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ASSISTED REPRODUCTION

Elective transfer of two embryos: Reduction of multiple gestations while maintaining high pregnancy rates

Donna Dowling-Lacey · Estella Jones · Jacob Mayer · Silvina Bocca · Laurel Stadtmauer · Sergio Oehninger

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Abstract *Purpose*: To determine if the elective transfer of two embryos reduced the incidence of multiple gestations while maintaining high pregnancy rates. *Methods*: IVF patients and recipients of oocyte donation with an elective day-3 transfer of 2 or 3 embryos were studied. *Result*(*s*): In IVF, the elective transfer of 2 embryos resulted in similar pregnancy rate but significantly reduced the overall incidence of multiple gestations (20% versus 39%) when compared to the elective transfer of 3 embryos. Twin gestations decreased from 28% to 19%, and triplets significantly decreased from 9% to 1%. In oocyte donation, the elective transfer of 2 embryos resulted in similar pregnancy rate but also significantly reduced the overall incidence of multiple gestations (26% versus 48%), with twins decreasing from 34% to 24%, and with a significant reduction of triplets (13% versus 2%). *Conclusions*: In IVF and oocyte donation, the elective transfer of 2 embryos resulted in similar pregnancy rates and significantly reduced multiple gestation rates when compared to the elective transfer of 3 embryos.

Keywords Embryo quality · Implantation · IVF · Multiple pregnancies · Oocyte donation

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D. Dowling-Lacey · E. Jones · J. Mayer · S. Bocca · L. Stadtmauer · S. Oehninger (✉)
The Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, 601 Colley Avenue, Norfolk, Virginia 23507, USA
e-mail: oehninsc@EVMS.edu

Introduction

The high incidence of multiple pregnancies in the USA has been associated with the increasing use of assisted reproductive technologies (ART) and other infertility treatments that involve a variety of ovarian stimulation regimens. It has been stated that multiple gestations resulting from infertility treatment constitute an "ongoing epidemic" that lead to health and economical stress [1]. Multiple gestations are associated with increased risk to the health of both mother and fetuses in comparison to singleton pregnancies. These risks include premature birth, growth retardation, increased infant mortality and costly medical treatment and follow up [1, 2].

In IVF, a reduction in the number of embryos transferred is a reasonable solution to the alarming incidence of multiple pregnancies. The number of embryos transferred is a significant risk factor for multiple gestations independent of patient's age [1]. It is a common practice of ART clinics to transfer more than one embryo. This is empirically performed in order to compensate for the negative impact of extra corporeal ART procedures, including suboptimal *in vitro* culture conditions, which may impinge stress upon the embryos [3]. It is also the result of the inability to choose embryos with optimized developmental and implantation competence, in spite of the fact that recent advances have resulted in an improvement in the selection of embryos of high quality [4–7]. As a result, new trends in embryo transfer practices have been associated with diminished incidence of multiple gestations in the USA [8].

The elective uterine transfer of 2 embryos has the potential to reduce the incidence of high order multiple gestations and still maintain high pregnancy rates. This has been demonstrated in European studies [9–12]. Giannini et al. [13] conducted research on the transfer of 2 versus 3 or more embryos and concluded that a 2-embryo transfer is as effective as the

transfer of 3 or more embryos in terms of pregnancy outcome. Gardner et al. [14] conducted a prospective randomized trial with the transfer of 1 or 2 blastocysts in a highly selected patient population. It was concluded that a single blastocyst transfer is an effective method of eliminating multiple births while maintaining high pregnancy rates.

To reduce the impact of multiple pregnancies, some countries have passed laws or instated regulations. For example, Belgian legislation reimburses laboratory expenses for up to six IVF cycles up to the age of 42, in exchange for restriction of the number of embryos replaced [15]. The United Kingdom restricted the number of embryos transferred to two [16]. Laws in Germany and Switzerland restrict the culture of more than three embryos after the pronuclear stage [17, 18]. Cryopreservation is not allowed in these countries beyond the pronuclear stage and so, if only one or two embryos are transferred, what happens to the remaining left in culture?

A proposed alternative is to keep only two embryos in culture and freeze the surplus pronuclear embryos. This practice demonstrated maintenance of acceptable pregnancy rates [10]. Finland has an elective single embryo transfer policy that reduced the incidence of multiple births [19]. The cumulative delivery rate per oocyte retrieval (including fresh and frozen embryo transfer cycles) was 52.8%, with a twin rate of 7.6%. Sweden also has a 2-embryo transfer policy and proposes the transfer of one embryo for patients with an increased risk of multiple births [20]. Another Swedish group compared the transfer of 2 embryos to the transfer of 1 embryo, and the remaining embryo being cryopreserved [21]. If pregnancy was not achieved following the fresh single embryo transfer, the other embryo was thawed and another single embryo transfer was performed. Pregnancy rates were slightly reduced; however, multiple pregnancy rates were significantly reduced from 33.1% to 0.8%. Van Montfoort et al. [22] of the Netherlands also found that the transfer of an elective (fresh) single embryo prevents all multiple gestations; nevertheless, slightly lower pregnancy rates were also recorded.

According to the 2004 CDC report [23], in the year 2002 in the USA the mean number of embryos transferred was 2.7 for women <35 years, 3.0 for women 35–37 years, 3.3 for 38–40 years and 3.5 for 41–42 year-old patients. Approximately 62% of fresh non-donor IVF transfers included 3 or more embryos transferred. Donor egg cycles had a mean of 2.7 embryos transferred. The incidence of multiple-fetus pregnancy for non-donor IVF patients was 36% (29.4% twins and 6.8% triplets). Fresh donor egg transfers had an overall multiple gestation rate of 44.7% (37.8% twins and 6.9% triplets).

Recently published ASRM guidelines [24] have recommended the transfer of no more than 2 embryos when the patient is under 35 years of age, and the elective transfer of 2–3 embryos for 35–37 year-old patients. The transfer of 2 embryos should be considered in all good prognosis cases if the woman is 35 years or younger. No more than 4 embryos should be transferred to 38–40 year-old patients; in good prognosis cases within this group consideration should be given to transfer 3 embryos.

In the United States there are no laws restricting the number of embryos transferred. Discussions on the number of embryos transferred according to the age of the patient have been conducted [25, 26]. The age of the patient as well as embryo quality should be considered before selecting the number of embryos for transfer. The elective transfer of 1 or 2 good quality embryos could possibly lower multiple gestations rates as well as maintaining high pregnancy rates. A Cochrane review of randomized European studies by Pandian et al. [27] demonstrated that single embryo transfer significantly reduces the risk of multiple pregnancies compared to double embryo transfer, but also decreases the chance of live birth in the fresh IVF cycle. The authors suggested that subsequent replacement of a single frozen embryo might achieve a live birth rate comparable with double embryo transfer. As such, debate continues about the feasibility and cost-effectiveness of single embryo transfer in the clinical setting.

In light of these considerations, our program has aimed to gradually and steadily decrease the number of transferred embryos both in IVF as well as in the oocyte donation programs [28]. The objective of the present study was to examine pregnancy rates in couples in which 2 selected embryos were transferred and to analyze the impact of this policy on the incidence of multiple gestations.

Material and methods

A retrospective analysis was conducted on IVF and oocyte donation data that included patients treated in our program from 2002 through 2005. Inclusion criteria for IVF were: female patients <39 years of age, with a basal cycle day 3 serum FSH level <10 IU/L (indicative of a good ovarian reserve), and with an elective day-3 transfer of 2 or 3 selected embryos. All etiologic diagnoses, and standard IVF or ICSI cases were included [28]. Ovarian stimulation was accomplished with the use of recombinant FSH and adjuvant therapy with a GnRH analogue following published protocols [28]. Patients fulfilling inclusion criteria but in whom no embryo selection was performed were excluded from the study.

Basal FSH serum hormone levels were measured with a microparticle enzyme immunoassay (MEIA-IMX: Abbott Laboratories, Abbott Park, IL). The intraassay coefficient of variation was 4.3% and the interassay coefficient of variation was 4.9% for FSH. The lower limit of sensitivity for FSH

was 1.0 mIU/mL and the regression equation to convert RIA to IMX was 0.46 X RIA-2.2.

The IVF study group included a total of 356 fresh embryo transfer cycles. Two IVF subgroups were examined: (i) patients with an elective transfer of 2 embryos (ET2, in these cases more than 2 embryos were available on day 3, and embryos were chosen for transfer based on the highest morphological score and cleavage status), and (ii) patients with an elective transfer of 3 embryos (ET3, in these cases more than 3 embryos were available on day 3, and embryos were chosen for transfer based on the highest morphological score and cleavage status). The patient and her attending physician made the final decision as to the number of embryos to be transferred (two or three).

Oocyte donors were $\leq$34 years of age and with a basal cycle day 3-serum FSH level $<$9 IU/L. All recipients with an elective transfer of two embryos (ODET2) or three embryos (ODET3) on day 3 were analyzed for a total of 259 oocyte donation embryo transfer cycles. In all cases more than 2 or 3 embryos were available on day 3.

Uterine embryo transfers were performed in the morning of day 3. The selection of embryos to be transferred was based on the criteria of Veeck [7] and included a grading scale (1 through 5) based on morphological features (1-best, 5-poor) and cleavage assessment [7]. According to our policy, the surplus embryos of good quality ($\geq$4 blastomeres, grades 1–3) were cryopreserved on day 3 [28]. The implantation rate was calculated as the number of gestational sacs/number of embryos transferred. The clinical pregnancy rate was calculated based upon the ultrasound confirmation of a heartbeat per embryo transfer.

Statistical analysis

Chi Square and unpaired *t*-tests with two-tailed analysis were used as appropriate. Results are presented as mean $\pm$ standard deviation (SD). *P* values $<$0.05 were considered significant.

Results

IVF: Elective transfer of 2 versus 3 embryos

There was no difference in age (ET2 = 31.3 $\pm$ 2.9 years versus ET3 = 31.9 $\pm$ 2.8 years, $P > 0.05$) or in basal cycle day 3 serum FSH levels (ET2 = 5.6 $\pm$ 1.6 IU/L versus ET3 = 5.9 $\pm$ 1.5 IU/L, $P > 0.5$) among groups. Over 2/3 of patients in both groups underwent treatment as their first IVF attempt. The total numbers of embryos available for selection at the time of transfer were 9.6 $\pm$ 4.5 for ET2 and 8.5 $\pm$ 3.8 for ET3.

Table 1 Elective transfer of two embryos (ET2) compared to the elective transfer of three embryos (ET3) in IVF: pregnancy and implantation rates, and incidence of multiple gestations

	ET2	*ET3*	*P* value
Pregnancy rate (%)	(91/158) 58%	(90/188) 51%	NS
Implantation rate (%)	(111/316) 35%	(133/564) 24%	0.0003
Multiple pregnancy rate (%)			
Overall	(18/91) 20%	(35/90) 39%	0.008
Twins	(17/91) 19%	(27/90) 27%	NS
Triplets	(1/91) 1%	(8/90) 9%	0.04

Table 1 presents pregnancy outcomes. There was no difference in clinical pregnancy rates among groups (ET2: 58% versus ET3: 51%, not significant -NS). The implantation rate was significantly higher in group ET2 (35% versus 24%, $P = 0.0003$). The overall incidence of multiple pregnancies (20% versus 39%, $P = 0.008$) and incidence of triplet gestations (1% versus 9%, $P = 0.04$) were significantly lower in ET2 versus ET3 group. The incidence of twin gestations (19% versus 28%) was lower in patients with ET2, but the difference did not attain statistical significance.

Oocyte Donation (OD): Elective transfer of 2 versus 3 embryos

There was no difference in age (ODET2 = 26.9 $\pm$ 3.1 years versus ODET3 = 26.9 $\pm$ 3.3 years, $P > 0.05$) or in basal cycle day 3 serum FSH levels (ODET2 = 6.2 $\pm$ 1.7 IU/L versus ODET3 = 6.0 $\pm$ 1.2 IU/L, $P > 0.5$) among groups. Over 3/4 of patients in both groups underwent treatment as their first oocyte donation attempt. The total numbers of embryos available for selection for transfer were 11.1 $\pm$ 5.8 for ODT2 and 9.9 $\pm$ 4.2 for EDT3.

There were no significant differences in pregnancy and implantation rates in oocyte donation cycles with ODET2 versus ODET3 (Table 2). Recipients of oocyte donation with ET2 had a significantly lower overall incidence of multiple gestations than patients with ET3 (26% versus 48%, $P = 0.03$). The incidence of twin gestations was lower in patients with ET2 (24% versus 34%), but the difference did

Table 2 Elective transfer of two embryos (ET2) compared to the elective transfer of three embryos (ET3) in oocyte donation (OD): pregnancy and implantation rates, and incidence of multiple gestations

	ODET2	*ODET3*	*P* Value
Pregnancy rate (%)	(54/118) 46%	(77/141) 54%	NS
Implantation rate (%)	(69/236) 29%	(123/498) 29%	NS
Multiple pregnancy rate (%)			
Overall	(14/54) 26%	(36/77) 48%	0.03
Twins	(13/54) 24%	(26/77) 34%	NS
Triplets	(1/54) 2%	(10/77) 13%	0.05

not attain statistical significance. The incidence of triplet gestations was significantly lower in patients with ET2 (2% versus 13%, $P = 0.05$) (Table 2).

Discussion

This study demonstrated that in an IVF population of women <39 years of age with good ovarian reserve, and irrespective of the ovarian stimulation protocol or infertility diagnosis, as well as in recipients of oocyte donation, the elective transfer of 2 embryos on day 3 of *in vitro* culture resulted in similar pregnancy rates when compared to the elective transfer of 3 embryos. Importantly, the elective transfer of 2 embryos resulted in a significantly lower incidence of multiple pregnancies with a significant reduction of triplet gestations.

Patients with an elective transfer of 2 or 3 embryos had a similar pregnancy rate (58% and 51%, respectively, not significant). Although this was not a randomized study, the two groups of patients were adequately matched regarding the main predictors of success, i.e., age and basal serum FSH levels (an estimate of the ovarian reserve). Patients with an elective transfer of 2 embryos had a significantly lower overall incidence of multiple gestations than patients with an elective transfer of 3 embryos (20% versus 39%). This was associated with a clear trend indicative of a lower incidence of twins (19% versus 28%), and with a significant reduction of triplet gestations (1% versus 9%) (Table 1).

Similar results were observed in recipients of oocyte donation. The two groups of donors were also matched in age and basal serum FSH levels. In this clinical scenario, the elective transfer of 2 embryos resulted in a significantly lower overall incidence of multiple gestations than in recipients receiving an elective transfer of 3 embryos (26% versus 48%). The incidence of triplet gestations was significantly lower in patients with a selective transfer of 2 embryos (1% versus 13%). Twin gestations were also lower in the group with elective transfer of 2 embryos (24% versus 34%), although the difference was not significant (Table 2).

Globally, the transfer of 2 selected embryos was associated with decreased multiple gestations, with a dramatic impact on the incidence of triplet gestations. Therefore, the implementation of the two-embryo transfer policy constituted an efficient and simple measure with possible profound medical and socio-economic repercussions both in the IVF and oocyte donation populations.

In some countries, dual embryo transfer has been the standard for several years. In the Nordic countries, this practice made triplets almost disappear, although the twin birth rate remained stable [29]. Some studies have suggested that IVF twins (including the presence of vanishing twins) may carry a considerable higher risk than IVF singletons regarding identified short- and long-term outcome measures [30]. As a consequence, a general change in the embryo transfer policy employing single embryo transfer to twin-prone patients has been recommended. The Finnish experience provides evidence that the implementation of single embryo transfer in combination with a well functioning program of embryo cryopreservation, reduced the twin birth rate to $< 10\%$ while maintaining satisfactory pregnancy rates between 30–40% [30–32]. However, while elective single embryo transfer may be the method of choice in qualified cases, only a small proportion of IVF patients may be candidates [33].

In conclusion, the results of the present study demonstrated that in a defined IVF population and in recipients of oocyte donation receiving an elective transfer of 2 embryos on day 3 of *in vitro* culture, pregnancy rates were comparable to those patients undergoing a transfer of 3 selected embryos. Importantly, the elective transfer of 2 embryos resulted in a significant decrease in the incidence of multiple gestations, including a drastic reduction of high order multiple pregnancies regardless of embryo quality. Improved methods for selection of embryos with highest implantation competence are needed in order to continue to test the efficiency of elective single embryo transfer in good prognosis patients.

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