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Case Report

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Selectively targeted blastocyst embryo transfer: optimizing ivf pregnancy results while minimizing risks and side effects

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Abstract

Research Question: *Is single embryo transfer a reasonable option.*

Design: *Material and methods: This is a retrospective cohort study conducted in a single IVF center. These patients consisted of 96 patients using egg donors and 92 patients using their own eggs for a total of 188 patients undergoing IVF-ICSI.*

Results: *Among 96 patients who received donor eggs, 59 women (61%) were positive for pregnancy tests, resulting in 40 live births (68% per Pregnancy). On the other hand, of 92 patients who received their own egg, 34 patients (37%) were positive in terms of pregnancy tests, resulting in 15 live births (44% per pregnancy). Overall, of all 55 live births, 24 were multiple pregnancies. Interestingly, all multiple pregnancies occured in patients <35 years of age. All of these multiples resulted from the transfer of two blastocysts of grade A.*

Conclusion: *These results corroborate and enhance the various reproductive society recommendations that a selective single embryo transfer at the blastocyst stage and above would be a feasible option in patients less than 38 years of age, especially in patients using egg donors younger than 33 years of age, while resulting in a reasonably high successful singleton live birth rate. This is based on the predicted calculation of a 42.5% singleton live birth per egg donor pregnancy, and a 28% singleton live birth per pregnancy for patients using their own eggs.*

Summary: *Patients who had Blastocyst or above grade A had a 63.7% biochemical or clinical positive pregnancy test result and a 40.7% Live birth. Whilst, patients with blastocysts of other grades or lower stages of development, resulted in 30.7% positive biochemical or clinical pregnancy with 10.7% live birth.*

Keywords: Embryo Transfer, Blastocyst, Embryo Selection, Singleton Pregnancy, Public Health, Reproductive Safety

Abbreviations: IVF: In Vitro Fertilization; ART: Assisted Reproductive Technology; HFEA: Human Fertilisation & Embryology Authority.

Introduction

Materials and Methods

IVF, as an assisted reproductive technology (ART), has allowed millions of individuals to hope for pregnancy since July 26, 1978, when the "first test-tube baby" in the UK resulted in a live neonate birth after an embryo transfer into the uterus [1]. Although IVF has carried some legal and ethical problems worldwide [2-5], it has been adopted as part of national policies in many countries around the world with infertility dilemmas [6]. Even though IVF has triumphantly raised pregnancy rates in infertile individuals, it has also increased the risk for the mother and babies primarily due to the increased incidence of multiple pregnancies [7]. Multiple oocytes are retrieved using controlled ovarian hyperstimulation and consequently multiple embryos are transferred in In-Vitro-Fertilization (IVF) to improve the final pregnancy outcomes [8]. Multiple pregnancies with twins and more carry substantial risk to the mother and the babies. These increased risks include heterotopic pregnancy, diabetes, hypertension, severe bleeding/hemorrhage, spontaneous abortion for the mother, congenital anomalies, preterm rupture of the membranes (PROM), prematurity, and neonatal deaths for the babies. Multiple pregnancies also increase healthcare costs by twenty to one hundred times more [9].

It is confirmed that the number and the stages of embryos transferred in IVF considerably affect the rate of live birth and determine singleton or multiple pregnancies. A significant challenge is picking the most suitable embryo for transfer to achieve a successful result. Although great strides in this endeavor have been made, yet after more than forty years since the first successful IVF, the exact embryo selection and number for transfer have remained elusive, and the rate of multiple pregnancies after IVF hovers around 20% or more globally [10]. The available evidence, therefore, has been considered by the relevant guidelines such as the American Society for Reproductive Medicine (ASRM) and Society for Assisted Reproductive Technology (SART) [11], European Society for Human Reproduction (ESHRE), and Human Fertility and Embryology Authority (HFEA) in United Kingdom, in continuing efforts to reduce the likelihood of high-order multifetal pregnancy as an adverse consequence of assisted reproductive technologies [12]; Human Fertilisation & Embryology Authority [13]. Although reducing the number of embryos transferred in IVF can potentially diminish fetus number, it can also reduce the chances of a successful pregnancy outcome due to the transfer of non-progressive embryos to a viable live birth [14]. However, since embryo selection and implantation rate of blastocysts stage embryos is substantially superior to cleavage stage embryos [15], the number of embryos needed to be transferred in IVF can be significantly reduced, which in turn causes an increase in live births, and a decrease in multiple pregnancies. This retrospective report tries to provide additional empirical evidence on the stage, grade, and number of embryos used for transferring to be applied by relevant societies as a reference for writing later versions of the guidelines.

Participants/Materials, Settings, Methods

All IVF patients studied were in a private setting from August 27, 2019 Through December 27, 2021. After initial patient screening for other health issues, stimulation of the ovaries was started on the second day of menses according to standard protocols, and dosages were adjusted accordingly, dependent on subsequent estradiol levels. Once at least three follicles reached a minimum of 18 mm, Induction of ovulation was triggered with either hCG® 10,000 IU (Karma Pharmatech, GmbH) given IM or with Ovidrel® 250 microgram (EMD SERONO, Merck KGaA, Darmstadt, Germany) 35 hours prior to oocyte pick up. After oocyte retrieval, progesterone in oil 50 mg IM was given for fourteen days. If beta hCG was positive, it continued until the end of the 8th week of pregnancy. If egg donors were used, the recipients were prepared first by down-regulation with an intramuscular injection of Diphereline® 3.75 mg (Ferring pharmaceuticals). Once serum estradiol was <60 pg/ml, the endometrium was synchronized with the donors by giving estradiol valerate® (Herderstr, 31, Germany) 5 mg every three days until donor oocyte retrieval. Progesterone in oil 50 mg IM was added on the day of donor retrieval, and a similar dosage was used, as stated above, for patients using their own oocytes. Embryo transfers were generally done at the blastocyst stage on day five after retrieval or after five days of progesterone in oil administration in FET cycles.

Oocyte Insemination

After oocyte retrieval, cumulus-oocyte complexes were digested with hyaluronidase enzyme (Life Global®, Guilford, CT, USA) and mechanical pipetting. Only MII oocytes, by first polar body, were used for ICSI. For the ICSI procedure, processed sperm suspension was added to the drop containing polyvinylpyrrolidone (PVP®; Life Global). Then, the sperm cell with good morphology was selected and immobilized through mechanical pressure. An MII oocyte was held on the holding pipette, and an immobilized sperm cell was aspirated and injected into the cytoplasm of the MII oocyte. They were then incubated in culture media for 3-5 days.

Evaluation of Fertilization, Cleavage Rate, and Embryo Quality Score

The injected oocytes were incubated into 30 µl droplets of total global medium (Life Global) under equilibrated mineral oil at 37 °C, 5% CO2, in a humidified atmosphere. The fertilization rate was calculated as the percentage of resulting zygotes with two pronuclei from the total number of the injected MII oocytes 16-19 h after sperm injection. After 72 h following ICSI, embryos were scored for their cleavage and quality. Embryo morphology was evaluated by assessing blastomere number, size, and percentage of fragmentation. A scale of A to C was employed to classify the embryos. Score 3 was used to grade A embryos with symmetrical blastomeres of equal size and no cytoplasmic fragmentation or slight cytoplasmic fragmentation (<10%). Score 2 was given to grade B embryos that had blastomeres with even sizes and moderate cytoplasmic fragmentation. Finally, grade C embryos

that had unequal sizes of blastomeres and severe fragmentation (>50%) were given a score of 1. In each grade, we evaluated the embryos' cumulative quality for each ICSI: the sum of scores of embryos to the total number of cleaved embryos. Cleavage rate was calculated by the number of cleaved embryos on day 3 to the total number of fertilized embryos was computed [33]. Embryos were graded as reported above. Up to 2 highest grade embryos were transferred according to published guidelines using either a Cook® or Wallace® Catheter.

Statistical Analysis

Statistical analyses were performed using IBM SPSS© Statistics version 26.0 (IBM; Armonk, NY). Descriptive statistics was comprised of means and standard deviations for continuous variables. Categorical variables were presented as counts and percentages. The student's test was used for two-group comparisons of normally distributed, continuous variables. Analysis of variance (ANOVA) with post hoc Scheffé's multiple range test was used for comparisons of normally distributed, continuous variables between more than two groups, while the Kruskal-Wallis H test was used for non-normally distributed variables. Chi-square tests were used for comparisons of categorical variables. Crude odds ratios and 95% confidence intervals were also calculated. A two-sided p-value OF <0.05 was considered statistically significant.

Result

188 procedures were performed at the Infertility Center of the Payambaran IVF center in Tehran, Iran, over the span of one and a half years. The diagnosis of infertility was varied. One or two embryos grown to the blastocyst stage with grade A or B were transferred for the majority of cases unless the patient had multiple prior failed attempts at IVF or did not have high-quality blastocysts. In this case, up to three embryos in the blastocyst or lower stages of development were inevitably transferred. Although the embryos prior to transfer were in different stages and quality grades, the blastocysts of expanded or hatched grade A were given the highest priority for transfer. Figures 1a and 1b indicate typical microscopic images of such blastocysts and one blastocyst of a lower grade (Figure 1a-1c).

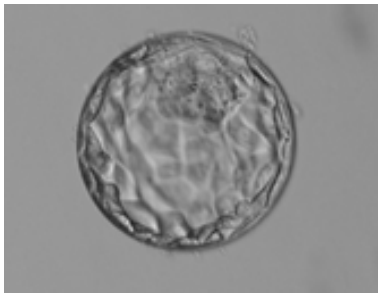

Figure 1A: Expanded Blastocyst Grade A on Day 5.

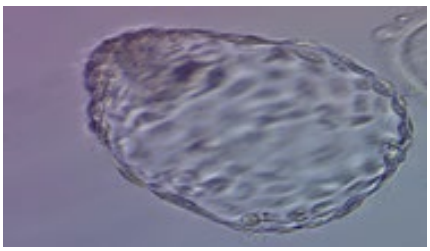

Figure 1B: Hatched Blastocyst Grade A on Day 5.

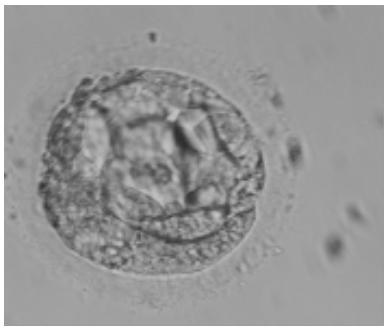

Figure 1C: Blastocyst Grade B on D 5.

The pregnancy test results indicated that 63.7% were clinically or biochemically positive among all the cases with blastocysts of grade A, and 36.3% were negative. Whilst, patients with blastocysts of other grades, resulted in 30.7% positive and 69.3% negative pregnancies (P Val=0.000) (Table 1a and 1b). Over all 50.5% of women had positive pregnancies, and 49,5% were negative.

Table 1a: Crosstab: Correlation of the stage and grade of embryos transferred with pregnancy outcome. relation of the stage and grade of embryos transferred with pregnancy outcome.

			Result		Total
			Negative	Positive	
Stage-Grade	Blastocysts grade A	Count	41	72	113
		% Within Stage grade	36.3%	63.7%	100.0%
	Other	Count	52	23	75
		% Within Stage Grade	69.3%	30.7%	100.0%
Total		Count	93	95	188
		% Within Stage Grade	49.5%	50.5%	100.0%

Table 1b: Chi-Square Tests.

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	19.699a	1	.000	-	-
Continuity Correction[b]	18.399	1	.000	-	-
Likelihood Ratio	20.104	1	.000	-	-
Fisher's Exact Test	-	-	-	.000	.000
Linear-by-Linear Association	19.594	1	.000	-	-
N of Valid Cases	188	-	-	-	-
a) 0 cells (0%) have expected count less than 5. The minimum expected count is 37,10. b) Computed only for a 2x2 table. Comparing success rate using blastocysts grad A with the rest of the embryos.					

Also, the effect of transferring the blastocysts of grade A was analyzed concerning the live birth rate in a different analysis. The data revealed that transferring blastocysts of grade A results in 40,7% live birth, while for embryos of other stages and grades, these rates changed to 10.7% for live birth and 89.3% that did not achieve a live birth (Table 2a and 2b).

Table 2a: Crosstab: Correlation of the stage and grade of embryos transferred with live birth rate.

			Live Birth		Total
			0.00	1.00	
Stage-Grade	Blastocysts grade A	Count	67	46	113
		% within Stage_grade	59.3%	40.7%	100.0%
	Other	Count	67	8	75
		% within Stage_grade	89.3%	10.7%	100.0%
Total		Count	134	54	188
		% within Stage_grade	71.3%	28.7%	100.0%

Table 2b: Chi-Square Tests: Comparing the rate of live birth using blastocysts grade A with the rest of the embryos.

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	19.872a	1	0.000	-	-
Continuity Correction[b]	18.431	1	0.000	-	-
Likelihood Ratio	21.822	1	0.000	-	
Fisher's Exact Test	-	-	-	0.000	0.000
Linear-by-Linear Association	19.766	1	0.000	-	-
N of Valid Cases	188	-	-	-	-
A) 0 cells (0%) have expected count less than 5. The minimum expected count is 21,54. b) Computed only for a 2x2 table.					

The age distribution of the patient enrolled in this study was between 20 and 47 years. Among these, 67.5 % were younger than 35, 12.23% were between 35 and 37, 8.51% were between 38 and 40, and 11.7% were between 41 and 45 years of age. This division is based on the age of the oocytes, which includes either age of the patients or the oocyte donors. The donors in this study were between 20 to 32 years of age (average=28 years). However, the average age of the patients who used their own oocytes was about 36 years of age (between 24 to 47 years). Of all these, 60.1% received blastocysts of grade A, and the rest received lower stage and grade embryos. No more than two embryos were transferred to patients who required oocyte donation. Since the oocyte donors are healthy and younger than the rest of the patients, we have used their results for comparison of success rate.

A comparison was made between four age groups to understand the effect of age on pregnancy and birth rate. Table 5 summarizes the pregnancy and birth rates among four age groups. The figures showed that blastocysts-based IVFs had a high efficiency among patients aged 35 and less, i.e., 57%, in terms of pregnancy rate. Despite this high efficiency, 39% and 17% of the IVFs result in livebirth for patients aged <35 and 35-37 years of age, respectively. There seems to be a significant drop in the success rate for patients over 37 years of age (Table 3).

Table 3: Relationship between age and live birth rates.

		Age			
		<35	**35-37**	**38-40**	**41-47**
Count		127	23	16	20
Age rate (%)		67.55	12.23	8.51	11.7
pregnancy rate (%)	Positive	57	52	25	27
	Negative	43	48	75	73
Birth rate (%)	Yes	39	17	6	5
	NO	61	83	94	95

Another analysis was performed to better understand the birth rate among patients with positive pragnancies. The data obtained from the analysis showed a significant decrease in the live birth rate with increasing age. For the age group less than 35 years of age, live births were reported as 68%, which was the highest live birth rate. Patients who were 41 to 45 years old possessed the lowest live birth rate, which was reported as 17% (Table 4).

Table 4: Comparing pregnancy result and birth rates.

		Positive pregnancy			
		<35	**35-37**	**38-40**	**41-47**
Birth rate (%)	Yes	68	33.3	25	17
	No	32	66.7	75	83

Among 96 patients who received donor eggs, 59 women (61%) were positive for pregnancy, resulting in 40 live births (64% per Pregnancy). On the other hand, of 92 patients who received their own egg, 34 patients (37%) were positive in terms of pregnancy, resulting in 15 live births (44% per pregnancy). Overall, of all 55 live births, 23 were multiple births. Interestingly, all multiple pregnancies occured in patients <39 years of age. All of these multiples resulted from the transfer of two blastocysts of grade A (Table 5). Two sets of triplet pregnancies resulting from transfer of only two blastocysts were also delivered.

Table 5: Comparing live birth rates and multiple pregnancies between patients with own eggs versus patients receiving donor eggs.

		start	**Positive pregnancy (+ Beta hCG)**	**Multiple pregnancy**	**Live birth**
Donor Egg (Age 20-33 Avg 27)	count	96	59	19 –> 4 Sab	40
	Percent (%)/Positive Pregnancy		61% Per Start	25% Per positive Pregnancy	68% Perpositive Pregnancy
Own Egg (Age 24-47 avg 36)	count	92	34	10	15
	Percent (%)/Positive Pregnancy		37% Per start	29% Per positive Pregnancy	44% Per positive Pregnancy

Discussion

Over the past forty years, IVF has evolved into a highly successful treatment for female and male infertility, reaching peak live birth rates by 2001-2002 [16]. This procedure has produced more than 8 million children worldwide (Yovich, 2020), and in the US alone, some 80,000 children are born yearly through more than 440 IVF clinics accounting for 1.9% of children born nationwide. However, the rates have since plateaued and started declining in most regions of the world. By 2012-2013, fresh cycle live birth rates were highest in the US (29%) and lowest in Japan (5%). During 2012–2013 Australia/New Zealand and Japan reported the lowest multiple delivery rates of 5.6% and 4%, respectively, while the US had the highest of 27%. Preterm delivery rates in all regions ranged between 9.0 to 16.6% for singletons, 53.9 to 67.3% for twins, and 91.4 to 100% for triplets and higher order multiples [17].

Due to the increased incidence of multiple pregnancies, there are increased maternal and neonatal complications, including higher rates of infant prematurity (with its associated co-morbidities), fetal growth restriction, and neonatal mortality [18,19]. Furthermore, there are reports of catastrophic complications in the setting of multiple gestations in IVF cycles, such as a case of postpartum hysterectomy due to placenta accreta in a triplet pregnancy [20].

In most industrialized countries, including the UK, fertility treatments are monitored by independent, comprehensive

regulators such as Human Fertilization and Embryology Authority (HEFA) in UK [13]. These countries have set a legal limit on the number of embryos transferred in a single cycle (21). In some countries (e.g., Austria since 2015, and Belgium in patients under 36 years of age), this may be as few as one or as many as three (as in Germany), though two are recommended in guidelines for women under the age of 37 years. Most limits are age-dependent (as in UK or Netherlands), though one is preferred. No more than two embryos can be transferred in France or Sweden. Currently, Europe moves towards complete statutory regulation of assisted reproduction [22]. In the US, however, there is no governmental regulation of fertility treatments. Therefore, many clinics are not following current ASRM and SART's guidelines for embryo transfer [11] on the grounds that fewer embryos would lead to low pregnancy results and live births. Legislators in the US have largely avoided fertility issues, and more recently, the contentious battle over abortion has created a political minefield around any issue concerning embryos [11].

In addition, it is worth noting that multiples also cost heavily for the healthcare system. The average cost of delivery with a singleton in the USA is $21,458 compared to $104,831 for twins and $407,199 for triplets, which is likely due to the high risk of preterm delivery in multiple gestations and the resulting complications leading to NICU admission [23].

This review of our experience in our center describes how transferring blastocyst(s) influences the pregnancy rate and birth rate among women twenty to forty-seven years of age. Unlike the majority of studies and guidelines that addressed the effect of age on the fertility rate using transferring embryos of different stages, our study highlights the impact of transferring up to two embryos in the blastocyst stage with grade A quality on the pregnancy rates and birth rates.

According to our analysis of the data obtained from 188 patients at the Infertility Center of the Payambaran Hospital, the pregnancy and birth rates are also remarkably influenced by age of the oocyte in addition to stage and grade of blastocyst(s).

A two-embryo transfer policy is now common in most European countries [24]. Elective single embryo transfer (SET) is today part of these embryo transfer policies (by legislation and/or guidelines/ voluntary agreement) in five EU countries [25]. Even though no mention of an expectant live delivery rate is stated, SET is also in agreement with the latest SART guidelines [11]. Currently, more accurate and reliable embryo selection prior to the transfer can be made by the blastocyst stage and grade versus a less reliable selection at lower stages of embryo development due to natural selection of the viable embryos in the extended embryo culture [26]. This enables us to better select one of the best embryos for transfer with a high rate of successful singleton live birth rate. In fact, in donor egg cycles we would expect a 33% singleton live birth rate per start or a 42.5% singleton live birth per donor egg positive result ((68% /2)=34% + (34 x 25%) =8.5%). In patients using their own eggs a 16% singleton live birth rate per start or a 28% ((44% /2)=22%+ (22 x 29%)=6%) singleton live birth per positive result would be expected.

Nevertheless, in spite of these efforts at identifying a single best blastocyst embryo for transfer, embryo splitting after blastocyst transfer has been reported previously [27-30].

This is a preliminary retrospective review of the IVFs done by a single physician at a single IVF facility for nearly two and a half years. A more comprehensive data gathering and analysis has now been initiated and will be the subject of future reports with larger cohorts.

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Declarations

Ethics Approval

This study was deemed as not meeting the criteria for human subject research and therefore did not require IRB approval.

Consent to Participate

Not applicable.

Conflict of Interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare no competing interests.

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