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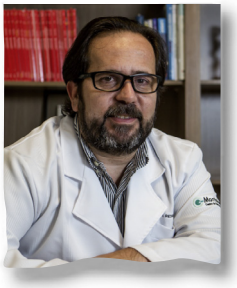
ARTICLE

Outcomes of elective cryopreserved single or double embryo transfers following failure to conceive after fresh single embryo transfer

Pedro Augusto Araújo Monteleone *, RJ Mirisola, SP Gonçalves, Edmund C Baracat, Paulo C Serafini

Centro de Reprodução Humana, Disciplina de Ginecologia, Departamento de Obstetrícia e Ginecologia, Hospital das Clinicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo 05403-000, Brazil
* Corresponding author. *E-mail address:* pedro@monteleone.med.br (PAA Monteleone).



Pedro Augusto Araújo Monteleone received his medical degree in 1994. He undertook a a fellowship in Obstetrics and Gynecology at Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (USP), Sao Paulo, Brazil, and received a specialization degree in 1997. He started working within the reproductive medicine field in 2001. He is currently Clinical Head of the Human Reproduction Division of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (USP), and Monteleone – Human Reproduction Centre. His main research interest is single embryo transfer.

Abstract The main adverse effect of IVF is the high multiple pregnancy rate resulting from the transfer of two or more embryos. The objective was to evaluate pregnancy rates in infertile women with a good prognosis who failed to conceive in a fresh elective single embryo transfer (eSET) and had a second cycle with elective double vitrified-warmed embryo transfer (eDFET) compared with elective single vitrified-warmed embryo transfer (eSFET). A total of 142 intracytoplasmic sperm injection cycles using a conventional protocol were evaluated. Good-prognosis patients underwent eSET in a fresh cycle, and those who failed to conceive underwent a second vitrified-warmed embryo transfer: eDFET ($n = 102$) or eSFET ($n = 40$). Embryos were transferred and vitrified on day 5 of development. Patients who received eDFET had fewer implantations (30.9%) than eSFET (52.5%; $P = 0.004$); pregnancy rates were similar (eDFET: 35.3%, eSFET: 42.5%). Patients with the eSFET had one monozygotic twin (5.9%), and 22.2% of eDFET patients had multiple pregnancies. Patients with a good prognosis who failed to conceive in the first fresh eSET did not have an advantage when receiving eDFET in the second cycle, as pregnancy rates were similar; 22.2% of patients in the eDFET group had multiple pregnancies.

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KEYWORDS: Infertility, IVF, Single embryo transfer, Double embryo transfer, Multiple pregnancies

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Introduction

The demand for assisted reproductive techniques has increased in the past 3 decades owing to a number of factors. These include more older women wishing to become pregnant, more sexually transmitted diseases, higher prevalence of obesity and medical issues such as endometriosis and polycystic ovary syndrome. Despite its success, IVF causes high rates of multiple pregnancies resulting from the transfer of two or more embryos after ovarian stimulation with the aim of increasing the chance of a pregnancy (Naasan et al., 2012; Kupka et al, 2014; Ishihara et al., 2015). Multiple pregnancies are the main adverse effect of IVF and are associated with a high risk of complications to both the mothers and fetuses, as well as potential long-term health issues for both. Multiple pregnancies raise the rate of premature births and intrauterine growth retardation, which contribute to the significantly higher rate of morbidity and mortality (Zollner and Dietl, 2013). Prematurity is six times more frequent; therefore, birth weight is significantly lower, which exposes twins to prematurity-related disorders (respiratory, cardiovascular, infectious) and long-term complications (especially neurological disabilities) (Giuffre et al., 2012).

It is therefore in the interests of public health to reduce multiple pregnancy rates in IVF cycles. The fewer number of embryos transferred is encouraged, as is the subsequent reduction in multiple pregnancies. The average number of embryos transferred, however, varies widely among countries. Reasons for this are multifactorial but consumer affordability will affect access to assisted reproduction techniques (Chambers et al., 2014). In general, the proportion of elective single embryo transfers (eSET) has increased (mean of 23.4% of cycles), and higher rates are seen in Sweden and Finland, which reported eSET rates in 2010 of 73.3% and 67.5%, respectively, with no reduction in pregnancy rates (around 30%) (Kupka et al, 2014). Other countries, such as the USA and Brazil presented lower eSET rates of 10% (Ishihara et al., 2015).

In a meta-analysis, eSET was shown to reduce the risk of multiple pregnancies and decrease live birth rates compared with elective double embryo transfer (eDET) (Baruffi et al., 2009; Pandian et al., 2009). Other studies have shown that when eSET is carried out, and the subsequent cryopreserved embryo transfers are taken into account, the cumulative pregnancy and live birth rates are similar to eDET (McLernon et al., 2010; Pandian et al., 2013). Therefore, in 2009, the Human Fertilization and Embryology Authority introduced a policy to encourage routine use of eSET, with a resulting reduction of multiple births from 24% in 2009 to 10% in 2012 (Harbottle et al., 2015). More recently, in IVF patients with good prognosis, specifically women aged younger than 37 years in their first or second IVF cycle and along with good-quality embryos, eSET is recommended by the Practice Committee of American Society for Reproductive Medicine (Practice Committee of American Society for Reproductive Medicine, Practice Committee of Society for Assisted Reproductive Technology, 2013).

In clinical practice, when an eSET results in failure, the decision to use eDET of cryopreserved embryos is common. On this basis, we hypothesized that an eDET is not beneficial in improving pregnancy rates in good-prognosis patients, even after an eSET failure. Hence, the aim of this study was to evaluate the pregnancy outcomes of IVF cycles of patients who failed to conceive in the fresh eSET and underwent a following elective double-vitrified-warmed embryo transfer (eDFET) or an elective single- vitrified-warmed embryo transfer (eSFET) and to compare the rates of pregnancies and multiple pregnancies.

Material and methods

In this retrospective observational study, IVF cycles were evaluated at the Human Reproduction Centre, Hospital das Clínicas, Faculdade de Medicina, Universidade de Sao Paulo, and a private assisted reproduction centre in Sao Paulo, Brazil (Monteleone, Centro de Reproduçao Humana) between 2007 and 2015. All of the procedures in this study are part of routine care in the assisted reproductive centre, and written informed consent was obtained from all patients before treatment. Patients consented to the treatment procedures and retrospective data use in scientific publications (Ethics Committee Proc. Number 1.151.345).

Study groups

Patients were designated for eSET according to the criteria of the study centre and were considered to have a good prognosis if they met the following criteria: patients aged between 18 and 38 years undergoing first or second fresh IVF cycle; at least four oocytes collected characterizing no poor responders at ovarian stimulation; and good-quality blastocysts available for transfer with at least two surplus good-quality blastocysts cryopreserved after transfer.

In Brazil, the law states that patients younger than 38 years can transfer a maximum of three embryos. The risks and benefits of transfer of one or more embryos were explained and the couples then decided on the number of embryos to transfer. Two hundred and thirty-four patients received a fresh eSET, and 58 become pregnant (24.8%). Of the 176 patients who failed to conceive, 142 underwent a second cycle by frozen embryo transfer (FET). It was defined as eSFET (n = 40) and eDFET (n = 102) patients who had at least two spare good-quality blastocysts that were cryopreserved and who had one or two cryopreserved good-quality blastocysts transferred, respectively (**Figure 1**).

IVF protocol

Briefly, pituitary blockage was obtained either with a GnRH agonist (Lupron kit™, Abbot SA Societé Française des Laboratories, France) or a GnRH antagonist (Cetrotide®, Serono, Switzerland). Ovarian stimulation was accomplished using recombinant FSH (rFSH, Gonal-F®, Serono, Switzerland). When at least two follicles reached a diameter of 18 mm, follicular maturation was triggered with an injection of 250 μg recombinant HCG (rhCG, Ovidrel®, Serono, Switzerland). Oocyte retrieval was carried out after 35–36 h by transvaginal ultrasound-guided aspiration; the luteal phase was supported by 90 mg of daily progesterone (Crinone®, Serono,

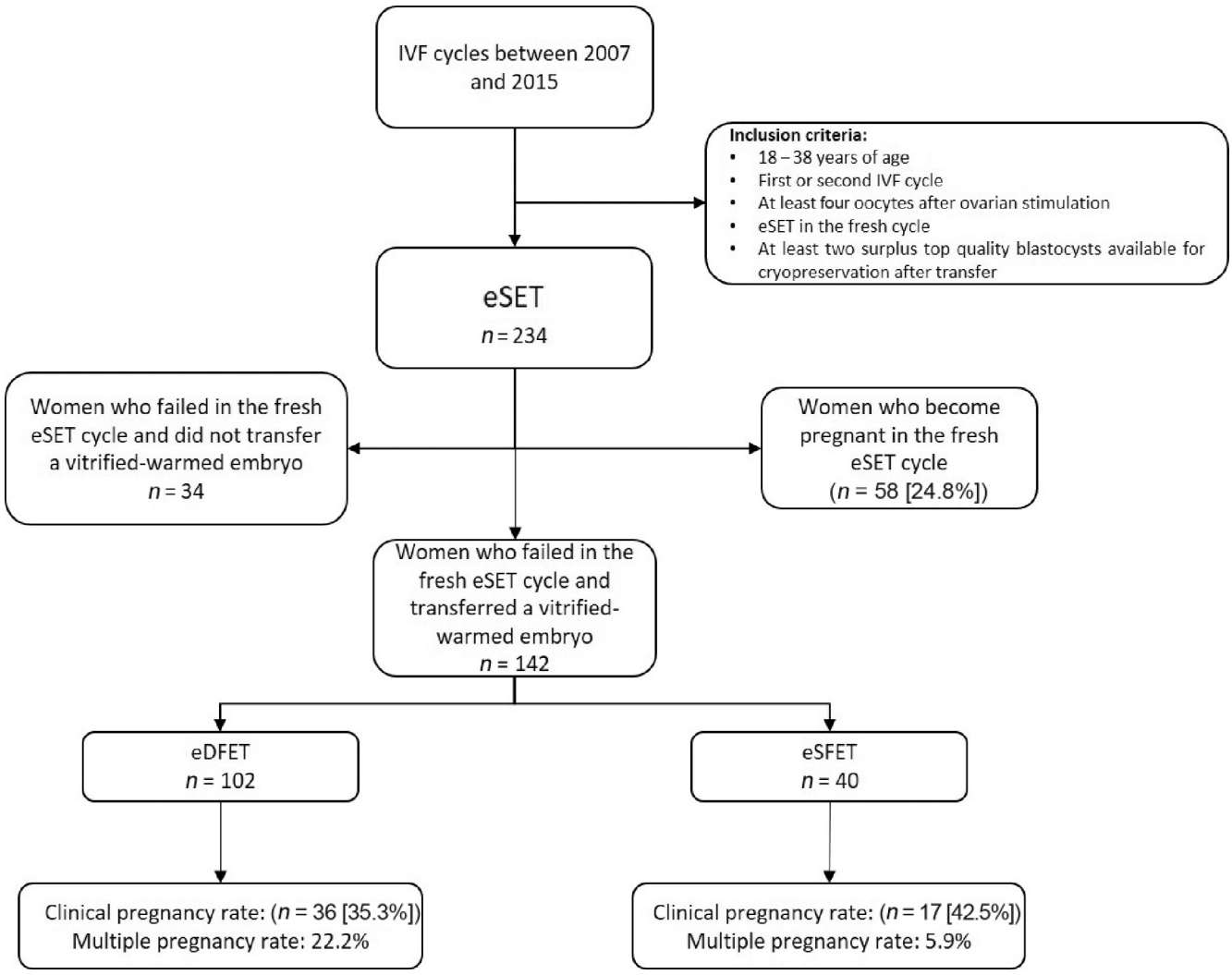

Figure 1 Study design description. eDFET, elective double vitrified-warmed embryo transfer; eSET, elective single embryo transfer; eSFET, elective single vitrified-warmed embryo transfer.

Switzerland) via the vaginal approach, starting on the day of oocyte retrieval. All of the oocytes were fertilized by intracytoplasmic sperm injection (ICSI) (Palermo et al., 1992).

Fertilization was assessed 18 h after ICSI; a normal fertilization was indicated when two clearly distinct pronuclei were present. Embryos were cultured in 40 μL drops using Global culture medium (Life Global, USA) supplemented with 10% human serum albumin (HSA, Irvine Scientific, USA), under a layer of paraffin oil (OVOIL, Vitrolife, USA). Embryos were incubated under 37°C triple gas incubators (90% N2, 5% O2 and 6% CO2). Embryo quality was evaluated daily under an inverted microscope until the blastocyst stage on day 5 of development. The following parameters were recorded during embryo development: the number of blastomeres; the fragmentation percentage; variation in blastomeres symmetry; the presence of multinucleation; and defects in the cytoplasm and zona pellucida. Blastocysts on day 5 were morphologically evaluated, taking into consideration the extent to which the volume of the embryo is occupied by the blastocoel and the number and organization of cells in both the inner cell mass and trophectoderm (Gardner et al., 2000). Blastocysts that were considered good quality were those presenting expanded (grades 3 or 4), inner cell mass grades A or B, and trophectoderm A or B. Only good-quality blastocysts were transferred on day 5 or vitrified. Vitrification of embryos used Vitrification Freeze kit (Irvine Scientific, USA) with Cryotip device (Irvine Scientific, USA) following the manufacturer instructions. Warming used Vitrification Thaw kit (Irvine Scientific, USA).

The endometrium was prepared using 100 μg of oestradiol valerate (Estradot, Novartis, Switzerland) for 14 days plus 600 μg of vaginal micronized progesterone (Utrogestan, Farmoquimica, Brazil) 5 days before the transfer. Blastocysts were warmed, evaluated for survival and morphology and transferred accordingly in the 5 day after the starting use of progesterone. The best quality embryos are warmed first. Clinical pregnancy was defined by the presence of gestational sac with heart beat at 2 weeks after the confirmation of biochemical pregnancy by serum beta-HCG measurement for fresh or cryopreserved embryos transfers.

Statistical analysis

The implantation rate was calculated by the ratio between number of gestational sacs and number of embryos transferred; the pregnancy rate was calculated by the number of patients presenting clinical pregnancy (defined by presence of gestational sac with heart beat) divided by the number of patients with embryos transferred. Also, the cumulative pregnancy rate was calculated, defined as pregnancy rate per patients after a fresh single embryo transfer or by an elective cryopreserved single embryo transfer.

SPSS 22 (IBM SPSS Software, USA) was used for data analyses. Patients' demographic data were evaluated using descriptive statistics, which included information on means and frequencies. Continuous variables were compared using mean comparisons tests (student t-test) and Pearson chi-squared compared frequencies. Regression analyses were used to evaluate the association between the variables. Factors examined in the multivariate models included patient age and number of oocytes collected. Results were reported as odds ratios and *P*-values. $P \leq 0.05$ was considered to be statistically significant.

Results

The demographic characteristcis of the patients included in the study are presented in **Table 1**. Patients in the eDFET and eSFET groups were similar in age (34.2 ± 3.4 years and 34.5 ± 2.6 years, respectively), basal-FSH (6.8 ± 7.9 IU/ml and 5.8 ± 1.9 IU/ml, respectively) and FSH dose administered (1712.1 ± 254.5 IU and 1710.6 ± 192.7 IU), respectively. Although the number of oocytes collected (10.6 ± 5.2 and 13.9 ± 5.3; $P = 0.001$), surplus embryos (5.7 ± 3.2 and 7.7 ± 4.0; $P = 0.003$) and embryos cryopreserved (5.5 ± 2.8 and 7.3 ± 3.3; $P = 0.002$) were higher in the eSFET group, all of the women in both groups were considered to have good prognosis for pregnancy.

The clinical outcomes of the vitrified–warmed blastocyst transfers were evaluated. Patients who received the eDFET had a lower implantation rate, although clinical pregnancy rates were similar. On the other hand, women who received the eSFET had one monozygotic twin pregnancy (5.9%), and the eDFET group had 22.2% of multiple pregnancies (**Figure 2**).

A multiple logistic linear regression model was built to evaluate the influence of the number of embryos transferred in the vitrified-warmed cycle on the pregnancy outcome, adjusted for possible confounders, such as the women's age and number of oocytes collected. The model showed that transferring two embryos on a second cycle (eDFET) did not increase the chance of becoming pregnant (**Table 2**).

Discussion

This retrospective study showed no differences in pregnancy rates between the two strategies, eDFET or eSFET, after one failure of fresh elective single embryo transfer. Otherwise,

Table 1 Demographic characteristics of the patients according to groups.

	eSFET	eDFET	*P*-value
Age (years)	34.5 ± 2.6	34.2 ± 3.4	NS
Infertility time (years)	2.3 ± 2.2	2.2 ± 1.2	NS
basal FSH (IU/mL)	5.8 ± 1.9	6.8 ± 7.9	NS
FSH dose administered (IU)	1710.6 ± 192.7	1712.1 ± 254.5	NS
Number of oocytes collected	13.9 ± 5.3	10.6 ± 5.2	0.001
Second metaphase rate (%)	81.6	85.1	NS
Normal fertilization rate (%)	86.5	83.6	NS
Surplus embryos	7.7 ± 4.0	5.7 ± 3.2	0.003
Number of embryos cryopreserved	7.3 ± 3.3	5.5 ± 2.8	0.002
Embryos survival rate after warming (%)	90	89	NS

Values presented as mean ± SD or percentage.
eDFET, elective double vitrified-warmed embryo transfer; eSFET, elective single vitrified-warmed embryo transfer; NS, not statistically significant.

Table 2 Multiple logistic regression analysis to evaluate the association of the number of embryos transferred on a vitrified-warmed cycle and the chance of pregnancy, adjusted for women's age and number of oocytes collected.

	Coefficient	*Standard error*	P-*value*	*Odds ratio*
Number of embryos transferred	−0.127	0.400	NS	0.881
Women's age (years)	−0.018	0.056	NS	0.982
Number of oocytes collected	0.057	0.033	NS	1.058
Constant	−0.334	2.159	NS	0.716

NS, not statistically significant.

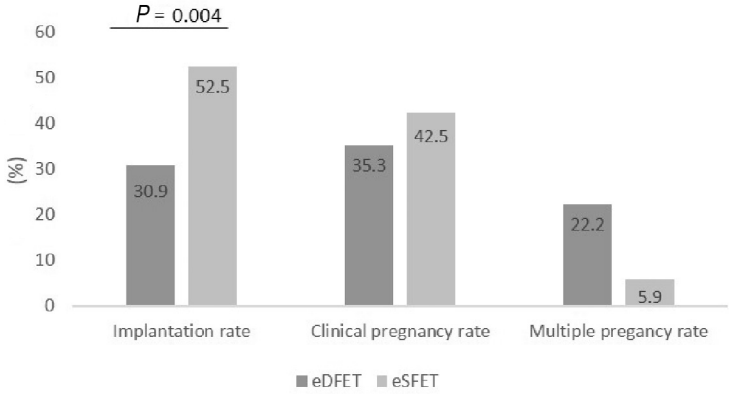

Figure 2 Clinical outcomes in women who failed to conceive in the first fresh elective single embryo transfer and underwent a vitrified-warmed transfer of elective single vitrified-warmed embryo transfer or double vitrified-warmed embryo transfer. eDFET, elective double vitrified-warmed embryo transfer; eSFET, elective single vitrified-warmed embryo transfer.

the multiple pregnancy rates after an elective single embryo transfer was much lower (5.9%) compared with double embryo transfer (22.2%) and was comparable to the observed rate in spontaneous pregnancies (Hamilton et al., 2015).

A recent study evaluating single and double elective embryo transfers for oocyte donation cycles and had similar results to our study (Clua et al., 2015). It is important to highlight that both studies included only patients who had a good prognosis; in addition, the embryos selected for transfer were chosen from a cohort that included at least two good-quality embryos. Embryo quality is an important factor to predict single and multiple pregnancies in IFV–ICSI (Lee et al., 2006), and when good-quality embryos are available, eSET is the best option for patients with a good prognosis.

When patients undergo eDFET, even though the embryo competency can be diminished on average as the best quality embryos are transferred preferentially, it is still sufficient to give an almost one-quarter multiple pregnancy rate. Those embryos, however, were morphologicaly selected and the association of genetic analysis could help in the best embryo choice. Moreover, in IVF–ICSI blastocyst transfers, the incidence of monozygotic twins was increased (Gee et al., 2014). In spite of the risk of monozygosity, in our sample, no high order pregnancies occurred in the eDFET group, and only one monozygotic twin pregnancy occurred in the eSFET.

The clinical pregnancy rate for patients who received a fresh eSET (25.0%) was lower than cryopreserved eSET patients (42.5%); yet the cumulative pregnancy rate was 32.1%. As previously reported, eSET in a fresh IVF cycle yields a lower live birth rate than eDET, but this difference is overcome by an additional cryopreserved SET cycle (Clua et al., 2015; McLernon et al., 2010; Pandian et al., 2013). Also, many studies have reported the deleterious effect on the endometrium during the window of implantation, which occurs in ovarian stimulation cycles. Multi-follicular development and final oocyte maturation induction with exogenous hormones may also have adverse consequences for reproductive outcomes, owing to the altered endogenous hormone conditions produced. The supra-physiological levels of progesterone and oestrogen often reached at the time of ovulation induction may have significant implications for reproduction outcomes (Evans et al., 2014). On the basis of the current literature, many assisted reproduction programmes are recommending cryopreservation of embryos, as a subsequent frozen embryo transfer cycle would allow better endometrial synchronization with the embryo. Theoretically, a frozen embryo transfer cycle would ameliorate the effects of the elevated oestrogen and progesterone, as it decreases pregnancy rates during the initial fresh cycle due to endometrium–embryo asynchrony (Healy et al., 2016). Some studies confirm that good-quality blastocysts transferred in frozen embryo transfer had a significantly greater chance of implantation and clinical pregnancy compared with blastocysts of matched quality transferred in fresh embryo transfer, suggesting reduced endometrial receptivity in fresh embryo transfer (Ozgur et al., 2015).

As our study included good-prognosis patients, most of them had a high number of oocytes collected and hence, high oestrogen levels, corroborating with lower pregnancy rates in fresh compared with FET. Also, the sample included in this study represents the establishment of the eSET programme in our clinic based on patients' objections to eSET after failure to conceive, so the number of patients included is still small. With the continuity of the programme and increased skills to select good-prognosis couples with indication for eSET, we expected those pregnancy rates to increase. Many factors potentially determine the transfer of two embryos other then SET. Compulsory protocols for eSET do not exist in most countries, and the number of embryos transferred is based on a shared decision-making process involving both patients and medical professionals, allowing individualization of patient care, and consideration of patients' preferences. Decisions about how many embryos should be transferred may be biased owing to economic pressures, insurance coverage requirements (Jain et al., 2002) and limited patient knowledge of risk factors (Ryan et al., 2004).

Despite all the concerns about multiple gestation, the American Society for reproductive Medicine recommendation suggests transferring up to two embryos for good-prognosis patients (Practice Committee of American Society for Reproductive Medicine, Practice Committee of Society for Assisted Reproductive Technology, 2013). As a result, these patients face two options: the first is a two embryo transfer with an approximate one-quarter chance of twins, or an eSET, which if not successful, can be immediately followed by a vitrified-warmed cycle with and equal chance of pregnancy

and greatly diminished chance of twins. After a failed eSET, patients were more reluctant to try a new SET. Despite the risks of multiple gestations, the patient has a right to make self-determined choices (WMA General Assembly, 1964) even against medical advice because having twins is not necessarily an undesired option for the infertile couple (Gleicher and Barad, 2006; Kalra et al., 2003; Ryan et al., 2004).

Lack of knowledge about essential eSET aspects and multiple pregnancy-related complications and the absence of a reimbursement system by medical coverage companies can make patients opt for a DET (van Peperstraten et al., 2008b; Van Peperstraten et al., 2008a). Nevertheless, assuming that patients have reached their decision after receiving proper and complete informed consent, one cannot deny that they have the right to make such a choice.

Because the aim of the IVF services is to achieve high pregnancy rates (Van Peperstraten et al., 2008c), the suboptimal success rates of cryopreservation and lack of a SET protocol and skills to select couples suitable for eSET can be an issue influencing the devision to undergo an eSET. The principal motivation for an eSET is the prevention of multiple pregnancies and possibly to increase the chance of a healthy singleton live birth. The difficulty of achieving a balance between maintaining an acceptable pregnancy rate and the prevention of multiple pregnancies is most likely why the implementation of eSET in clinical practice has not yet been achieved (van Peperstraten et al., 2008b). Therefore, eSET represents an appropriate transfer choice in selected IVF patients.

The retrospective nature of this study limited the number of cases in the group receiving eSFET. Regardless of professional approach encouraging patients to undergo an eSFET after failure to conceive, the acceptance is low, justifying the small number of patients in that group. Another limitation of this study is that patients who underwent eSFET transfer had significantly more oocytes collected and embryos cryopreserved than women who underwent cryopreserved double embryo transfer. Therefore, the patients could be systematically different in total reproductive potential, favouring those who underwent eSFET. On the other hand, the multiple regression model was adjusted to number of oocytes collected to avoid this bias, and one or two embryos transferred did not influence the pregnancy chance in a vitrified-warmed cycle.

Overcoming the barriers for SET in practice is a challenge. Prognostic models to enable the medical professional to apply eSET properly (Hunault et al., 2007) would minimize the lower success rate reported with eSET in an unselected population (van Montfoort et al., 2006). Our findings, however, emphasize the advantages of SET in a system where an eSET protocol is well-established, which include the definition of good-prognosis patients, with high-quality embryos available and an efficient embryo cryopreservation technique, which motivates and reinforces the recommendation for SET. Hence, for patients with a good prognosis who failed to conceive in the first fresh eSET, no advantage was found in undergoing an eDFET compared with eSFET in a second cycle.

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