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Effect of Adding a Delayed Blastocyst to a Good Quality One during Embryo Transfer on ICSI Cycle Outcomes

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Abstract

Objectives: Study the effect of adding a delayed blastocyst to a transferred good quality one on ICSI cycle outcomes. Study design: Prospective cohort study. Participants/materials, setting, methods: 90 infertile patients aged from 20 - 35 years due to mild male factor, unexplained infertility or tubal factor. Patients with PCOS, endometriosis, RIF, poor responder and azoospermia were excluded. Setting: Duration 6 months from October 2019 to April 2020 in a private IVF center in Egypt. 30 cases were subjected to elective single embryo transfer and the other 60 with two embryo transfers, one good quality and another poor quality blastocyst. Results: (clinical pregnancy rate) was comparable between the two groups, being 47.7% and 36.7% in SET and DET groups respectively (p = 0.361). Miscarriage and ectopic pregnancy rate were observed in 10% of the cases pregnant after SET (group I) and in only 6.7% of the cases pregnant after DET (group II) denoting no significant statistical difference between the two groups. The highest incidence was missed miscarriages in the two groups and ectopic pregnancy was observed only in one case in group II (DET). Twin pregnancy occurred in 22% of pregnant females in the second group. Late pregnancy complications in the form of preterm labour, premature rupture of membrane and preeclampsia occurred mainly in pregnant females in the second group 18%. Conclusion: Neither adding a delayed blastocyst negatively affected the good one nor affected the live birth rate but increased multiple pregnancy rates.

Keywords

Embryo Transfer, ICSI, Delayed Blastocyst, Pregnancy Rate

1. Introduction

IVF has become the most successful treatment of infertility. With continuous improvement of IVF protocols, implantation rates have greatly improved pushing us to shift our focus from implantation rates to properly counselling patients and implementing the practice of single embryo transfers in order to reduce the associated risks of multiple and high order pregnancies. Moreover, it is known from many publications that obstetric outcome after IVF is less favorable than natural conception [1].

Historically, multiple embryos were transferred to compensate for low rates of implantation. European and American registries of assisted reproduction consider a twin pregnancy rate of >25% unacceptable and recommend reducing this incidence to 10% or lower [2]. Since the optimal outcome of an IVF cycle is the birth of a healthy singleton rather than a positive pregnancy test [3], thus implementing single embryo transfer (SET) is the only way to help us achieve this.

The definition of elective SET was stated by Gerris *et al.* in 2004 and in all randomized controlled trials (RCT) as transfer of one good quality embryo in cases where at least two or more good quality embryos were available and it was decided to intentionally transfer only one [4]. Transferring embryos one at a time would minimize the potential for all embryos to be transferred to a nonreceptive uterus as there exists variation in cycle-to-cycle endometrial receptivity in the form of endometrial thickness [5], progesterone levels [6] and histological configuration of the endometrium [7].

There is an everlasting concern of which is more important when applying SET strategy, decreasing overall implantation rates or reducing multiple pregnancies and their associated co-morbidities. The answer to this lies in considering the cumulative pregnancy rate which overcomes this decrease in implantation rate by an additional frozen single embryo transfer of the same stimulated cycle [8]. Thus the principle to balance this strategy and achieve optimal live birth rates is based on a strong blastocyst cryopreservation programme, selecting the best quality embryo for transfer and minimal prognostic criteria in selected patients.

The American Society of Reproductive Medicine (ASRM) assigned minimal good prognostic criteria to be available in SET candidates such as <35 years, more than one top-quality embryo available for transfer, excess embryos available for cryopreservation, outcome of any previous IVF cycles and availability of euploid embryos [9].

Moreover selecting the best embryo for transfer is an important factor influencing IVF outcome. In spite of its subjectivity, morphological evaluation is still the most widely used tool in embryo selection. Based on the ESHRE consensus, embryos at cleavage stage are classified as good, fair, and poor quality according to fragmentation rate, blastomere size and number, and the presence or not of multinucleated blastomeres [10].

A preposition to improving embryo selection is extending culture to blasto-

cyst stage, but its grading is still far from standardized as blastocyst quality is still largely assessed based on appearance rather than viability. In spite of this Gardner classification until now remains largely unchallenged. It takes into account the expansion grade (score 1 - 6) and the development of the inner cell mass (ICM) and the trophectoderm (TE) each graded from (A-C) [11].

Outcome of blastocyst transfer is nearly 50% as this allows a natural selection and embryo assessment beyond genomic activation [12]. Blastocyst transfer provides better synchronization with uterine environment [13], spares exposure of early stage embryos to supra physiological concentrations of gonadotropins [14], allows time for reduction of uterine pulsatility post HCG injectiom to decrease expulsion [15] and greatly reduces the incidence of aneuploidies from 59% to 35% [16].

Many studies have also proposed that when transferring two or more embryos along of different qualities, the poor ones may have a potential to impair implantation of the good ones through activating an oxidative stress response in endometrial stromal cells [17]. On the other hand others proved otherwise [18].

IVF coverage, country legislations, guideline recommendations, and patients' education, counselling and culture are of utmost importance to apply SET.

2. Objectives

To compare ICSI outcome between cases transferring a single good quality embryo and cases transferring good and delayed embryo(s) on day 5 in the same cycle.

3. Materials and Methods

3.1. Study Design

This is a Prospective cohort study conducted in a private assisted reproductive centre in Alexandria, Egypt. The sample size included all patients attending the center from the period of 10/2019 to 5/2020 who met our inclusion criteria undergoing ICSI were included in the study after signing informed consent.

3.2. Inclusion and Exclusion Criteria

Female age limit was from 20 to 35 years. All patients were complaining of primary infertility and they were scheduled for intracytoplasmic sperm injection (ICSI). Any gynaecological pathology was treated before starting. Patients with Recurrent implantation failure, severe male factor, endometriosis, uterine cavity anomalies and polycystic ovaries (PCO) were excluded from the study.

3.3. Treatment Protocols

Flexible antagonist protocol was applied to all patients. All patients' follicular cohorts were synchronized using timed administration of an oral contraceptive (OC) containing 0.03 mg of ethynil estradiol (EE) and 0.15 mg of levonorgestrel (LNG). Then gonadotropins were administrated starting from day 2 cycle with a

dose assigned according to patient's age, BMI, AMH and day 3 FSH. Gonado-tropin dosage will be modified according to the follicular response. Approx-imately after the 6th day of gonadotropin injection when follicular size reaches more than or equal to 14 mm, subcutaneous administration of the GnRH anta-gonist (e.g., Cetrorelix) begins. Patient is routinely monitored via trans-vaginal sonography (TVS) and hormonal profiling of estrogen and progesterone levels of patients. When at least 3 follicles reach >18 mm, trigger and final maturation was induced using 10,000 units of HCG. After 35 hours of HCG injection the mature oocytes were retrieved under ultrasound guidance puncture with light general anasthesia.

3.4. Oocyte Retrieval, Grading and Fertilization

Retrieved oocytes were isolated from follicular fluid, denuded and classified into GV, MI and MII according to nuclear maturity. Each Oocyte of MII grade and of good quality was injected with a single immobilized sperm within 3 - 4 hours after retrieval based on semen quality. Embryo development was monitored once at 16 - 18 h for fertilization and pronuclear formation and at 28- 30 h for early cleavage scoring. Embryos were incubated and monitored daily for blasto-mere number, size, symmetry and degree of fragmentation. The decision to cul-ture to day 5 using sequential media was based on the quantity and quality of embryos on day 3 *i.e.* at least 8 cells. Day 3 embryos were excluded from the study and only harvested day 5 embryos were used.

3.5. Blastocyst Grading

On day 5 blastocyst quality and expansion were checked by 2 senior embryolo-gists based on the Tripartite Scoring of Blastocyst proposed by Gardner and Schoolcraft. Grades from 1 - 6 were given to blastocysts based on the degree of blastocoel expansion and status of hatching. The numeric grading is followed by two Alphabetic grading (from AA to CC), for assessing the Inner Cell Mass (ICM) and Trophectoderm cells [11]. If necessary, assisted hatching with laser zona drilling (20 mm) was performed in embryos selected for embryo transfer. Patients who had only one blastocyst on day of transfer were also excluded as the single blastocyst transfer was nonelective.

3.6. Embryo Transfer and Cryopreservation

On the day of transfer, all patients were counselled properly on the quality and number of embryos they will transfer. After the decision, embryo transfer was done based on morphology using transcervical soft tip catheter under ultrasound guidance. Immediately after embryo transfer, catheters were flushed and the media inspected microscopically to confirm absence of embryos. Day 5 blasto-cysts were considered good quality while day 5 morula and cleavage embryos were considered poor. Cryopreservation was done for good quality supernume-rary embryos with less than 30% cytoplasmic fragmentation.

3.7. Luteal Phase Support and Pregnancy Test Timing

Luteal Support will be given in the form of 100 mg IM progesterone in oil daily and 400 mg progesterone pessaries. Serum Beta HCG will be tested after 14 days of transfer. In case of a positive pregnancy test, a transvaginal ultrasound will be performed 28 - 32 days after the embryo transfer and repeated as required. Clinical pregnancy will be confirmed if a fetal heartbeat was observed by transvaginal ultrasound.

3.8. Outcomes

Primary outcomes were clinical pregnancy rate which will be determined by observation of a gestational sac with fetal heart beat by transvaginal ultrasound at 6 weeks of pregnancy [18] and implantation rate which is defined as the number of gestational sacs observed at echographic screening at 6 weeks of pregnancy divided by the number of embryos transferred [19]. Secondary outcome were miscarriage and ectopic rate.

4. Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level [20] [21].

The used tests were:

1) Chi-square test

For categorical variables, to compare between different groups.

2) Fisher's Exact or Monte Carlo correction

Correction for chi-square when more than 20% of the cells have expected count less than 5.

3) Student t-test

For normally distributed quantitative variables, to compare between two studied groups.

4) Paired t-test

For normally distributed quantitative variables, to compare between two periods.

5) Mann Whitney test

For abnormally distributed quantitative variables, to compare between two studied groups.

6) Spearman coefficient

To correlate between two distributed abnormally quantitative variables.

Study cohorts: 90 patients will be included in the study. The study group will consist of 30 patients transferring single good quality embryo only [group 1]. The other group will include 60 patients transferring good and poor quality embryo(s) [group 2].

5. Results

Demographic data and cycle dynamic differences between the two groups were comparable between the two groups. The mean age for the whole study population was 31.13 ± 3.76 (p = 0.600). Most of the cases were overweight (BMI 25 - 29.9) and had their AMH > 1. The most common indication of ICSI in the two groups was the tubo-peritoneal factor which presented 26.7% in group I and 31.7% in group II. Thus, no significant statistical difference was found in any of these factors.

Comparing between the two groups regarding cycle characteristics as the number of oocytes retrieved, percentage of MII mature oocytes yielded in each group and fertilization rate in each was shown in Table 1. Thus, again none of these factors presented any significant statistical difference between the two groups.

Table 2 shows the comparison between the two studied groups regarding the quality of the transferred embryos. In both groups, the majority of good quality embryos were blastocysts grade 4AA. Thus, avoiding the quality of the good quality embryo being transferred to be a confounding factor between the two groups (p = 0.089).

The implantation rate was significantly different between the two groups, being 100% in pregnant ladies after SET (group I) while it was only 50% in pregnant ladies after double embryo transfer (DET) (group II) (p ≤ 0.001). Despite this difference the other outcome (clinical pregnancy rate) was comparable between the two groups, being 47.7% and 36.7% in SET and DET groups respectively (p = 0.361), Table 3.

Table 1. Comparison between the two studied groups according to oocytes retrieved, maturity and fertilization rate.

No. of oocytes	SET (n = 30)	DET (n = 60)	U	p
Retrieved	**377**	**934**		
Min. - Max.	1.0 - 27.0	6.0 - 40.0		
Mean ± SD.	12.57 ± 6.83	15.57 ± 7.61	741.0	0.173
Median	13.50	14.0		
M2 oocytes	**275**	**618**		
Min. - Max.	1.0 - 23.0	4.0 - 30.0		
Mean ± SD.	9.17 ± 5.45	10.30 ± 5.08	803.50	0.407
Median	8.50	9.0		
Fertilization rate (%)				
Min. - Max.	33.3 - 100.0	20.0 - 100.0		
Mean ± SD.	68.48 ± 18.12	72.2 ± 18.04	786.50	0.331
Median	71.0	72.05		

U: Mann Whitney test; p: p value for comparing between the studied groups.

Table 2. Comparison between the two studied groups according to ET1.Q.

ET1.Q	SET (n = 30)		DET (n = 60)	
	No.	%	No.	%
6 AA	1	3.3	2	3.3
5AA	5	16.7	1	1.7
4AA	14	46.7	21	35.0
4AB	0	0.0	3	5.0
4BB	0	0.0	2	3.3
3AA	1	3.3	6	10.0
3AB	1	3.3	0	0.0
3BB	1	3.3	0	0.0
2AA	1	3.3	5	8.3
2AB	1	3.3	2	3.3
2BB	0	0.0	2	3.3
1AA	5	16.7	16	26.7
χ^2 (^{MC}p)	15.325 (0.089)			

χ^2: Chi square test; MC: Monte Carlo; p: p value for comparing between the studied groups.

Table 3. Comparison between the two studied groups according to clinical pregnancy and implantation rate (%).

	SET (n = 30)		DET (n = 60)		Test of Sig.	p
	No.	%	No.	%		
Clinical pregnancy						
Negative	16	53.3	38	63.3	$\chi^2 = 0.833$	0.361
Positive	14	46.7	22	36.7		
Implantation rate (%)	(n = 14)		(n = 22)			
Min. - Max.	100.0 - 100.0		33.30 - 100.0			
Mean ± SD.	100.0 ± 0.0		56.05 ± 22.75		U = 28.0*	<0.001*
Median	100.0		50.0			

χ^2: Chi square test; U: Mann Whitney test; p: p value for comparing between the studied groups; *: Statistically significant at p ≤ 0.05.

Other secondary outcomes were also observed in our study including missed miscarriage, miscarriage and ectopic pregnancy. It was presented in Table 4.

Rate of twinning in DET group (group II) only was 5 of 60 cases in DET group (group II) had multiple gestations (8.3%). The 5 cases were dichorionic diamniotic. No cases of twins were registered in SET group (group I).

Complications to multiple gestation, that occurred only in group II (DET), in neonates ranged from preterm birth and low birth weight to NICU admission

Table 4. Comparison between the two studied groups according to other outcomes.

Other outcomes	SET (n = 30)		DET (n = 60)		χ	^{FE}p
	No.	%	No.	%		
No	27	90.0	56	93.3	0.310	0.682
Yes	**3**	**10.0**	**4**	**6.7**		
Missed miscarriage	2	6.7	3	5.0	0.106	1.000
Miscarriage	1	3.3	0	0.0	2.022	0.333
Ectopic	0	0.0	1	1.7	0.489	1.000

χ^2: Chi square test; FE: Fisher Exact test.

and long-term developmental delay. While in mothers it ranged from premature preterm rupture of membranes to preeclampia and gestational diabetes mellitus. The highest incidence was incubation and NICU admission for preterms *i.e.*, <37 weeks (5%) and preeclampsia in the mothers 3.3% (Table 5).

The relation between age and clinical pregnancy rate was comparable between the two groups with a p value of 0.899 denoting no significant statistical difference.

On the other hand, most cases with a positive clinical pregnancy rate were of AMH > 1 but still no significant statistical difference was found here either (p = 0.394) (Table 6).

6. Discussion

Demand for ART services increased placing pressure on IVF technologies to be more efficient. ICSI is a multistep invasive technique and its outcome is related to many factors raising an inevitable dilemma about which is the best approach in each step. One of the main issues during *in vitro* fertilization is the embryo quality yielded in every patient [22].

We carried out an extensive literature search and complied with existing guidelines. The overall quality of received data was good and there were few missing values. Our findings were adjusted for confounding factors such as maternal age, BMI, AMH and stage of the embryo which was all day 5 fresh blastocyst transfers. There is now a mounting body of evidence in favour of deferring the transfer of blastocysts and keeping them in culture until full expansion is achieved and transferring them in subsequent warming cycle [23].

With the great improvement in IVF outcomes comes the major arising complication of twins and higher order pregnancies with all their existing comobidities and mortalities. SET made it possible to decrease the multiple birth rates considerably, while keeping the overall pregnancy rate fairly constant.

Generally single embryo transfers are not a common practice in many developing countries due to many patient and physician factors. But the everlasting concern was, does implementing this strategy compromise pregnancy rates or

Table 5. Distribution of the studied cases according to pregnancy course complications for multiple pregnancy in DET group (n = 60).

Pregnancy course complications	No.	%
PROM	1	1.7
PTLP	2	3.3
Incubation	3	5.0
PET	2	3.3
GDM	0	0.0

Table 6. Relation between clinical pregnancy and AMH in each group.

AMH	Clinical pregnancy				χ^2	p
	Negative		Positive			
	No.	%	No.	%		
SET (n = 30)	(n = 16)		(n = 14)			
<1	12	75.0	4	28.6	6.467*	0.011*
>1	4	25.0	10	71.4		
DET (n = 60)	(n = 38)		(n = 22)			
<1	25	65.8	3	13.6	15.227*	<0.001*
>1	13	34.2	19	86.4		
p₁	0.749		0.394			

χ^2: Chi square test; p: p value for comparing between negative and positive in each group; *: Statistically significant at $p \leq 0.05$.

not. Moreover, is it suitable with all patients and does embryo quality play a role in its success or not.

Our study was designed to question a common issue in the clinical practice of physicians dealing with IVF. What should we do with a poor-quality embryo? Is it right to transfer it along with a good quality embryo or will it impair its implantation potential?

Our cohort demonstrated that the live birth rates were similar either when a poor-quality embryo was transferred along with a good quality embryo or when a good quality embryo was transferred alone. Therefore, transferring a poor quality embryo with a good quality embryo did not decrease nor increases the chances for women to conceive, On the contrary it only increased the incidence of multiple gestations denoting that a poor quality embryo still has a chance for implantation [24]. This coincides with four recent studies by Wintner *et al.* in 2017 [18]. Dobson *et al.* in 2018 [25], Berkhout *et al.* in 2017 [26] and yanaihara *et al.* in 2008 [27].

Also a published RCT in 2006 on single versus double blastocyst transfer that included 48 patients randomized for one or the other regardless the embryo quality, showed no statistically significant difference in the pregnancy rates between the two groups [28]. Moreover, a systematic review and meta-analysis was

done in 2010 yeilding similar results as our study in all collected trials. Gerris *et al.* [29], Martikainen *et al.* [30], Lukassen *et al.* [31] and Moustafa *et al.* [32] showed that the multiple pregnancy rates decreased significantly in the SET group (from 23% to 5%) without a significant decline in the clinical pregnancy rates (from 39% to 34%).

Our results contradict a recent study performed by El-danasouri *et al.* and Richardson *et al.*, they found that transferring an impaired quality embryo along with a good quality embryo significantly lowered both the pregnancy and implantation rates than transferring the good quality embryo alone [18] [33].

Additionally, miscarriage rates were similar in the two groups. This is similar to other studies which have shown that once a clinical pregnancy was achieved with poor quality embryo, it had a similar chance of reaching live birth as a high-quality embryo [24].

Day 5 blastocyst transfers were used in this study as recent studies support embryo selection at the blastocyst stage as pregnancy rate of up to 50% can be obtained from it. In addition, a Cochrane Review demonstrated a significant difference in pregnancy rate in favour of blastocyst transfer in good prognostic patients in 9 of 18 randomized control trials reviewed [12].

The decision to perform DET rather than SET is not justified for patients that do not have two high-quality embryos available and any potential increase in pregnancy rate should be weighed against an increased multiple gestation rate in DET compared to SET.

In women under 35 years of age, transferring one fresh embryo and then, if needed, one frozen-and-thawed embryo dramatically reduces the rate of multiple births while achieving a rate of live births that is not substantially lower than the rate that is achievable with a double-embryo transfer [8]. In our study, we focused mainly on outcomes after a single fresh IVF cycle, cumulative outcomes of fresh followed by frozen single embryo transfers of the same stimulated cycle were not analysed in this study and is for future research.

According to our results, single embryo transfers (SET) should be the initial recommendation for selected patients with minimal prognostic criteria. Yet, when two embryos are transferred, women can be reassured that the quality of the second embryo does not seem to affect the pregnancy rate.

Lastly, Single embryo transfer challenges IVF providers to put the "art" back into ART. There is clearly an art to selecting appropriate patients, an art to selecting the best embryo for transfer and most importantly, there is the art of counselling patients. In order to move successfully toward single embryo transfer, we must truly master the art of the doctor–patient relationship.

Strength of the study: it was a prospective cohort study. Clinical pregnancy rate and implantation rates were calculated. All the study population was homogenous and we excluded confounding factors.

Limitations, reasons for caution: small sample size may affect the conclusions. Follow up for cumulative pregnancy rate in both groups is needed. Frozen cycles were not tracked to predict the cumulative outcome in our study. Ongoing

pregnancy rate and live birth rates were difficult to track and recruit. Our study results were somewhat limited by the way the embryos were graded. Selecting the best embryo for transfer is still an ongoing challenge as it is still a subjective assessment, as morphological evaluation is still the most widely used tool in embryo selection. Perhaps more advanced methods to evaluate embryos, such as time lapse and genetic screening or pre-gestational screening (PGS) will provide better selection.

Wider implications of the findings: Encouragement of elective single blastocyst transfer policy for wider scale may improve cycle outcome and decrease risk of multiple pregnancy.

7. Conclusion

Elective SET is the most appropriate for those with a good prognosis: age < 35 years, more than one top-quality embryo available for transfer, first or second treatment cycle or previous successful IVF cycles. DET with one delayed embryo plus one good quality embryo, at the blastocyst stage, does not increase the live birth rate but only increases multiple births. A delayed embryo does not negatively affect a good quality embryo, when transferred together in a double embryo transfer.

Authors' Contributions

ME, YE analyzed and interpreted the patient data regarding the quality of embryos at embryo transfer. EK, ME, YE and PR performed the ICSI procedure and the embryo transfer, and were contributors in writing the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

This study was approved from the Ethics Committee, Faculty of Medicine, Alexandria University. The number is: 0105646.

Written informed consent for publication of their clinical details and/or clinical images was obtained from the patient. A copy of the consent form is available for review by the Editor of this journal. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest

The authors declare no conflict of interest.

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