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### Full Length Research Paper

## Pregnancy Rates in Patients Undergoing Intracytoplasmic Sperm injection with Embryo transfer After 4 and 5 days of Culture in a Closed Incubation System

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#### ABSTRACT

**Background and Objective:** to reduce the developmental arrest potential risk of in vitro cultured embryos, the majority of embryo transfer (ET) had been performed on day 3. The culture conditions had been improved by the development of the sequential media. This permits blastocyst transfer on the day 5. The day 5 embryo transfer provides significantly higher clinical pregnancy rate with a reduction of the risk of multiple pregnancies. However, it still has potential risks to developmental arrest of embryos. The current study aimed to evaluate the pregnancy rate of day 4 ET compared to day 5 ET. **Methods:** A total of 153 fresh intracytoplasmic sperm injection- Embryo transfer (ICSI-ET) cycles were retrospectively analysed. Their files were checked between March 2017 to March 2019. They were 54 for day 4 ET, and 99 for day 5 ET. The cycles with any genetic issues were excluded. Both groups were compared for rates of matured oocytes, fertilization, good embryos, and clinical pregnancy rates. **Results:** There was no significant difference between day 4 and day 5 ET groups regarding female's mean age and rates of matured oocytes. The pregnancy rate of day 4 ET (46.3%) was lower than day 5 ET (48.5%), with no significant difference between groups. **Conclusion:** Day 4 ET can be practiced to avoid cancellation of ET in day 5, attributed to suboptimal circumstances in the in-vitro fertilization (IVF) laboratory, but the reduced quality and lower pregnancy rate must be taken into consideration

### Introduction

The culture environment used for gametes in embryo transfer (ET) affects the clinical pregnancy rate. The optimal conditions of the culture are concerned by many clinical researchers for embryo development before ET. Some recommended ET on day 2 or day 3 to avoid suboptimal culture conditions related to prolonged culture time [1-3]. However, it had the potential risk of reduced embryo quality due to activated embryonic genome, with unpredictable pregnancy outcome [4]. This could be improved by increased number of transferred embryos on day 3 or 4. However, it can increase the multiple pregnancies risk [5,6].

Recent advances in culture media permits the transition from early cleavage ET to blastocyst stage transfer in assisted reproductive technology (AR) practice (7). The ET postponing to later stages can permit for better embryo selection and could be associated with significant increase of successful implantation rate and reduction of multiple pregnancies (8). The number of transferred embryos can be reduced with the ability to select the most capable embryos. Thus, maintain an acceptable clinical pregnancy rate with limitation of high order multiple pregnancies (9). However, the ET at the blastocyst stage, at day 5, still had the potential risks for developmental arrest and decreased embryo quality in vitro, irrespective of the presence of advanced sequential culture media (10).

Based on the pregnancy rates in those cycles, we hypothesized that day 4 ET would have equivalent benefits of embryo selection for transfer to day 5 ET. In addition, day 4 ET could have the advantage of flexibility with busy transfer unit, without affecting ongoing clinical pregnancy rates.

#### The aim of the work

The current study aimed to evaluate the value of day 4 ET in comparison to day 5 ET regarding pregnancy rate.

## Methodology

### Study design and patients

From 2017 to 2019, a total of 153 fresh ICSI-ET cycles were analyzed: 54 cycles on day 4 and 99 cycles on day 5 ET. Cycles with any genetic factors (PGD) were excluded. The collected data were female's, infertility criteria, number of retrieved oocytes, the rate of matured oocyte, the fertilization rate, and rate of good embryo. The pregnancy rate between day 4 and day 5 ET was compared.

### In vitro culture and oocyte retrieval

Ovarian stimulation was completed using gonadotropin-releasing hormone agonist/antagonist, human menopausal gonadotropin (hMG), and recombinant human follicle stimulating hormone (FSH). Human chorionic gonadotropin (hCG) was administered after achievement of optimal follicle development, as appraised by serial transvaginal ultrasound and determination of serum estrogens. Oocyte retrieval was done via a transvaginal approach under guidance of ultrasound 34 hours after injection of hCG. The intracytoplasmic sperm injection (ICSI) was performed with the male partner's spermatozoa. Fertilization was assessed after 16 to 18 hours. After insemination by ICSI, the fertilized oocytes were cultured with Global Total Media (Life Global Europe, Rue de la presse 4, 1000 Brussels Belgium) until ET. After ET, the remained good grade embryos (if any) were cryopreserved for the next cycles.

### Embryonic grading

Grading quality of embryos was done using grading system on day 4 as described by Tao's et al. [11] and grading of embryos on day 5 according to Gardner's blastocyst grading system [12]. Briefly, the I-grade represented the best quality, while grade III represents "poor" quality.

### Endometrium thickness

Thickness of endometrium was 11 mm for all cycles included in this study.

### Assessment of pregnancy

Pregnancy was assessed by serial serum  $\beta$ -hCG levels, at 12 days after the oocyte retrieval.

### Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 16 (SPSS Inc., Illinois, Chicago, USA). Quantitative data were presented by their arithmetic means and standard deviation, while qualitative data were presented by their frequency and percentages. Groups were compared by student samples "t" test, and Chi square tests for quantitative and qualitative data respectively. P value < 0.05 was considered significant.

## Results

Cycle characteristics and fertilization outcomes are shown in Table (1). The number of cycles were 54 for day 4 ET and 99 for day 5 ET. The mean female age was 30.6 $\pm$ 5.9 and 28.6 $\pm$ 5.7 years in day 4 and day 5 ET groups respectively, with non significant difference between groups. Intracytoplasmic sperm injection (ICSI) was performed for all cycles in both groups. The mean number of retrieved oocytes did not differ significantly between day 4 and day 5 ET (10.2 $\pm$ 5.5 and 12.2 $\pm$ 6.8, respectively). However, the oocyte maturation rate was significantly lower in day 4 ET than day 5 ET (60.2% vs 70.09% respectively). But fertilization rate was significantly higher in day 4 than day 5 ET (86.7% vs 81.1%, respectively). The male factor infertility was reported in 46.2% and 45.4% in day 4 and day 5 ET, respectively.

Of the transferred embryos on day 5, 59.9% were of good quality and 66.9% of the embryos transferred on day 4 were good. The proportion of good ET embryos was significantly different (p<0.01) between the two groups. The mean number of embryos transferred on day 4 was significantly higher (p<0.01) than that on day 5. There was no significant difference in the pregnancy rate between the two groups (46.3 % vs. 48.5%) (Table 2).

**Table (1).** Cycle characteristics and fertilization outcomes

	D4 E.T	D5 E.T	P-value
Number of cycles	54	99	
Female age (yr.)	30.6 $\pm$ 5.9	28.6 $\pm$ 5.7	>0.05
ICSI (%)	100 %	100%	-
No of retrieved oocytes	10.2 $\pm$ 5.5	12.2 $\pm$ 6.8	>0.05
Oocyte maturation rate (%)	334/554 (60.2)	853/1217 (70.09)	<0.01*
Fertilization rate (%)	348/401 (86.7)	764/941 (81.1)	<0.01*
Cause of infertility	Male factor Female factor	25(46.2) 19(35.1)	45(45.4) 31(31.3)
			>0.05

**Table (2):** Comparison of embryo quality by day of embryo transfer

	Day 4 ET	Day 5 ET	P value
Number of culture embryos	6.5±3.5	7.6±3.9	<0.01*
Good embryo rate	235/351 (66.9)	454/757 (59.9)	<0.01*
Number of transferred embryos	3.1±0.9	2.5±0.8	<0.01*
Pregnancy rate	25(46.3%)	48(48.5%)	>0.05

### Discussion

The selection of the most viable embryo for transfer is an important issue in ICSI-ET cycles. The advances in the embryo culture media permits a longer period of culture prior to ET. Blastocyst transfer has many advantages. It improves embryo-uterine synchrony and increased the chance of selecting suitable embryos for ET with lower chromosomal abnormalities [13,14]. However, day 5 ET at the blastocyst stage has probable risks of cycle cancellation due to developmental arrests and decreased the quality of embryos in vitro, even with the recent advanced sequential culture media. Montag et al. [15] proposed that extended embryo culture is not helpful when the embryo selection option at later developmental stages is not available. The majority of ET in ICSI-ET cycles is done with blastocyst stage. It appears that the day 4 ET has rarely been attempted by specialists in advanced reproductive techniques (ART) even if such ET have reached the morula stage with certain advantages [16]. Day 4 ET is advantageous for the following: the embryo is returned to normal environment (the uterus), The embryos reside in the uterus for the maximum time period, with shortest in-vitro residence before implantation. In addition, the uterine contractility is decreased at this time; all of these advantages maximize the potential for successful implantation [17]. In 2002, Tao et al. [18] published a retrospective analysis comparing day 4 and 3 ET in which the researchers proposed and followed up a grading system for morula/compacted embryos. They concluded that, day 4 ET had an implantation rate comparable to or higher than day 3 ET.

In the current work, ET had been performed in the morula stage at day 4 in accordance with Tao's. Cleavage stage embryos -(e.g., 4- to 8-cell stage embryos) have slow development on day 4- were transferred in the absence of morula/compaction stage embryos. There was no significant difference in the pregnancy rate between the two groups (46.3 % vs. 48.5%). This is in line with Glujovsky et al. (19) who reported that, there is no evidence favoring day 4 over day 5 or day 5 over day 4 ET, specifically in cumulative pregnancy rates either from fresh or frozen-thawed cycles after a single oocyte retrieval. Thus, it remains unclear whether the day of ET had an impact on the cumulative live birth or pregnancy rates. Future randomized controlled trials are recommended. In addition, Feil et al. (3) reported non significant differences between day 4 ET and day 5 transfers regarding *single* ET. The largest prospective study carried out by Huisman et al. (20) comparing day 4 and 5 ET revealed comparable pregnancy rates in all groups with pregnancy rates of 25.8% after day 4 transfers ( $n=475$ ) and 27.8% after day 5 transfers ( $n=694$ ). In a retrospective analysis of 440 in vitro fertilization (IVF) cycles with day 4 and 307 cycles with day 5 ET also there was no significant difference in pregnancy rates (21).

### Conclusion

In summary, the current work results revealed that, day 4 ET is effective as day 5 ET regarding pregnancy rates. Thus, it is recommended to individualize the day of ET on the basis of physician and patient's preferences, available resources and flexibility of the centers.

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