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Delivery rates after elective single cryopreserved embryo transfer related to embryo survival

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ABSTRACT

Objective: The objective of this study was to assess if eSCET (elective Single Cryopreserved Embryo Transfer) outcome is related to blastomere survival rate. The final objective was to avoid multiple pregnancies and offer the best chances to women to achieve pregnancy even during their frozen–thawed embryo transfer (FET) cycles.
Study design: Patients were included in this prospective observational study if they met the following criteria: (i) women age <37 years old; (ii) IVF of ICSI cycle rank ≤2, (iii) eSET proposed during fresh embryo transfer cycle and (iv) ≥1 good quality cryopreserved embryos available (<20% fragmentation and 4–5 blastomeres at day-2 or 7–9 blastomeres at day-3). Live birth rates (LBR) were compared into eSCET groups according to embryo survival (partially damaged or intact transferred embryo).
Results: We observed among selected patients, that partial loss of blastomeres (1 blastomere for day-2 embryos, 1 or 2 blastomeres for day-3 embryos) following FET cycles did not affect LBR compared with intact embryo.
Conclusion: These results underline the relevance of eSCET as a strategy to reduce multiple pregnancies frequency without reducing LBR.

Introduction

Multiple pregnancies are a major drawback of assisted reproductive technologies (ART), leading to serious obstetrical risks. Higher risk of prematurity, intrauterine growth retardation, low birth weight, perinatal death, neurologic sequelae (cerebral palsy), psychomotor impairment and malformations have been reported in twin or triplet infants [1–3]. In addition, multiple pregnancies are associated with maternal risks. Indeed, Women with multiple pregnancies have an increased risk of hypertensive disorders, diabetes, hemorrhage and postnatal illness [3,4]. Consequences of multiple births on family well-being should also be taken into consideration since higher risk of stress, depression, divorce and financial issues have been observed in this situation [5]. Moreover, higher health care costs per child have been highlighted in case of multiple pregnancies [6–8]. It is now well established that the use of fertility drugs and ART dramatically contribute to the increase in multiple birth rate [3,5]. In IVF/ICSI (*in vitro* fertilization/intracytoplasmic sperm injection) cycles, the best option to avoid multiple pregnancies and their adverse consequences is the reduction of the number of transferred embryos [9,10]. Over the past few years, a strategy so-called elective single embryo transfer (eSET), involving the transfer of a single good quality embryo when at least two are available, has been developed in selected populations. Clinical and embryo criteria justifying eSET have been established by several ART centers [11–14]. Most of randomized trials having compared eSET *versus* DET (double embryo transfer) strategies, concluded that eSET led to a significant decrease in the incidence of multiple pregnancy, while keeping acceptable delivery rates. However, the overall pregnancy rates after eSET was significantly lower when compared to those obtained after DET [11–14]. Nevertheless, in

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selected populations transferring a single fresh-embryo, and if needed, one frozen–thawed embryo transfer (FET), dramatically reduces the rate of multiple births while achieving a live birth rate (LBR) not substantially lower than the rate obtained with DET [8,15]. Edgar et al. reported that cryopreserved embryos having totally survived the freeze–thaw process showed implantation rates that were similar to those observed with fresh embryos [16]. These results reinforce the hypothesis that an eSET followed if needed by the transfer of one intact frozen-thawed embryo probably do not reduce the overall chances of pregnancy. Furthermore, Hyden-Granskog et al. have proposed the single embryo transfer as a strategy to avoid multiple pregnancy in FET cycles [17]. However, blastomere loss is common during embryo freezing and thawing procedures, especially following slow freezing (SF) [16], sole procedure allowed in France until 2011. Several lines of evidence indicate that partial cell loss from embryos at early cleavage stage may affect implantation rates [18–20]. As a consequence, when a FET is scheduled, a cellular loss observed after thawing of an embryo will usually lead to increase the total number of transferred embryos, therefore increasing the risk of multiple pregnancies.

The eSET strategy has been included in our routine practice since 2005. We reported that eSET performed on day 2 or 3, and followed by a FET if needed, significantly reduced the occurrence of twin pregnancies without altering the clinical pregnancy rates in comparison with a DET strategy [21]. Our strategy was initially to thaw the best quality frozen embryo available from patient eligible for eSET. This embryo was single transferred into the uterus if it remained intact following SF procedure. In case of at least one blastomere loss, subsequent embryos were thawed until two were available to be transferred. Following this strategy studying a limited series of 34 FET with a double embryo transfer, even if a good ongoing pregnancy rate of 41.2% (14/34) was observed, a twin pregnancy rate of 21.4% (3/14) was surprisingly observed. These twin pregnancies were all obtained transferring at least one partially damaged embryo loosing (i) one cell from day 2 or (ii) one or two cells from day 3 embryos after thawing (unpublished personal data). Keeping the goal to avoid multiple pregnancies, these unsatisfying results led us to assess prospectively whether an elective single cryopreserved embryo transfer (eSCET) including FET cycles performed with either partially damaged or intact embryos impact LBR.

Materials and methods

Study groups

A prospective observational study was conducted between January 2007 and March 2012 (Fig. 1). Eligible women were offered an eSCET if they met the following inclusion criteria: (i) age <37 years old at the time of controlled ovarian hyperstimulation (COH), (ii) IVF or ICSI cycle rank ≤2, (iii) eSET proposed during fresh embryo transfer cycle and (iv) ≥1 good quality cryopreserved embryos available (<20% fragmentation and 4–5 blastomeres on day-2 or 7–9 blastomeres on day-3, adequately sized with no multinucleation). Women with IVF/ICSI rank >2 who fulfilled the other inclusion criteria were also eligible only if they gave birth to a healthy child after fresh or frozen-thawed embryo transfers of their last cycle. Patients may have been included twice. The present study was approved by the Local Ethical Committee of Jean Verdier University Hospital. Furthermore, each included couple signed an informed consent form and agreed to participate before enrolling in the study. FET cycles were sorted into two groups according to embryos characteristics after thawing (Fig. 1). FET cycles performed using an intact embryo were assigned to group 1, while transfers from frozen–thawed embryo that lost (i) 1 cell on day-2 and (ii) 1 or 2 cells on day-3 were assigned to group 2.

Semen preparation

Semen was prepared, using a two-layer density technique (45% and 90%) of PureSperm (Nidacon International, Göteborg, Sweden) diluted in Ferticult HEPES culture media (FertiPro N.V, Beernem, Belgium). After a 20 min centrifugation at 300 × *g*, semen pellet was washed using Ferticult HEPES media (FertiPro N.V) and then centrifuged 5 min at 600 × *g*. The supernatant was carefully

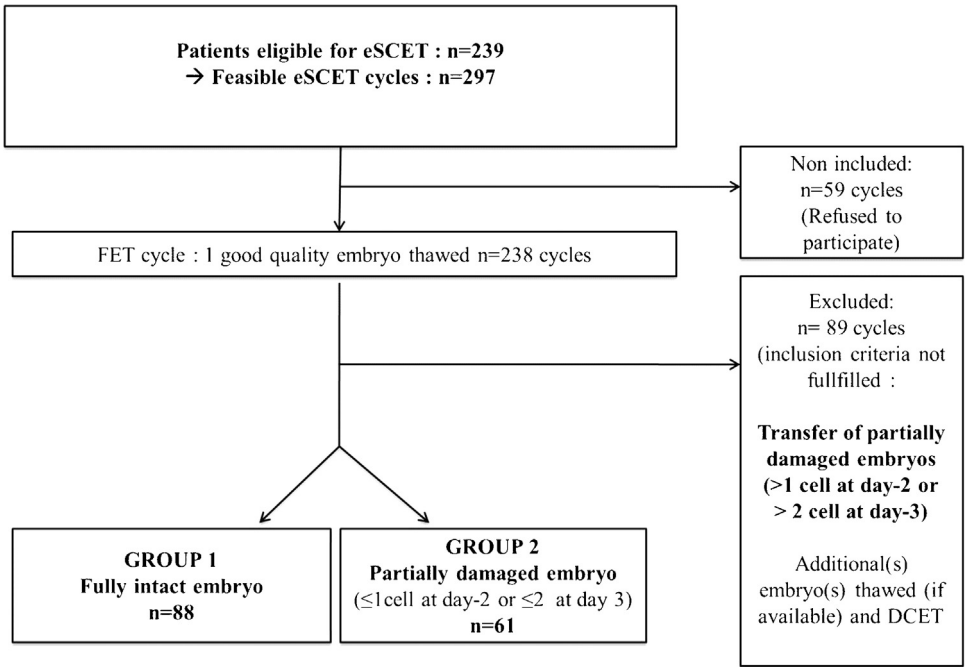

Fig. 1. Flow chart (eSCET: elective single cryopreserved embryo transfer; FET: frozen-thawed embryo transfer; DCET: double cryopreserved embryo transfer).