al., 2002). From 1 July 2003, SET and elective SET has been mandatory by Belgian law in first and second cycles, respectively, for young patients (<36 years). In the study centre, SET was also advised in consecutive cycles and extended to patients up to 38 years of age (De Neubourg et al., 2006).

From 1 July 2003, the Belgian government linked reimbursement of the laboratory costs of six IVF/ICSI cycles to a restriction of the number of embryos transferred, related to age of the patient and rank of the cycle (De Neubourg et al., 2006; Ombelet et al., 2005). From the moment the number of embryos for transfer was legally restricted, the multiple pregnancy rate decreased from 24.4% to 13.4% for all IVF/ICSI patients in Belgium (Belrap, 2002, 2006).

When fewer embryos are transferred, the number of embryos available for cryopreservation increases (De Neubourg et al., 2002).The additional effect of cryopreservation has been described in first and second cycles (Gerris et al., 2003; Lundin and Bergh, 2007; Thurin et al., 2004). In the study centre, patients had always been encouraged to undergo frozen–thawed cycles when possible. It became a routine procedure since reimbursement of the laboratory costs. Therefore, the additional effect of frozen–thawed cycles was calculated from 1 July 2003.

The aim of the study was to counsel new assisted reproduction patients about their realistic prospects of achieving a live birth.

Materials and methods

Patient selection

In the study period from 1 January 2001 until 31 December 2005, 1047 patients had their first IVF/ICSI treatment in the study centre. They were all included in the analysis. These patients underwent 2164 oocyte retrieval cycles. A subanalysis was performed for the period starting 1 July 2003 when the laboratory costs for IVF/ICSI were reimbursed and frozen–thawed cycles resulting from these egg retrievals were consecutively and consequently performed. All cycles leading to oocyte aspiration were included in the analysis.

IVF/ICSI procedure

The IVF/ICSI treatment was performed as described by De Neubourg et al. (2002). Briefly, all patients were treated with GnRH agonist down-regulation in the long agonist protocol and recombinant FSH or HMG was administered for ovarian stimulation. All fresh transfers were performed at day 3 after oocyte aspiration. Elective SET and SET were progressively applied as described above.

Cryopreservation protocol

Day-3 embryos were suitable for cryopreservation if they had at least six cells, ≤20% fragmentation and if no multinucleation was visible.

Freezing procedure

Embryos were frozen in vials following a slow-freezing protocol (Lassalle et al., 1985) using a mixture of propane-1,2-diol (PrOH)/dimethylsulphoxide (DMSO) (both from Sigma–Aldrich, USA) as a cryoprotectant (Russell, 1991). The freezing solution consisted of Hepes-buffered Earle's Saline (BioWhittaker, USA) supplemented with 4% (v/v) human serum albumin (HSA; Red Cross, Belgium). During three incubation steps of 5 min at room temperature, the embryos were exposed to: HSA, 0.37 mol/l PrOH/0.37 mol/l DMSO in HSA and 0.75 mol/l PrOH/0.75 mol/l DMSO in HSA. Subsequently each embryo was transferred individually to a cryovial (Simport, Canada) containing 0.75 mol/l PrOH/0.75 mol/l DMSO in HSA with 0.1 mol/l sucrose (Sigma–Aldrich). Freezing was carried out in a controlled-rate freezer (Kryo10 series II, Planer, UK) starting at 0°C. The initial cooling rate was −1°C/min until a temperature of −6.5°C was reached and manual seeding was performed. After a hold of 5 min cooling was continued at a rate of −0.3°C/min to −40°C after which the temperature was lowered at a rate of −3°C/min to −80°C. Finally the vials were plunged and stored in liquid nitrogen.

Thawing procedure

Embryos were thawed individually by taking one vial out of the liquid nitrogen and immersing this into a water bath containing ice water. When the freezing solution had melted completely, the embryo was aspirated out of the vial and passed through a series of decreasing concentrations of cryoprotectant in thawing solutions which consisted of 4% (v/v) HSA with 0.2 mol/l sucrose: 0.5 mol/l PrOH/0.5 mol/l DMSO, 0.37 mol/l PrOH/0.37 mol/l DMSO and 0.18 mol/l PrOH/0.18 mol/l DMSO where each step persisted for 5 min. Subsequently the embryo was incubated in thawing solution without cryoprotectant for another 5 min. During the dilution of cryoprotectant the embryo was kept cool by placing the incubation dishes on a refrigerant brick. Rehydration was completed by a 5-min incubation in sucrose-free HSA at 37°C. When the embryo was considered to have survived the freeze–thaw process (at least 50% of the blastomeres intact and no obvious damage to the zona pellucida) it was transferred to M3 medium (MediCult, Denmark) and cultured overnight before subsequent transfer to the patient.

Embryo transfer in a frozen–thawed cycle was performed in a spontaneous cycle with human chorionic gonadotrophin for ovulation triggering when a mature follicle of $\geq$18 mm and endometrium $\geq$7 mm was detected on ultrasound. For anovulatory patients, the endometrium was prepared with oestradiol valerate 2×2 mg/day and micronized progesterone (600 mg/day). Embryo transfer (usually SET) was planned 4 days after human chorionic gonadotrophin administration or administration of progesterone.

During the whole study period, no changes in stimulation protocols or laboratory aspects were made.

Statistical analysis

Data analysis was performed using the Statistical Package for Social Sciences version 14.0 (SPSS Inc., USA). The significance level was set at a *P*-value <0.05.

All new assisted reproduction patients who started IVF/ICSI treatment and had egg retrieval in the study period were analysed and the end-point was live-birth delivery. Patients who experienced miscarriage or loss of pregnancy prior to 24 weeks and who continued the treatment remained included in the analysis. On the other hand, patients were excluded from further analysis after one live-birth delivery.

Survival analysis was performed with the Kaplan–Meier method, using the sequence of cycles as well as duration of treatment (in months) as unit of analysis. In survival analysis, comparison between groups was made using the log-rank test (Mantel–Cox) with pairwise comparison. For time scale analysis, the starting point was arbitrarily set at 1 month before the date of the first IVF/ICSI cycle leading to egg retrieval.

Survival analysis was performed using a restrictive and a permissive method. In the restrictive method, patients were only censored if they achieved a continuing pregnancy. Patients who discontinued IVF treatment were not censored and remained included in the population 'exposed to risk' for six cycles and with a negative result for each cycle. In contrast, in the permissive method, all patients who discontinued IVF treatment, were censored from the time that they were out of follow-up.

All cryocycles that originated from one oocyte retrieval were added to the cycle of origin. Live births after a frozen–thawed cycle were referred to the fresh cycle they originated from. The additional effect of cryopreservation cycles is calculated by comparison of the survival analysis with and without inclusion of the cryopreservation cycles.

Only pregnancies related to the IVF/ICSI treatment were included.

Results

Description of the patients' characteristics

Data on outcome of the IVF/ICSI cycle as well as delivery were available for 1043 of the 1047 (99.6%) patients. The mean age of the patients was 32.5 years (range 18.8–43.8).

Table 1 shows age distribution, indication for IVF/ICSI, distribution of IVF and ICSI treatment and fertilization rates. Patients had a total mean of 2.2 cycles (range 1–18) and were under treatment for a mean of 6.7 months (range 1–51) with 16% of patients treated for more than 1 year.

Of all patients, 54.9% had one live birth. The live-birth delivery rate of twins was 6.7%. There were eight stillbirths in this cohort of patients.

Table 1 Description of patients' characteristics.

Parameter	*Sample data*
Number of patients	1047
Age	
Mean age in years (range)	32.5 (18.8–43.8)
<30 years	317 (30.3)
30–35 years	493 (47.1)
>35 years	237 (22.6)
Oocyte retrievals	
One	467 (44.8)
Two	289 (27.7)
Three	133 (12.8)
Four	76 (7.3)
Five	42 (4.0)
Six	20 (1.9)
More than six	16 (1.5)
Live-birth delivery	575 (54.9)
Live-birth delivery of twins	70 (6.7)
Cause of infertility	
Male factor	546 (52.1)
Female factor	228 (21.8)
Mixed	135 (12.9)
Unexplained	138 (13.2)
Mean number of oocytes	10.6
Type of treatment	
IVF	503 (48.0)
ICSI	544 (52.0)
Fertilization rate (per oocyte)	
IVF	8393/13162 (63.8)
ICSI	9091/13893 (65.4)

Values are number (%) unless otherwise stated.
ICSI = intracytoplasmic sperm injection.

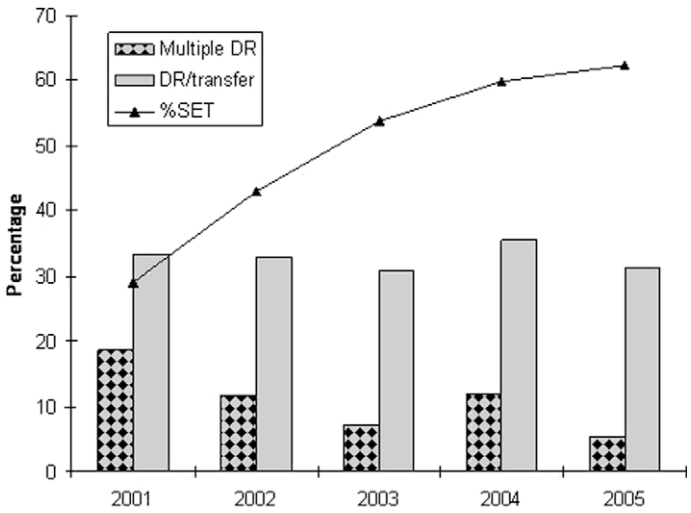

Figure 1 Evolution of delivery rate (DR), multiple delivery rate and percentage of single-embryo transfer (SET) from 2001 to 2005.

Description of the IVF/ICSI cycles

The overall delivery rate per cycle was 31.6% (683/2164) with a mean of 1.32 embryos transferred. The overall twin delivery rate was 11.3% (77/683) per cycle with an evolution of 18.6% in 2001 to 5.8% in 2005. There were no higher-order multiple pregnancies. Data from each individual year are depicted in **Figure 1** and show a decline in twin deliveries in accordance with the increase of SET. **Table 2** shows the proportion of SET per cycle. The percentage of SET declined from 67% to 16% from the first to the sixth cycle.

Cumulative live-birth delivery rate per cycle

Figure 2 shows that the cumulative live-birth delivery rates per patient after three and six IVF/ICSI cycles were 51% and 58%, respectively.

Cumulative live-birth delivery rate over time

Time of achieving success leading to delivery was also calculated. After 12 months of treatment, 47% of patients were pregnant and anticipated a live-birth delivery; this number was 54% after 24 months and increased to 59% after 3 years. By 'anticipated', it is meant that it is known that the outcome was a live birth but it could not be denominated as such because many of them had not yet delivered at that time.

Figure 3 illustrates that the cumulative live-birth delivery rate is age dependent. For patients <30 years of age, between 30–35 and >35 years of age, the results after 1 year were 56%, 47% and 34%, respectively.

Table 2 Proportion of single-embryo transfers per cycle rank.

	Cycle					
	1	*2*	*3*	*4*	*5*	*6*
Single-embryo transfer (%)	67	45	27	16	12	16

Cryopreservation cycles

In the study period starting 1 July 2003, the live-birth delivery rate was 30.5% (312/1024) per IVF/ICSI cycle and 19.5% (52/266) for frozen–thawed cycles when analysed on a per cycle basis for the whole programme. There was a significant difference in the live-birth rate after frozen–thawed embryo transfer between patients who previously conceived (30%) compared with patients with no previous pregnancy (13.6%; OR = 2.17; 95% CI 1.54–3.05) during this period.

Of all planned cryopreservation cycles, 73.9% had embryo transfer. The embryo survival rate (≥50% of blastomeres intact) was 50.1%.

Of the 601 patients who started IVF/ICSI treatment, 250 had embryos frozen (41.6%) with a mean of 2.8 embryos frozen per cycle. However, there was a significant difference between patients who had embryos frozen: 57% of the patients who became pregnant and delivered after a fresh

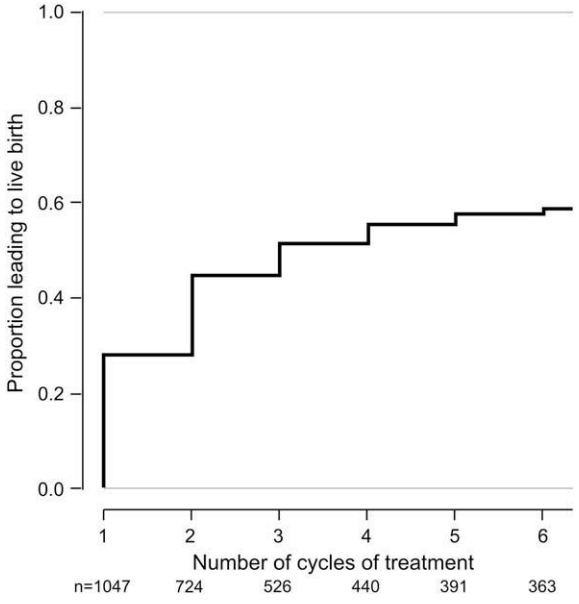

Figure 2 Cumulative live-birth delivery per treatment cycle.

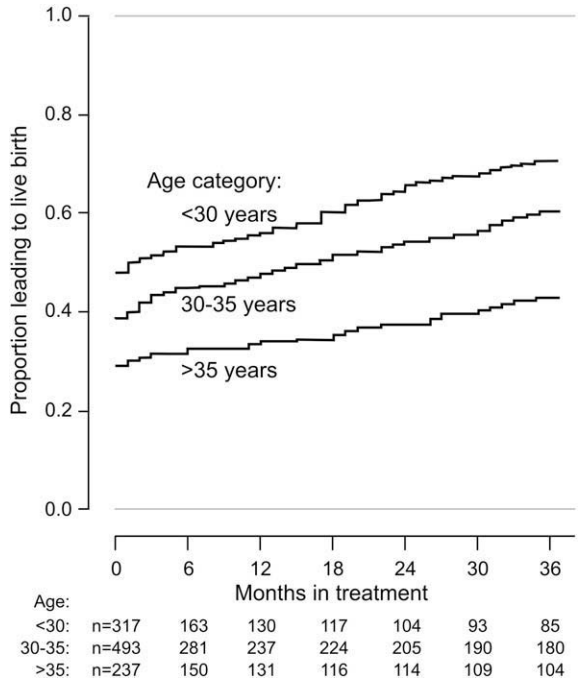

Figure 3 Cumulative live-birth delivery according to age and time in treatment.

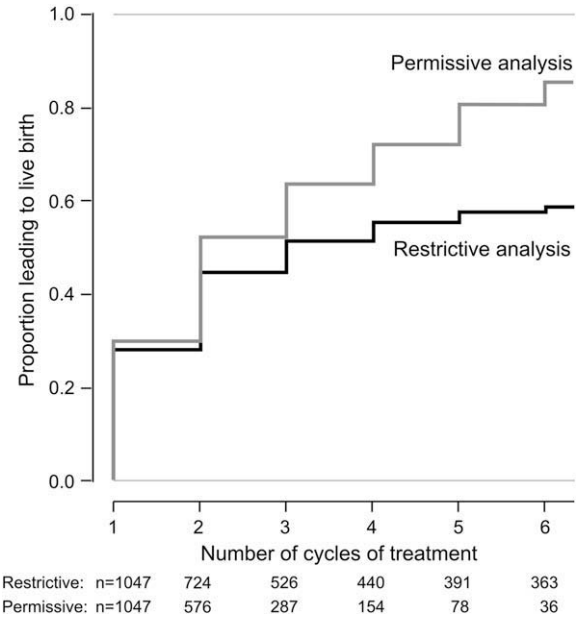

Figure 4 Cumulative live-birth delivery per treatment cycle without and with censoring of drop-outs.

IVF/ICSI cycle had embryos frozen whereas in patients with no delivery only 33.8% had embryos frozen (OR = 2.59; 95% CI 1.79—3.75).

When analysed per patient, the additional effect of performing cryopreservation cycles consecutively was only 5% on the total delivery rate after six cycles when analysed per patient starting IVF/ICSI treatment (**Table 3**) and this additional effect was already reached after the first cycle.

Restrictive versus permissive method of survival analysis

To investigate the influence of the method chosen to perform the survival analysis, a comparison was made between the 'restrictive' method and a 'permissive' method where all patients who discontinued IVF treatment, were censored. Using the 'permissive' method, the cumulative live-birth delivery rate increased from 58% to 85% after six cycles (**Figure 4**).

Discussion

There still is a lot of debate on the standard of success in assisted reproduction treatments. Pregnancy rates per cycle (started cycle, oocyte retrieval, embryo transfer) are easy to calculate and to use to compare different aspects of the IVF treatment, stimulation regimes, laboratory aspects, transfer strategies or performance of centres. This information, however, is not always sufficient to the patient entering an IVF/ICSI programme. Since 2001, when SET was progressively introduced, 51% of patients delivered after three cycles and 58% after six cycles. As far as is known, this is the first survival analysis of a complete IVF/ICSI programme with a substantial amount of SET and with a twin live-birth delivery of only 6.7%. Witsenburg et al. (2005) reported a cumulative live-birth rate of 59.1% per patient, reached after seven cycles, with a multiple-birth rate of 25.7%. In a Swedish study, the cumulative live-birth rate was 55.5% after three completed cycles with 23% twin live births (Olivius et al., 2002). In 1999, Engmann et al. described a cumulative live-birth rate of 48.2% after three cycles of treatment. Live-birth rates after five cycles were reported to be 45% for women 20—34 years and 28.9% at 35—39 years (Tan et al., 1992). Elizur et al. (2006) reported

Table 3 Cumulative live-birth delivery rate without and with frozen/thawed cycles.

	Cycle					
	1	*2*	*3*	*4*	*5*	*6*
No. of patients	601	378	267	230	217	208
Term live-birth deliveries (%)						
Frozen—thawed cycles excluded	28	43	48	49	50	51
Frozen—thawed cycles included	33	48	53	54	55	55

a cumulative delivery rate of 87% after up to 14 cycles. The paper by Malizia (2009) described cumulative live-birth rates in IVF in more than 6000 patients and showed cumulative live-birth rates of 51% and 72% with the conservative and optimistic analysis, respectively, after six cycles. The multiple-birth rate, however, was 29%.

After 1 year of IVF treatment, 47% of all patients had left the programme because of ongoing pregnancy leading to delivery. These results compare favourably to the study by Heijnen et al. (2007). They reported ongoing pregnancy leading to delivery of 43.4% after mild treatment with SET or 44.7% with standard treatment and double-embryo transfer in patients younger than 38 years with no previous IVF treatment or after the birth of a healthy child after IVF. The cumulative delivery rates in the current study compare favourably to results listed in the literature especially when the very restrictive type of analysis and the unselected patient population is taken into account.

It may be assumed that financial restraints are not the main reason for the majority of patients to discontinue treatment in Belgium. Patients are allowed reimbursement of laboratory costs up to six IVF/ICSI cycles. It has previously been shown that psychological distress is the most important reason for drop out (Olivius et al., 2004; Smeenk et al., 2004; Verberg et al., 2008). Apart from financial reasons and psychological distress, informative censoring has been suggested as an important reason for interruption of the treatment although this could not be confirmed by De Vries (1999) and Roest et al. (1998).

Based on the method of survival analysis used in the study of Heijnen et al. (2007), the cumulative proportion of live-birth deliveries were also calculated in a 'restrictive' way. However, it can be argued that patients who withdraw from further treatment have to be considered as 'failure' rather than 'lost to follow-up'. As shown in **Figure 4**, the method chosen to perform the calculation, influences the results substantially. The method presented in the study by Heijnen et al. (2007) is too restrictive while the classic handling where drop-outs are censored is too permissive. In the context of the current study, it is assumed that, in most cases, withdrawal from further treatment does mean failure of the treatment and does not just mean lost to follow-up for any reason.

Although high cumulative live-birth rates were described after SET and consecutive frozen–thawed cycles in first (Le Lannou et al., 2006) and first and second cycles (Lundin and Bergh, 2007), the present study could not detect an important contribution of frozen–thawed cycles on the cumulative live-birth rate after six cycles. Surprisingly, the additional effect after transfer of frozen–thawed embryos was only 5% per patient and was already obtained after the first cycle. Although the number of cryopreserved embryos per cycle is higher with SET when compared with double-embryo transfer (De Neubourg et al., 2002), the cumulative live-birth delivery rate is not substantially altered through the addition of frozen–thawed cycles. This may be related to the study population because it was found that patients who conceived had a higher chance of having embryos available for cryopreservation and had a higher pregnancy rate after subsequent frozen–thawed cycles and these results are not reflected in the survival analysis. These data suggest that the augmentation effect of cryopreservation cycles, although generally believed as being beneficiary for the whole IVF/ICSI population, may be limited to a subgroup of patients with a 'good prognosis'. These aspects should be further evaluated in future studies on cryopreservation and vitrification techniques.

A Belgian law on assisted reproduction (Wet betreffende de medisch begeleide voortplanting, 2007) obliges the performance of frozen–thawed cycles whenever frozen embryos are available before a new fresh cycle can be started. On the basis of the data in this study, one may wonder how valid this obligation is from a treatment point of view, particularly when few embryos are available for thawing and cycles may end with no transfer. From the patients' point of view, it is often regarded as a waste of time. Good clinical judgement is overruled by strict regulations particularly in the case of older women or patients experiencing repeatedly poor outcome after frozen–thawed cycles.

Bringing down the number of multiple pregnancies and deliveries needs care to maintain an acceptable cumulative live-birth rate and prospect of success for the majority of patients. This implies both the identification of the patient at risk for multiple pregnancy as well as the patient who is at risk for interrupting the treatment due to the distress of the treatment (De Neubourg and Gerris, 2006).

In conclusion, the progressive implementation of SET in combination with an excellent refunding of the majority of the costs makes IVF/ICSI a successful and attractive treatment to obtain a singleton delivery.

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Declaration: The authors report no financial or commercial conflicts of interest.

Received 14 July 2009; refereed 21 September 2009; accepted 22 January 2010.