

Research Article

Clinical Pregnancy Rates after Elective versus Non-Selective Single Embryo Transfer in PGS Cycles

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Abstract

Objective: The objective of this study was to evaluate ongoing clinical pregnancy rates after elective single embryo transfer (eSET) versus non-elective single embryo transfer (non-elective SET) and compare them to ongoing pregnancy rates after double embryo transfer (DET) in IVF cycles with preimplantation genetic screening (PGS).

Design: A retrospective study of SNP PGS outcome data from blastocysts biopsied on day 5 or day 6 was conducted to identify differences in ongoing clinical pregnancy rates between study groups.

Settings: Large private IVF practice.

Materials and methods: 676 cycles of IVF treatment (591 patients) with PGS between January 2013 and July 2016 followed by 658 FETs were included in the study (569 SETs and 89 double embryo transfers). 4102 embryos were vitrified after the trophectoderm biopsy, and selected embryos were subsequently thawed for a hormone replacement frozen embryo transfer (FET) cycle. 415 SETs were elective (two or more euploid embryos were available) and 154 SETs were non-elective (only one euploid embryo was available). Cumulative live birth rates were assessed by Kaplan-Meier function.

Results: Our data demonstrated no statistically significant difference in clinical outcomes between the study groups: the ongoing pregnancy rate after an elective SET was 61.0% (253/415) and 53.3% (82/154) after a non-elective SET. Moreover, the ongoing pregnancy rates in a group of patients who had elective SET were not statistically different among different age groups (ongoing PR ranged from 42.3% to 56.1%). Similar results were obtained in a group of patients with non-elective SET (ongoing PR ranged from 56.1% to 66.1%). An increase in the total number of available euploid embryos from 2 to ≥ 5 embryos did not affect ongoing pregnancy rates after SET: 58.2% (53/91), 68.4% (67/98), 52.9% (36/68) and 61.4% (97/158), respectively, $\chi^2=2.087$, $p=0.1486$. Cumulative live birth rate after two consecutive SETs is equivalent to live birth rate after DET: 74.3% and 72.9%, respectively.

Conclusion: Analysis of the data proved the effectiveness of single embryo transfers in IVF PGS cycles regardless of maternal age or total number of euploid embryos available for transfer. In order to maintain high ongoing pregnancy rates and reduce multiple gestation rates, single embryo transfer should be imperative in PGS cycles.

Keywords: PGS; Single embryo transfer; Assisted reproductive technologies; Blastocyst morphology

Introduction

The ultimate goal of any IVF cycle is a single healthy baby. Since the early days of IVF, the efficiency of the assisted reproductive technologies was one of the biggest concerns [1,2]. Historically, the simplest way to increase the ongoing pregnancy rate was to transfer multiple embryos. This approach presents a great risk, not only to the mother but also for the offspring. Based on the latest available data from Centers for Disease Control and Prevention [3] in 2014, 25% of all pregnancies after IVF in the USA were multiple gestation pregnancies and had at least five times higher chances of infant mortality [4]. Improvements in the process of culturing human embryos *in vitro* (low oxygen environment, bench top incubators, single step culture media and controlled air quality) coupled with further refinement of stimulation protocols and embryo transfer techniques significantly increased the number of good quality embryos available to establish a healthy pregnancy [5]. These recent technological advances along with improved freezing protocols [6,7] created a basis for efficient and reliable single embryo transfer in IVF cycles.

Currently in many IVF clinics around the world single embryo transfer has become a predominant option for patients using autologous eggs under 35 years old [8-10], in IVF cycles with donor eggs [11] and even for patients 35-38 years old in some IVF practices [12]. ASRM

Practice Committee guidelines [13] strongly encourage transferring a single embryo to all patients under 35 years old. HFEA in the UK set a goal to limit the multiple pregnancy rates to 10% over the course of the next few years to assure that the best clinical standards of care were provided to the patients [14]. In PGS cycles single embryo transfers have the potential even for wider utilization for two main reasons: multiple gestation pregnancies present a bigger neonatal and obstetric risk for older patients [15] and recent studies revealed high clinical pregnancy rates can be achieved after single embryo transfer in PGS cycles in all age groups [16,17]. Modern advances in molecular biology significantly improved efficiency and reliability of preimplantation genetic screening [18,19]. Consistent and accurate PGS results were

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Received November 18, 2016; **Accepted** December 13, 2016; **Published** December 20, 2016

Citation: Barash OO, Ivani KA, Willman SP, Hinckley MD, Wachs DS, et al. (2016) Clinical Pregnancy Rates after Elective versus Non-Selective Single Embryo Transfer in PGS Cycles. JFIV Reprod Med Genet 4: 195. doi: [10.4172/2375-4508.1000195](https://doi.org/10.4172/2375-4508.1000195)

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reflected in improved IVF outcomes and wider introduction of PGS in clinical IVF settings around the world [20,21]. The main purpose of this paper was to explore efficiency and obstacles for single embryo transfer in IVF PGS cycles.

Materials and Methods

A retrospective comparative study was conducted between January 2013 and July 2016. A total of 676 cycles of IVF treatment with PGS (591 patients) followed by 658 embryo transfers were included in the study. Mean maternal age was 37.46 ± 4.26 . In 569 cycles (average age – 36.89 ± 4.29) only one euploid embryo was transferred and in 89 cycles two euploid embryos were transferred (average age – 37.44 ± 4.29). Preimplantation genetic diagnosis cases for single gene disorder, translocation (balanced and unbalanced) and gender selection were excluded from the study. 569 SETs were divided into two groups: elective SETs – 415 transfers (two or more embryos were available at the time of the transfer) and non-elective SET – 154 transfers (only one embryo was available for the transfer).

Embryos were cultured in MINC benchtop incubators at 37°C in low oxygen culture system in a humidified atmosphere (7% CO₂ and 5% O₂). Quinn's Advantage sequential culture media supplemented with 10% Serum Protein Substitute under mineral oil was used according to the manufacturer's recommendations (Sage *In vitro* Fertilization, Inc., USA). Embryos were vitrified after the biopsy on day 5 or day 6 of embryo development (Irvine Scientific Vitrification kit) and selected embryos were subsequently thawed for a hormone replacement frozen embryo transfer cycle. All embryos were hatched on day 3 (~70 h post insemination). Only good and fair quality embryos that had at least 3-7 herniating cells and met the criteria for cryopreservation were considered for biopsy. The biopsy procedure was performed in Washing mHTF w/ HEPES (Life Global, USA) in 25 µl drops under oil in Falcon 351006 dishes (50 × 9 mm) on the heated stage of Olympus Ix 71 microscope equipped with Narishige micromanipulator (MM-91) and 1460 nm, 300mW Class 1 laser (Hamilton Thorne, Lykos).

A total of 4102 embryos were analyzed for euploidy rates and blastocyst morphology. Morphology of blastocysts was evaluated independently by two embryologists using Gardner classification [22] before trophectoderm biopsy. Embryos were divided into three groups based on blastocyst quality: good (AA/AB/BA), fair (BB) and borderline fair quality embryos (B-/B-) embryos. Euploidy rates were assessed in each study group by SNP (Illumina HumanCytoSNP-12 DNA Bead Chips in combination with an informatics-based algorithm).

Chi-square analysis was used to assess the difference in pregnancy rates. Univariate logistic regression analysis was used to evaluate association between pregnancy rates and maternal age. Continuous values were presented as means with standard deviation. The statistical analysis was performed using R statistical software version 3.3.1 - The R Foundation for Statistical Computing. A p-value of <0.05 was considered as statistically significant.

Results

The ongoing pregnancy rate in IVF PGS cycles was statistically significantly higher after transferring two embryos versus one embryo: 74.2% and 58.8% respectively, $\chi^2=7.55$, $p=0.006$. Live birth rate was also significantly higher when patients chose to transfer two euploid embryos versus one euploid embryo: 72.97% and 54.5%, respectively, $\chi^2=6.90$, $p=0.009$.

In the group of patients with SET only three pair of monozygotic twins were identified, multiple gestation rate – 0.9% (3/335). In a group

of patients with DET 32 pairs of twins and one triplet pregnancy were identified, multiple gestation rate – 50.0% (33/66) (Table 1).

All SETs were divided into two groups: elective SETs (eSET) and non-elective SETs. Our data demonstrated a statistically significant difference in average maternal age between patients who had elective SET versus non-elective SET: 36.6 ± 4.1 years old and 38.9 ± 3.2 , respectively, $p=0.01$.

The initial number of euploid embryos available for transfer was defined by many factors, but primarily by maternal age, ovarian reserve, and culture conditions in the embryology laboratory. Distribution of euploid embryos per cycle in a group of patient ≤ 37 years old is bell-shaped (normal or Gaussian distribution). Distribution of euploid embryos per cycle in a group of patient's ≥ 38 years old was asymmetrical and had a heavy tail (Figure 1).

The proportion of eSETs had a strong tendency to decrease with advancing maternal age: In patients ≤ 35 years old, 92.3% of all patients had two or more euploid embryos available for the transfer. In patients 35-37 years old, 80.3% of all patients have two or more euploid embryos available for the transfer. In patients 38-40 years old, 60.5% of all patients have two or more euploid embryos available for transfer. Furthermore, even in a group of patients ≥ 41 years old 44.2% of all patients had two or more euploid embryos available for transfer. The ongoing pregnancy rate was not statistically different between eSET and non-elective SET groups: 61.0% and 53.3%, respectively, $\chi^2=2.763$, $p=0.097$. Despite significant differences in average maternal age, miscarriage rates were similar in both study groups: 8.0% (22/275) and 8.2% (11/135), respectively. Likewise, the difference in live birth rate between patients with elective and non-elective SET was only 3.7%: 55.8% and 52.1%, respectively, $\chi^2= 0.43771$, $p=0.5082$ (Table 2).

Moreover, ongoing pregnancy rates were not statistically different among different age groups within patients, who had an elective SET (ongoing PR ranged from 42.3% to 56.1%). Similar results were obtained in patients with a non-elective SET (ongoing PR ranged from 56.1% to 66.1%) (Figure 2).

Analysis of the data revealed no association between an increased number of euploid embryos available for transfer and ongoing pregnancy rates in PGS cycles. 91 patients had only two embryos available for transfer - ongoing PR in this group was 58.2%, 98 patients had 3 embryos available for transfer- ongoing PR in this group was 68.4%, 68 patients had 4 embryos available for transfer - ongoing PR in this group was 52.3%, 158 patients had ≥ 5 embryos available for transfer – ongoing PR in this group was 61.4%.

	ETx1	ETx2	P-value
Total SETs	569	89	
Average age	36.89 ± 4.29	37.44 ± 4.29	NS
Positive HCG	77.7% (442/569)	87.6% (78/89)	$p<0.032$
Negative HCG	22.3% (127/569)	12.36% (11/89)	
Biochemical,%	16.7% (74/442)	10.3 (8/78)	NS
Clinical PR per ET, %	64.7% (368/569)	78.7% (70/89)	$p<0.009$
Miscarriage rate, %	9.0% (33/368)	5.7% (4/70)	NS
Ongoing pregnancies	335	66	
Ongoing PR per ET, %	58.8% (335/569)	74.2% (66/89)	$p<0.006$
Live birth rate,%	54.50%	72.90%	$p<0.009$
Twins	3	33 (1 triplet)	
Twin rate	0.9% (3/335)	50.0% (33/66)	$p<0.001$

Table 1: Clinical outcomes in IVF PGS cycles after single embryo transfer versus double embryo transfer.

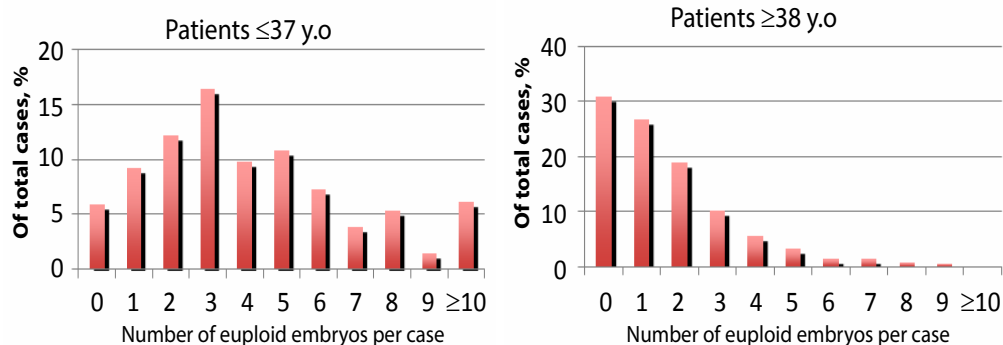


Figure 1: Distribution of IVF PGS cases by number of available euploid embryos in a group of patients ≤ 37 y.o (left graph) and ≥ 38 y.o. (right graph).

	eSET	non-elective SET	P-value
Total SETs	415	154	
Average age	38.9 \pm 3.2	36.6 \pm 4.1	p<0.01
Positive HCG	79.5% (330/415)	72.7% (112/154)	NS
Negative HCG	20.5% (85/415)	27.3% (42/154)	
Biochemical,%	13.3% (55/415)	12.3% (19/154)	NS
Clinical PR per ET, %	66.3% (275/415)	60.4% (93/154)	NS
Miscarriage rate, %	8.0% (22/275)	8.1% (11/135)	NS
Ongoing pregnancies	253	82	
Ongoing PR, %	61.0% (253/415)	53.3% (82/154)	NS
Live birth rate, %	55.80%	52.10%	NS

Table 2: Clinical outcomes in IVF PGS cycles after elective single embryo transfer versus non-elective single embryo transfer.

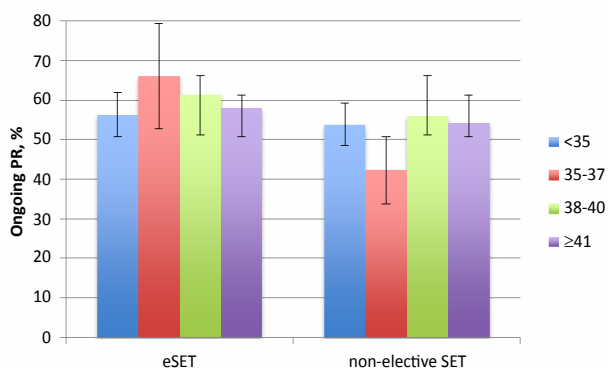


Figure 2: Ongoing pregnancy rates after elective versus non-elective single embryo transfer in IVF PGS cycles.

Morphological characteristics of the embryo play an important role in establishing a healthy viable pregnancy. In this study in patients with elective SETs good quality embryos were transferred in 283 FETs (68.2%), fair quality embryos were transferred in 96 FETs (23.1%) and borderline fair quality embryos were transferred in 36 FETs (8.7%). In patients with non-elective SET, good quality embryos were transferred in 68 FETs (39.0%), fair quality embryos were transferred in 52 FETs (33.8%), and borderline fair quality embryos were transferred in 34 FETs (22.1%).

Analysis of the data demonstrated the statistically significant impact of morphological characteristics of the embryo on ongoing pregnancy rates in both study groups. The highest ongoing pregnancy

rates were achieved with good quality embryos: 67.1% in patients with elective SET and 61.7% in a group of patients with non-elective SET, $\chi^2=0.7063$, $p=0.4$. Pregnancy rates after transferring fair quality embryos were lower in patients with elective SET as well as in patients with non-elective SET: 51.2% and 47.9%, respectively, $\chi^2=0.2166$, $p=0.64$. The lowest ongoing PR was achieved in PGS cases where only one borderline fair quality embryo was available for transfer – 38.2%, $p<0.05$.

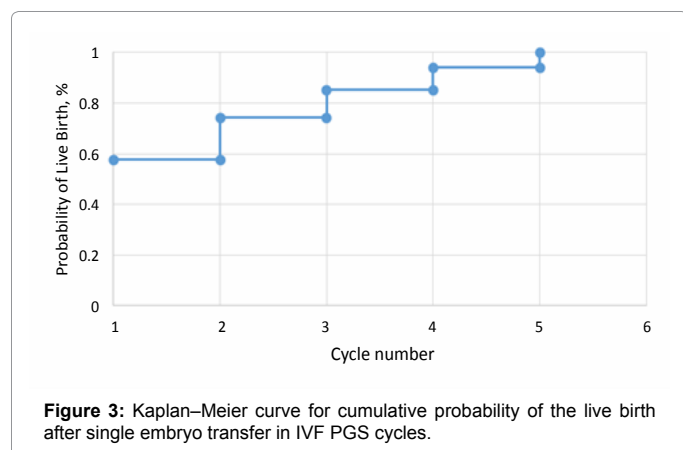
The cumulative probability of a live birth after two consecutive single euploid embryo transfers was 74.3%, after three consecutive single euploid embryo transfers – 85.13%, and after four consecutive single euploid embryo transfers – 94.05 (Figure 3).

Our data demonstrated no statistically significant difference in ongoing pregnancy rates after single embryo transfer between young (≤ 35 years old) and older patients (≥ 41 years old): 56.0% (79/141) and 55.8% (48/86), respectively, $p<0.05$. Furthermore, linear regression analysis showed that live birth rate is age-independent after transferring one euploid embryo in all age groups. Coefficients of linear regression were defined as: $y=0.0585x+54.964$.

Discussion

Comprehensive chromosomal screening in many ways had changed the way we perform and understand IVF nowadays. The recent publications have shown that several factors once thought to be important for several decades in conventional IVF may have lost some of their significance in modern PGS cycles [23,24]. The number of eggs retrieved the number of blastocysts per cycle or even maternal age and the number of euploid embryos available for the embryo transfer has lost their predictive capability because of genetic screening. Morphological and kinetic characteristics of the embryo still have a sizeable effect on clinical outcomes [25,26]. With the current state of the technology we can accurately and reliably assess chromosome count, but unfolding of genetic information and epigenetic characteristics of the embryo is still remains beyond the reach [27,28] and may have direct or indirect effect on embryo development.

Several publications have shown an association between morphological and kinetic characteristics of the embryo and the chromosomal status [29-31]. While such research and models are still in their early stages of conception, they may create a background for future non-invasive embryo evaluation *in vitro*. One of the most obvious pieces of evidence of the existence of an association between morphokinetics and chromosomal status is the fact that many practices



around the globe were very successful in a wide introduction of SET in non-PGS IVF cycles even in a patient group 35-38 years old.

With advancing maternal age euploidy rates have a tendency to substantially decrease – over 60% of all embryos in a group of patients \geq 38 years old have minor or major numerical chromosomal aberrations. For this group of patients, it is not feasible to maintain a high clinical pregnancy rate in non-PGS cycles by transferring only one embryo because a priori the ongoing pregnancy rate after SET can't be higher than expected euploidy rate.

Analysis of our data shows a significant difference in live birth rate after transferring one euploid embryo versus two euploid embryo: 54.7% and 72.9%, respectively, $\chi^2=8.04$, $p<0.05$. At the same time, the multiple gestation rate increased from $<1\%$ to 50%, which is well above the recommended ASRM and HFEA guidelines [4,14]. As a further matter, our data demonstrated that even patients \geq 41 years old can expect live birth rate of 54% after transferring just one euploid embryo, so advanced maternal age should not be considered as a reason for transferring multiple euploid embryos in IVF PGS cycles.

A high ongoing pregnancy rate in patients with non-elective SET reduce the need for batching of untested embryos, especially for older patients. While batching may be seen as a reasonable solution to avoid excessive charges for embryo biopsy and genetic analysis from the clinical point of view it may have limited value because of the heavy tails observed in the distribution of the normal embryos per cycle. This means that desired euploid embryo potentially can be found in a cohort of embryos from the first IVF cycle and subsequent IVF cycles may be unnecessary.

In addition, analysis of the data demonstrated no “cohort effect”- An increase in the number of available euploid embryos does not correspond with increased ongoing pregnancy rate per transfer.

The most common reason for DET is the patient anxiety about the suboptimal quality of the euploid embryos available for transfer. A growing amount of evidence shows that the overall proportion of good, fair, and poor quality embryos remains unchanged with advancing maternal age [32,33]. The number of usable blastocysts per cycle decreases with advancing maternal age due to decreased ovarian reserve, lower blastocyst conversion rate, and increased aneuploidy level. At the same time, we were able to show in this study that even after transferring one fair quality embryo, patients can expect an ongoing pregnancy rate in a range from 38.2% to 51.9%.

An essential part of this research is assessing cumulative live birth

rates after several consecutive single euploid embryo transfers by building Kaplan – Meier curves. Our data demonstrated that cumulative live birth rate after two consecutive SETs is equivalent to live birth rate after DET (74.3% and 72.9%, respectively), but from clinical standpoint presents a much better perspective to avoid complications caused by multiple gestations. These findings corresponded with previously published data accumulated in non-PGS IVF cycles [8].

Conclusion

The data presented here is a part of growing evidence that single embryo transfer should be considered as a standard of care in PGS cycles. Live birth rate after single embryo transfer proven to be age-independent, so only one euploid embryo should be transferred to all patients regardless of patient age. A small number of available euploid embryos or suboptimal morphological characteristics of the embryos should not be considered as a basis for transferring multiple embryos in PGS cycles. Cumulative live birth rate after two consecutive single embryo transfers is equivalent to live birth rate after double embryo transfer. The priority in IVF outcomes should be the birth of a single healthy baby.

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