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Randomised clinical trial comparing elective single-embryo transfer followed by single-embryo cryotransfer versus double embryo transfer

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ABSTRACT

Objective: To analyze the impact of the eSET followed by single-embryo cryotransfer versus double embryo transfer in older women (<38 years) without taking into account embryo quality.
Study design: This is a prospective randomised clinical trial performed on 194 couples attempting a first IVF cycle in a Public Hospital in Spain. The women in Group 1 received eSET plus a single-embryo cryotransfer, and those in Group 2 received a double embryo transfer (DET).
Results: In the intention-to-treat analysis, the cumulative live birth delivery rate in the eSET group was similar to the results obtained for the DET group (45.2% vs. 41.8%; $p = 0.60$). The rate of multiple gestation was significantly lower in the eSET group than in the DET group (0% vs. 26.4%; $p < 0.05$). The findings obtained in the per-protocol analysis were similar to those obtained in the intention-to-treat analysis. The per-protocol analysis revealed no significant differences in the rate of implantation (29.8% in eSET vs. 29.7% in DET; $p = 0.98$), in cumulative pregnancy rates per transfer (49.1% in eSET vs. 46.9% in DET; $p = 0.80$) or in the cumulative live birth delivery rate (38.6% in eSET vs. 42.2% in DET; $p = 0.69$). In the cycles with eSET, there were no twin pregnancies (0% in eSET vs. 27.6 in DET; $p < 0.05$).
Conclusions: For women aged under 38 years with good prognosis, without taking embryo quality as a criterion for inclusion, an eSET policy can be applied, achieving acceptable cumulative clinical pregnancy rates and birth rates.

Introduction

Multiple pregnancy is the most important adverse effect associated with assisted reproductive techniques, due to its prevalence [1], costs [2–5] and maternal and neonatal morbidity [6,7]. Elective single-embryo transfer (eSET) is the most effective strategy to reduce the multiple pregnancy rates in IVF/ICSI. Recent meta-analyses [8,9] have shown that this technique reduces the multiple pregnancy rate without significantly decreasing cumulative pregnancy rates (fresh and cryopreserved transfers). This result, moreover, is associated with significantly lower rates of fetal and neonatal mortality in comparison with double embryo transfer (DET) [7]. Current opinion is that eSET success rates depend on two factors: suitable patient selection and the efficiency of the embryo cryopreservation technique.

In 2004, the Spanish Fertility Society issued guidelines on the number of embryos to be transferred. However, the cases in which eSET is recommended are not clearly defined. This has resulted in a low acceptance of eSET in Spain [10].

In European public health systems, the implementation of eSET as an embryo-transfer strategy is very irregularly distributed; in some countries, such as Sweden, Finland and Belgium, it is well established while in others, like Spain, it is rarely applied [1,11,12]. There are various reasons for this. First, the perception that the

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implementation of eSET would lead to lower success rates in the public system [13]. For this reason, in some public systems it has been proposed that several consecutive cycles of fresh embryo transfer should be carried out without the corresponding cryotransfer cycles, as the implantation rate is assumed to be lower in the latter case. This approach would alleviate the possible injustice to couples included in the eSET strategy [14]. On the other hand, various factors inherent to the public health system (waiting lists, limits to the number of cycles, limited portfolio of services) could restrict the acceptance of eSET by the couples involved, who would not be prepared to face a lower success rate for the sake of reducing twin-pregnancy rates [11,15]. Finally, much of the evidence achieved in the clinical trials performed to date [16–19] cannot be applied to the field of assisted reproduction in public systems.

To raise the profile of eSET programmes in the public system, further RCTs are needed in this context, together with an increase in the population considered appropriate for inclusion, by raising the age limit and relaxing the embryo quality criteria applied, as suggested in the recent meta-analysis [9]. The present study is based on a RCT conducted to analyse the effectiveness of eSET strategy in a public hospital with a programme of embryo cryopreservation by vitrification and using broader inclusion criteria than in previous RCT.

Material and methods

The study was approved by the Clinical Research Ethics Committee of the Virgen de las Nieves University Hospital (Granada, Spain), and met the eligibility requirements of the protocol in relation to the study goals and current legislation on data protection (FIS 09/01968; Clin. Trial. No. NCT01909570). Written informed consent was obtained from all couples. The study was designed as a prospective, randomised clinical trial, conducted during the period January 2010–December 2012 and including a total of 194 women.

Study population

Patients fulfilling the following inclusion criteria were considered eligible to participate in the trial: (i) women aged under 38 years; (ii) body mass index 19–29 kg/m^2; (iii) Follicle stimulating hormone $<$15 mUI/mL on the third day of the cycle; (iv) first cycle of IVF/ICSI or second cycle after a prior attempt with a positive pregnancy test result. Patients were excluded from the study if they had been infertile for over five years, had previous uterine surgery (fibroids, endometriosis, hydrosalpinx), uterine malformations, repeated spontaneous abortions (two or more). Each couple included in the study received an extra cycle in the public health system.

Randomisation

Couples were randomly assigned to one of two groups. In Group 1 (eSET), the elective transfer of a single embryo was performed, and if no pregnancy was achieved, this was followed by a single-embryo cryotransfer cycle; in Group 2 (DET), a double-embryo transfer was performed. In addition, we analysed two other groups: patients excluded from the study and those who were eligible but chose not to participate. In both cases, the current hospital policy was followed, and two embryos were transferred. Randomisation was carried out on the day that patients agreed to participate, and performed by the embryologist who conducted the interview, using a computer-generated randomised numbers process. The primary outcome was the ongoing pregnancy per transfer rate achieved. Secondary outcomes were the multiple gestation rate and the live birth delivery rate.

Protocols

All patients received ovulation stimulation treatment, in order to achieve multiple follicular development. GnRH agonists were used in the long analogue protocol for 115 women (70.2%), and the antagonist protocol was applied to 49 women (29.8%).

The fresh embryo transfer was carried out on the second or third day (D + 2 or D + 3) after oocyte retrieval to allow proper evaluation and embryo selection. All transfers were performed with a full bladder under ultrasound guidance using the labotect embryo transfer catheter set (Labor-Tachnik-Gottigen, Germany). Excess mucus and debris were cleared from the ectocervix using sterile cotton swabs dampened with phosphate-buffered saline. The embryos were then deposited approximately 1.5 cm from the uterine fundus.

The difficulty of the transfers was determined by the physician performing the transfer, who scored it as easy, moderate, or difficult. The presence of blood and mucus in the catheter after transfer was also determined. To ensure proper embryo selection, with the best possible potential for implantation, the embryos were assessed according to the criteria of the Spanish Association for the Study of Reproductive Biology (ASEBIR) revised in 2008 [20].

The non-transferred embryo from the eSET group was vitrified on the third day (D + 3), using commercial vitrification medium (Origio Vitrification, Denmark) containing ethylene glycol and 1,2-propanediol in HTF culture medium fluid, and the Cryoleaf (McGill Cryoleaf, Origio, Denmark) storage device. Cryotransfer protocol: protocols used were natural cycle and substitution cycle. The day before cryotransfer, the embryo was devitrified using the devitrification kit (Origio Warming, Denmark) in accordance with the manufacturer's instructions.

Sample size

Sample size was calculated for ongoing pregnancy per transfer rate under the hypothesis of non-inferiority. A clinical pregnancy rate of 41% was estimated for DET, based on the results of a randomised controlled trial with inclusion criteria similar to ours [16]. Assuming fresh and post-desvitrification eSET to be equally effective, we calculated a non-inferiority margin of 50%. Therefore, a total of 112 patients were needed (56 in each arm) to detect 20.5% difference in the ongoing pregnancy rate with a power of 90%, with $p < 0.05$.

Statistical analysis

The statistical analysis was carried out according to: (a) the intention-to-treat criteria (175 patients); (b) the per-protocol criteria (121 patients). Categorical variables were analysed by the Chi square test or Fisher's exact test. In addition, the odds ratio and the 95% confidence interval were calculated for each group to assess the differences between them. The continuous variables were analysed by the independent *t*-test or the Mann–Whitney *U*-test, depending on the normality of the results. Normality of the distribution was assessed by the Shapiro–Wilk test. Mean differences and 95% confidence intervals were calculated for each group to assess the differences between them. All values were two-tailed with $p < 0.05$.

Results

Patients

A total of 194 women aged under 38 years were included in the study. Of these, 19 women were not randomised. Finally, 175

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