

RESEARCH ARTICLE

Comparison between Day 2 and Day 3 Embryo Transfer following *in vitro* Fertilization/Intracytoplasmic Sperm Injection

¹Hemant S Shintre, ²Hrishikesh D Pai, ³Deepa Talreja, ⁴Kinjal R Shah

ABSTRACT

Aim: To compare reproductive outcomes of day 2 and day 3 embryo transfer (ET).

Materials and methods: In this retrospective records study, all couples who underwent *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) and ET cycles at Lilavati Hospital & Research Centre over a period of 1 year were studied. Data were collected and analyzed by chi-square test and unpaired t-test by Statistical Package for the Social Sciences, version 16.

Results: There was no statistically significant difference between the clinical and demographic parameters of group day 3 and day 2 ET. In our study, clinical pregnancy rate was 45% in day 3 ET and 36.5% in day 2 ET group [odds ratio (OR) 1.43, p-value 0.49]. The ongoing pregnancy rate was 39.2% in day 3 ET and 26.9% in day 2 ET group (OR 1.75, p-value 0.26). We observed that the miscarriage rate was 5.9% in day 3 ET and was 5.8% in day 2 ET group (p-value 0.69, OR 1.02). We observed one case each of multiple pregnancy, ectopic pregnancy, and fetal anomaly (anencephaly) in day 2 ET group, while in day 3 ET group, no such case was detected.

Conclusion: There are chances that day 3 ET has better clinical and ongoing pregnancy rates than day 2 ET, but the difference is not statistically significant. Study showed similar miscarriage rates in both groups and very low incidence of complications like multiple pregnancy, ectopic pregnancy, and fetal anomaly. So, it is safe to schedule and transfer embryos either on day 2 or on day 3 for planning and programming cycles in coordination with patient and IVF team and for adjusting weekends (nonworking days).

Clinical significance: Many steps of IVF procedure became standardized. However, the optimum timing of ET is still debatable. Several studies comparing ET on day 2 vs day 3 after oocyte retrieval have been performed, but the conclusions are conflicting. Despite development in culture media allowing blastocyst transfer, many centers still practice day 2/3 ET.

Keywords: Clinical pregnancy, Day of embryo transfer, Embryo transfer, Intracytoplasmic sperm injection, *In vitro* fertilization, Ongoing pregnancy, Retrospective study.

How to cite this article: Shintre HS, Pai HD, Talreja D, Shah KR. Comparison between Day 2 and Day 3 Embryo Transfer following *in vitro* Fertilization/Intracytoplasmic Sperm Injection. Int J Infertil Fetal Med 2017;8(2):68-74.

Source of support: Nil

Conflict of interest: None

Date of received: 30-01-2017

Date of acceptance: 24-04-2017

Date of publication: August 2017

INTRODUCTION

Infertility is a public health problem associated with medical, emotional, social, and financial consequences. Recent study on infertility suggests that in India, approximately 15 to 20% of married couples in the reproductive age group suffer from infertility and its incidence is on the rise. Artificial reproductive techniques (ARTs) including IVF/ICSI and ET have been a major development in the treatment of infertility. But whether ET after IVF/ICSI has to be done on day 2 or day 3 still remains a controversial topic, which definitely has an impact on the final outcome of ART. The Cochrane review¹ on "Assisted reproductive technology: An overview of Cochrane reviews" states that there was insufficient good quality evidence to suggest day 3 vs day 2 ET as promising intervention and more evidence is needed.

Although the first human birth after IVF resulted from transfer of a blastocyst in 1978,² most transfers since then have involved earlier cleavage-stage embryos (day 2 and day 3 after fertilization), primarily for lack of culture media that could reliably sustain embryos. Uterus was supposed to provide the best environment for the survival of the embryo. Early replacement in the uterus may be advantageous for the embryos, by limiting the time spent in the *in vitro* environment of embryology laboratory.³

However, the identification of key regulators and a greater understanding of the changing physiologic requirements have fostered the development of culture media which will enable embryos to develop *in vitro*.⁴ Transfer of embryos to the uterus on day 2 is premature compared with situation *in vivo*, while transfer on day 3 after oocyte retrieval may be closer to the physiological time of

¹Resident, ²Honorary Consultant, ^{3,4}Fellow

¹⁻⁴Department of Obstetrics and Gynecology, Lilavati Hospital & Research Centre, Bandra (West), Mumbai, India

Corresponding Author: Hemant S Shintre, Resident Department of Obstetrics and Gynecology, Lilavati Hospital & Research Centre, Bandra (West), Mumbai, India, Phone: +919967304921, e-mail: hemantshintre@gmail.com

uterine entry of embryo. Transfer of embryos prior to the activation of the embryonic genome (at the four to eight cell stages) decreases the precision of embryo selection.⁵ Embryo morphology, along with other factors, is thought to be highly indicative of pregnancy outcome.⁶ Moreover, delaying ET would allow selection of the most viable embryos for transfer. It has been suggested that the longer time in culture improves the accuracy of selection of the best quality embryos for transfer⁷ as additional morphological features are available for identifying good embryos.⁸ Delaying transfer an extra day may increase the likelihood of successful implantation⁹ and also improve endometrial differentiation¹⁰ similar to the natural situation in which the embryos arrive in the uterus 4 to 5 days after ovulation.

Delaying transfer from day 2 to day 3 might have a positive effect on pregnancy outcomes. But this conclusion does not come true for all studies. There exists some studies supporting day 3 ET^{11,12} and the others are in favor of day 2.^{3,13} However, there are articles stating no statistically significant difference exists between day 2 and day 3 ET.¹⁴⁻¹⁶ The Cochrane review¹ on "Assisted reproductive technology: An overview of Cochrane reviews" states that there was insufficient good quality evidence to suggest day 3 *vs* day 2 ET as promising intervention and more evidence is needed. Based on the above-mentioned conflicting results, the study was aimed to compare their reproductive outcomes in terms of clinical pregnancy rate, ongoing pregnancy rate, miscarriage (spontaneous abortion) rate, multiple pregnancy rate, ectopic pregnancy rates, and fetal anomaly rate.

MATERIALS AND METHODS

Patient Population, Data Collection Technique, and Data Analysis

In this retrospective records study, all patients with infertility, who had their IVF/ICSI and ET cycles at tertiary IVF center: Lilavati Hospital & Research Centre over the period of 1 year (from July 2014 to June 2015) were studied. After obtaining approval from our hospital Ethics Committee, all the documented data from patients' medical records, including case papers, treatment sheets, investigation reports, ultrasound scans, follow-up case sheets, embryologists' notes and laboratory records, were retrospectively studied. Out of 360 couples with infertility, who had their IVF/ICSI and ET from July 2014 to June 2015, we got 103 patients fulfilling the inclusion and exclusion criteria. Couples with females age ≤ 40 years, anti-Müllerian hormone (AMH) level in the range of 2 to 3.5 ng/mL (normo-responders), who had undergone controlled ovarian stimulation protocol with gonadotropin-releasing hormone (GnRH) antagonist protocol and recombinant human chorionic gonadotropin (hCG) were used as trigger,

with follicle count of 8 to 14 on the day of trigger (normo-responders) and fresh ET cycle in which three good quality embryos were transferred. Patients who were poor/hyperresponders, had previous failed IVF cycles, canceled cycles, and frozen ET cycles were excluded from the study. Universal sampling technique was applied, and sample size of 103 patients, which included 52 patients of day 2 ET and 51 patients of day 3 ET, was decided for the study.

Parameters like age, body mass index (BMI), AMH values, causes of infertility, serum β -hCG levels, and ultrasound scan results were studied to compare the outcome of day 2 ET and day 3 ET. Follow-up sheets documenting results of IVF/ICSI and ET in terms of continuation or noncontinuation of pregnancy or any complications if occurred till the stage of ongoing pregnancy (12 weeks of gestation) were studied. Parameters like clinical pregnancy rate, ongoing pregnancy rate, miscarriage (spontaneous abortion) rate, ectopic pregnancy rate, fetal anomaly rate, and multiple pregnancy rates were decided to be the main outcome measures for this study.

Methodology: Ovarian Stimulation, Oocyte Retrieval, IVF/ICSI and ET

All the couples included in our study group were thoroughly examined, properly investigated to find out the cause of infertility, counseled for the appropriate treatment accordingly, and all the relevant findings were documented.

In these patients, first they underwent controlled ovarian stimulation with GnRH antagonist flexible protocol and careful monitoring of follicle number and size by ultrasonography (USG). Whenever the lead follicle size reached 18 mm or more, recombinant hCG was given as ovulation triggering agent. After 34 to 36 hours of trigger, oocyte retrieval was done under USG guidance under general anesthesia.

Oocytes were evaluated for maturity and mature oocytes were incubated in tissue culture dishes at 37°C and 5% CO₂ in air. The ICSI/IVF procedure was performed about 2 to 3 hours after retrieval. The ICSI procedure was used particularly as per indications (male factor infertility patients in specific) quoted in recent guidelines and literature^{17,18} and IVF was implemented for all other patients.

Then, oocytes were checked after about 16 to 20 hours (mostly 17 hours postfertilization) for pronuclei formation (first check) and the normally fertilized ones were transferred to cleavage media that was incubated overnight and no further changes of media was done till the day of ET. The embryos were cultured in groups of 3 to 4 in 50 μ L droplets of tissue culture media under mineral oil. Then embryonic development was assessed with inverted microscope for 42 to 44 hours after IVF/ICSI (second check). Then just before transfer, embryos were examined and

graded and good quality embryos according to embryo grading technique were selected and transferred either on day 2 or day 3. No specific criteria were applied to allot patients to day 2 ET or day 3 ET. The ETs were done either on day 2 or on day 3 according to cycle programming for coordinating between patient and IVF team as well as adjusting weekends (nonworking days).

In both day 2 ET and day 3 ET group, the three good quality embryos were selected and transferred 1.5 cm below fundus under USG guidance with labotect ET catheter/soft ET catheter. The surplus good quality embryos were frozen by open vitrification method. Adequate luteal phase support was given.

Patients underwent serum β -hCG test 14 days after ET and patients with positive test underwent transvaginal ultrasound scan 2 weeks later. The pregnancies were monitored with serial ultrasound scanning. All cases were followed up in our hospital and all significant events, findings were documented in follow-up sheets of patients. As we had 100% follow-up of patients till 12 weeks of gestation and after 12 weeks of gestation, many patients preferred nearby obstetricians for further obstetric care, it was difficult to follow-up cases till full term. In addition, the proposed duration of study was 1 year only. So we have studied each case till the stage of ongoing pregnancy (12 weeks of gestation).

Ethical and Humane Considerations

Our study was retrospective records study and ethical approval was sought from Ethical and Scientific Committee of Lilavati Hospital & Research Centre prior to the study.

Statistical Analysis

Data were analyzed by chi-square test and unpaired t-test using software package, Statistical Package for the Social Sciences, version 16 as well as Microsoft Excel for the statistical and graphical representation.

RESULTS

Out of 103 patients fulfilling the criteria, who had undergone IVF/ICSI and ET during the period of 1 year, 52 patients had undergone ET on day 2 and 51 patients had undergone ET on day 3.

There was no statistically significant difference between the parameters (age, BMI, AMH value, duration of infertility, type of infertility, cause of infertility, number of oocytes retrieved) and their distribution of group day 2 ET and day 3 ET. Table 1 shows the demographic and clinical characteristics of infertile couples.

Maximum number of patients (48.54%) were in the age group of 31 to 35 years. Maximum women (63.11%) were

Table 1: Patient demographics and clinical characteristics

| | Day 2 | Day 3 | p-value | Significance |
|--|----------------|---------------|---------|--------------|
| <i>Patient characteristics</i> | | | | |
| Age (years, mean \pm SD) | 32.2 \pm 3.4 | 32.7 \pm 4 | 0.48 | NS |
| BMI (kg/m ² , mean \pm SD) | 27.2 \pm 2.6 | 27 \pm 2.8 | 0.7 | NS |
| AMH (ng/mL, mean \pm SD) | 2.8 \pm 0.4 | 2.7 \pm 0.5 | 0.21 | NS |
| Duration of infertility (years, mean \pm SD) | 4 \pm 1.4 | 4.2 \pm 1 | 0.54 | NS |
| <i>Type of infertility</i> | | | | |
| Primary | 36 | 37 | 0.71 | NS |
| Secondary | 16 | 14 | | |
| <i>Cause of infertility</i> | | | | |
| Unexplained | 17 | 17 | 0.825 | NS |
| Female factor | 22 | 19 | | |
| Male factor | 11 | 14 | | |
| Combined (male + female) | 2 | 1 | | |
| No. of oocytes retrieved (mean \pm SD) | 9.4 \pm 4.2 | 8.8 \pm 3.9 | 0.44 | NS |

Statistical significance – if p-value \leq 0.05; SD: Standard deviation; NS: Nonsignificant

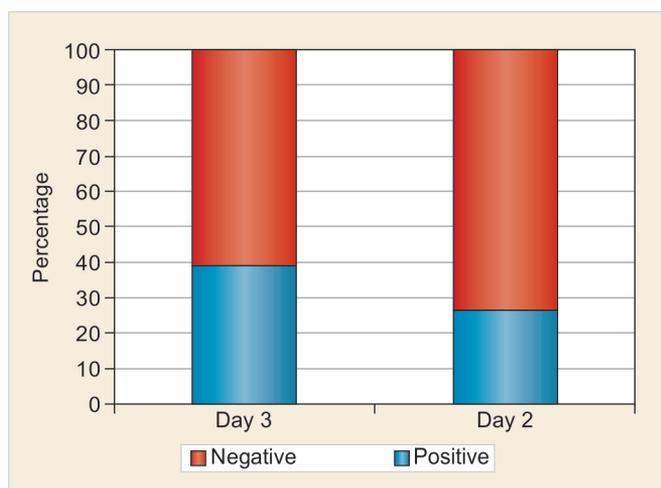
from overweight category (World Health Organization classification). While using Indian classification,¹⁹ 74.76% women were obese. Maximum patients (39%) had AMH value of 2 to 2.5 ng/mL. Among our study, maximum patients (71%) had duration of infertility since 3 to 5 years. Mean number of oocytes retrieved in day 2 ET group was 9.4, while in day 3 ET group was 8.8.

Majority of the patients (70.87%) who underwent IVF/ICSI and ET had primary infertility compared with patients with secondary infertility (29.13%). Among the patients, majority had female factor infertility (40%) followed by unexplained infertility (33%). Among female factor infertility causes, tubal block (37%) and polycystic ovary syndrome (20%) were the major causes. Among male factor infertility causes, azoospermia (48%) and oligospermia (36%) were the major causes of infertility.

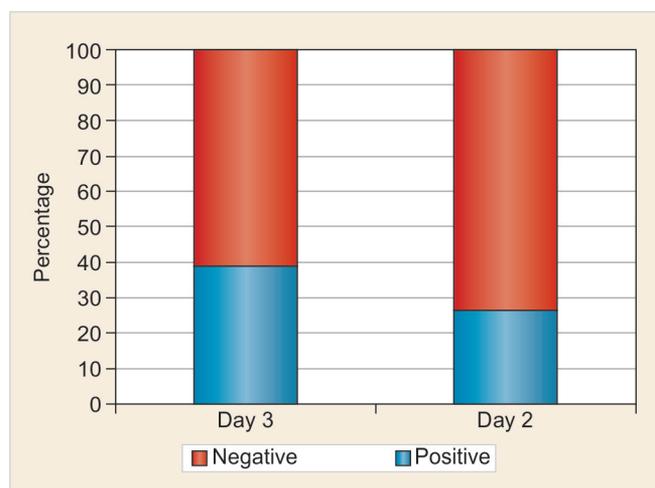
In our study, clinical pregnancy rate per ET cycle in day 3 ET was 45% (23/51) and in day 2 ET was 36.5% (19/52) (Graph 1). Odds ratio (OR) was 1.43 indicating clinical pregnancy rate in day 3 ET was just 1.43 times better than day 2 ET. The difference between clinical pregnancy rates of both groups was not statistically significant (p-value 0.49).

The ongoing pregnancy rate in day 3 ET group was 39.2% and in day 2 ET group was 26.9% (Graph 2). The OR was 1.73, indicating day 3 ET was 1.75 times better than day 2 ET with respect to ongoing pregnancy rate. But the difference was found to be statistically insignificant (p-value 0.26).

We observed that the miscarriage rate in day 3 ET group was 5.9% and in day 2 ET group was 5.8%. So



Graph 1: Clinical pregnancy rate per ET cycle in both groups



Graph 2: Ongoing pregnancy rate per ET cycle in both groups

Table 2: Compiled final outcome measures per ET cycle of both groups

| Final result | Group | | p-value | Difference | OR |
|------------------------------------|---------------------|---------------------|---------|------------|------|
| | Day 3 (51 patients) | Day 2 (52 patients) | | | |
| Clinical pregnancy | 23 (45%) | 19 (36.5%) | 0.49 | NS | 1.43 |
| Ongoing pregnancy | 20 (39.2%) | 14 (26.9%) | 0.26 | NS | 1.75 |
| Miscarriage (spontaneous abortion) | 3 (5.9%) | 3 (5.8%) | 0.69 | NS | 1.02 |
| Ectopic | 0 | 1 (1.9%) | NA | NA | NA |
| Fetal anomaly | 0 | 1 (1.9%) | NA | NA | NA |
| Multiple pregnancy | 0 | 1 (1.9%) | NA | NA | NA |

Statistical significance – if p-value \leq 0.05; NA: Not applicable; NS: Nonsignificant

they were almost similar with statistically insignificant difference (p-value 0.69, OR 1.02).

Multiple pregnancy rate was observed to be 1.92% (1/52) in day 2 ET group, while in day 3 ET group, all were singleton pregnancies. One case was found in day 2 ET group giving the ectopic pregnancy rate per ET cycle as 1.92%, while no case was found in day 3 ET group. In this study, we observed one case of fetal anomaly (anencephaly). In day 2 ET group deriving fetal anomaly rate per ET cycle was 1.92% (1/52). No cases of fetal anomaly were observed in day 3 ET group. Table 2 compiles the final outcome measures per ET cycle of both groups.

DISCUSSION

Clinical Pregnancy Rate

In our study, the clinical pregnancy rate per ET cycle in day 3 ET was 45% (23/51) and in day 2 ET was 36.5% (19/52). Odds ratio was 1.43, indicating clinical pregnancy rate in day 3 ET was just 1.43 times better than day 2 ET. The difference between clinical pregnancy rates of both groups was not statistically significant (p-value 0.49).

All the recent available studies also showed no significant difference between the two in terms of clinical pregnancy rate. However, some of these studies showed statistically insignificant, but still comparably better

clinical pregnancy rate in either of these two groups than the other one.

- Day 2 ET better than day 3 ET: Laverge et al³ – clinical pregnancy rate in day 2 ET (42.6%) is better than that in day 3 ET (28.8%). Cabar et al¹³ – day 2 ET clinical pregnancy rate was 40.3%, which is higher in comparison to day 3 ET of 32.8%. Odds ratio is 1.45, indicating day 2 ET was 1.45 times better than day 3 ET.
- Almost similar: Aboulghar et al¹⁴ – clinical pregnancy rates were almost similar between day 2 ET (50.9%) and day 3 ET (50.5%). Ashrafi et al²⁰ – clinical pregnancy rates were almost similar between day 3 ET (40.7%) and day 2 ET (38.9%). Mahdavi et al¹⁶ – day 3 ET clinical pregnancy rate was 18% and day 2 ET clinical pregnancy rate was 20%. Clinical pregnancy rate was shown to be higher in day 3 ET group (39.2%) compared to day 2 ET group (31.6%).
- Day 3 ET better than day 2 ET: Suzuki et al¹¹ – day 3 ET clinical pregnancy rate was 58.3% and day 2 ET clinical pregnancy rate was 37.5%. Meta-analysis (Cochrane review)¹² – in the ICSI trials, the study for clinical pregnancy rate gives result in favor of day 3 transfer [OR 1.40, 95% confidence interval (CI) 1.13–1.74]. The result of the chi-squared test for heterogeneity was 18.74 (p = 0.07). The subgroup analysis showed day

3 gave significantly better results than day 2 in case of ICSI than in case of IVF.

Ongoing Pregnancy Rate

In our study, the ongoing pregnancy rate in day 3 ET group was 39.2% and in day 2 ET group was 26.9%. The OR was 1.73, indicating day 3 ET was 1.73 times better than day 2 ET with respect to ongoing pregnancy rate. But the difference was found to be statistically insignificant (p-value 0.26).

Most of the other studies showed similar ongoing pregnancy rates in both groups with no statistically significant difference. Meta-analysis (Cochrane review)¹² – the meta-analysis did not provide evidence of a difference in ongoing pregnancy rate between day 3 and day 2 (OR 1.08, 95% CI 0.82–1.43). The result of the chi-squared test for heterogeneity was 9.71 ($p = 0.05$). Ertzeid et al²¹ was the only study to provide sufficient information in the published article to calculate the ongoing pregnancy rate (day 2 ET – 18.5%, day 3 ET – 22.6%); however, further information obtained from the authors allowed the inclusion of the Baruffi et al²² and Laverge et al³ trials for this outcome. Mahdavi et al¹⁶ showed day 3 ET ongoing pregnancy rate was 15.4% and day 2 ET ongoing pregnancy rate was 17%.

Miscarriage (Spontaneous Abortion) Rate

In our study, we observed that the miscarriage rate in day 3 ET group was 5.9% and in day 2 ET group was 5.8%. So they were almost similar with statistically insignificant difference (p-value = 0.69, OR = 1.02).

Literature reveals conflicting results with respect to miscarriage rate. Edwards et al²³ reported the incidence of miscarriages after day 3 ET to be higher. Mahdavi et al¹⁶ also reported higher miscarriage rate after day 3 ET (47.4%) compared with day 2 ET (29%). Laverge et al³ showed no significant difference between miscarriage rate of day 3 ET (8.3%) and day 2 ET (7.02%) groups. Meta-analysis (Cochrane review)¹² – the meta-analysis for the outcome miscarriage per woman did not provide evidence of a difference between day 3 and day 2 ET (OR 1.20, 95% CI 0.79–1.83). The result of the chi-squared test for heterogeneity was 6.82 ($p = 0.56$).

Multiple Pregnancy Rate

Our study showed that multiple pregnancy rate per ET cycle (%) was observed to be 1.92% (1/52) in day 2 ET group, while no case of multiple pregnancy was observed in day 3 ET group. Because our sample size is small, we cannot conclude that there is no risk of multiple pregnancy in day 3 ET group.

All the other studies showed that there was no significant difference between multiple pregnancy rate of two groups. Laverge et al³ reported multiple pregnancy rate per ET cycle in day 2 ET group derived was 16.6% and in day 3 ET group was 16.9%. Suzuki et al¹¹ in their study observed multiple pregnancy rate per ET cycle as 11.1% in day 2 group, while 14.3% in day 3 ET group. Aboulghar et al¹⁴ showed multiple pregnancy rate was 39.8% in day 2 ET group, while in day 3 ET group it was 31.9%. Meta-analysis (Cochrane review)¹² – the meta-analysis for the outcome multiple pregnancy per woman did not provide evidence of a difference between day 3 and day 2 transfer overall (OR 1.26, 95% CI 0.89–1.79). Mahdavi et al¹⁶ also showed similar rates (day 2 ET 9.7%, day 3 ET 7.5%).

Ectopic Pregnancy Rate

In our study, one case of ectopic pregnancy was found in day 2 ET group giving us ectopic pregnancy rate per ET cycle as 1.92%, while no case was found in day 3 ET group. The ectopic pregnancy was diagnosed on transvaginal ultrasound done on follow-up basis and was managed with conservative medical management with methotrexate. It is very difficult to confirm that there is no risk of ectopic pregnancy in day 3 ET or day 2 ET predisposing to ectopic pregnancy as sample size is small.

All other studies showed similar ectopic pregnancy rates in both groups. Laverge et al³ showed 0.5% ectopic pregnancy rate in day 2 ET group and 0.6% in day 3 ET group. Meta-analysis (Cochrane review)¹² – the meta-analysis did not provide evidence of a difference between day 3 and day 2 transfer (OR 0.99, 95% CI 0.24–3.99). The result of the chi-squared test for heterogeneity was 0.62 ($p = 0.73$). Mahdavi et al¹⁶ also showed similar rates (day 2 ET 6.5%, day 3 ET 5%).

Fetal Anomaly Rate

Limited evidence suggests that the prevalence of chromosomal abnormalities in children conceived with ART is not different from that in children conceived naturally. Nonetheless, concerns persist that the use of sperm from infertile men, and ICSI itself, might increase the risk for conceiving the child with chromosomal or genetic defect, because infertile men and women are more likely to have chromosomal abnormality.²⁴

In this study, we observed one case of fetal anomaly in day 2 ET group deriving fetal anomaly rate per ET cycle as 1.92% (1/52). The anomaly detected was anencephaly (neural tube defect) in case of mothers 38 years of age, which was picked up on 11 weeks early anomaly scan and induced abortion was done with proper documentation and valid informed consent. No case of fetal anomaly (0%) was detected in day 3 ET group. There is increased risk of

neural tube defects in very young mothers and in mothers 30 to 40 years or higher age. Also, it is related to many etiologies including folic acid deficiency, etc. In addition, sample size is also small. So we cannot conclude that day 2 ET is associated with more incidence of fetal anomalies and day 3 ET does not carry the risk of fetal anomaly at all. No recently available studies have compared fetal anomaly rate in these two groups.

It can be argued that a delay of 1 day is too short to better differentiate the quality of embryos. In recent years, therefore, a more extended delay of ET up to the blastocyst stage has been tried by several investigators. With blastocyst transfer, some reported higher (56%) clinical pregnancy rates²⁵ while some reported similar (38%) clinical pregnancy rates²⁶ as compared to our study. But blastocyst transfer risks the loss of embryos during prolonged culture and a lower number of blastocysts available for freezing.²⁷ So, blastocyst transfer is upcoming intervention in ART, but still day 2/day 3 ET is a promising technique practiced worldwide.

We have included patients who have AMH value of 2 to 3.5, i.e., normo-responders but the results may vary in poor responders. One study by Shahine et al²⁸ showed lower clinical pregnancy rates in both transfer groups of poor responders (day 2 ET 15.4%, day 3 ET 16.4%) than normo-responders, but showed no difference between day 2 ET group and day 3 ET group.

LIMITATIONS AND RECOMMENDATIONS

Although our study evaluates clinical and ongoing pregnancy rates as outcome measures, live birth rate is most reliable indicator of successful outcome of IVF/ICSI and ET as per the literature. But since our study period duration was short, i.e., 1 year, and patients were lost to follow-up till term, there were limitations for calculating live birth rate. So, similar study with inclusion of live birth rate parameter studied for longer duration of time is recommended. Since the number of patients in our study, i.e., the sample size was small, similar study with large sample size studied for longer duration of time are recommended. These studies will be more confirmatory for comparison of day 2 ET and day 3 ET. Since the sample size was small, we got very less number of cases of multiple pregnancy, ectopic pregnancy, fetal anomaly and we could not comment conclusively on comparison of these rare parameters. So, studies with large sample size studied for longer duration of time, including comparison of these parameters like multiple pregnancy rate, ectopic pregnancy rate and fetal anomaly rate, are recommended. Our study includes normo-responders (AMH value: 2–3.5 ng/mL) with mostly favorable factors (no history of previous failed cycles, fresh ET in the same cycle, age

≤40 years, adequate number of good quality embryos available). The results with poor or high responders with/without unfavorable factors (previous failed cycles, less number/poor quality embryos available, age >40 years) may vary. So studies including these subjects are recommended.

CONCLUSION

In conclusion, IVF/ICSI and ET can be addressed to almost all causes of infertility and in our study, we observed that almost all the causes of infertility including female factor infertility, male factor infertility, combined male and female factor infertility, and unexplained infertility underwent IVF/ICSI and ET treatment. Our study demonstrates that OR in day 3 ET and day 2 ET for clinical pregnancy rate was 1.43 and for ongoing pregnancy rate was 1.75, indicating chances that day 3 ET appears to allow selection of more viable embryos than day 2 ET. But the difference is not statistically significant. In day 3 ET group and day 2 ET group, similar miscarriage (spontaneous abortion) rates were seen. Our study showed very low incidence of complications associated with IVF:ICSI and ET like multiple pregnancy, ectopic pregnancy, fetal anomaly in day 2 ET group, and no cases of above-mentioned parameters in day 3 ET group. So, it is safe to schedule and transfer embryos either on day 2 or on day 3 for planning and programming cycles in coordination with patient and IVF team and for adjusting weekends (nonworking days). Since the number of patients in our study, i.e., the sample size is small, larger sample studied for longer duration will be more confirmatory and concluding.

CLINICAL SIGNIFICANCE

Despite development in culture media allowing blastocyst transfer, many centers still practice day 2/3 ET. Over the period of past many years, many steps of IVF procedure became standardized. However, the optimum timing of ET is still debatable. Several studies comparing ET on day 2 vs day 3 after oocyte retrieval have been performed, but the conclusions are conflicting. More research is needed on this controversial topic, which definitely has impact on final outcome of ARTs. Our study attempts to throw some light on this important topic.

REFERENCES

1. Farquhar C, Rishworth JR, Brown J, Nelen WL, Marjoribanks J. Assisted reproductive technology: an overview of Cochrane Reviews. *Cochrane Database Syst Rev* 2014;12:CD010537.
2. Steptoe PC, Edwards RG. Reimplantation of a human embryo with subsequent tubal pregnancy. *Lancet* 1976 Apr;1(7965):880-882.

3. Laverge H, De Sutter P, Van der Elst J, Dhont M. A prospective, randomized study comparing day 2 and day 3 embryo transfer in human IVF. *Hum Reprod* 2001 Mar;16(3):476-480.
4. Kovacic B, Vlasisavljevic V, Reljic M, Gavric Lovrec V. Clinical outcome of day 2 versus day 5 transfer in cycles with one or two developed embryos. *Fertil Steril* 2002 Mar;77(3):529-536.
5. Braude P, Bolton V, Moore S. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. *Nature* 1988 Mar;332(6163):459-461.
6. De Placido G, Wilding M, Stina I, Mollo A, Alviggi E, Tolino A, Colacurci N, De ML, Marino M, Dale B. The effect of ease of transfer and type of catheter used on pregnancy and implantation rates in an IVF program. *J Assist Reprod Genet* 2002 Jan;19(1):14-18.
7. Huisman GJ, Fauser BC, Eijkemans MJ, Pieters MH. Implantation rates after in vitro fertilization and transfer of a maximum of two embryos that have undergone three to five days of culture. *Fertil Steril* 2000 Jan;73(1):117-122.
8. Desai NN, Goldstein J, Rowland DY, Goldfarb JM. Morphological evaluation of human embryos and derivation of an embryo quality scoring system specific for day 3 embryos: a preliminary study. *Hum Reprod* 2000 Oct;15(10):2190-2196.
9. Dawson KJ, Conaghan J, Ostera GR, Winston RM, Hardy K. Delaying transfer to the third day post-insemination, to select non-arrested embryos, increases development to the fetal heart stage. *Hum Reprod* 1995 Jan;10(1):177-182.
10. Nikas G. Endometrial receptivity: changes in cell-surface morphology. *Semin Reprod Med* 2000;18(3):229-235.
11. Suzuki T, Shibahara H, Hirano Y, Ohno A, Takamizawa S, Suzuki M. Randomized study comparing day 2 versus day 3 elective transfer of two good-quality embryos. *Reprod Med Biol* 2004 Jun;3(2):99-104.
12. Gunby JL, Daya S, Olive D, Brown J. Day three versus day two embryo transfer following in vitro fertilization or intracytoplasmic sperm injection. *Cochrane Database Syst Rev* 2004;2:CD004378.
13. Cabar FR, Duarte Filho OB, Soares JB, Mizhari FE, Busso NE, Tognotti E. Embryo transfer day 2 and day 3: comparison of pregnancy rates of IVF cycles of normal responder patients with few available embryos. *Fertil Steril* 2009 Sep;92(3):S78.
14. Aboulghar MM, Aboulghar MA, Mansour RT, Serour GI, Amin YM, Abou-Setta AM. Pregnancy rate is not improved by delaying embryo transfer from days 2 to 3. *Eur J Obstet Gynecol Reprod Biol* 2003 Apr;107(2):176-179.
15. Kiani K, Ashrafi M, Madani T, Mirzaagha E, Shabani F. The pregnancy outcomes of day 2 versus day 3 embryo transfer: a cross-sectional study. *Reprod Biomed Online* 2010 Oct;20:S73.
16. Mahdavi A, Qashqaei A, Aleyasin A, Aghahosseini M, Safdarian L, Rezaeean Z, Fallahi P. Embryo transfer in days 2 to 4 following intracytoplasmic sperm injection: a prospective cohort study. *Med J Islam Republ Iran* 2015 Sep;29:262.
17. Rao, KA.; Carp, H, editor. *The infertility manual*. 3rd ed. New Delhi: Jaypee Brothers; 2009.
18. Gardner, DK.; Weissman, A.; Howles, CM.; Shoham, Z. *Textbook of assisted reproductive techniques*. 4th ed. Nashville (TN): Pathenon Publishing Group; 2012.
19. Misra A, Chowbey P, Makkar BM, Vikram NK, Wasir JS, Chadha D, Joshi SR, Sadikot S, Gupta R, Gulati S, et al. Consensus statement for diagnosis of obesity, abdominal obesity and the metabolic syndrome for Asian Indians and recommendations for physical activity, medical and surgical management. *J Assoc Physicians India* 2009 Feb;57:163-170.
20. Ashrafi M, Kiani K, Mirzaagha E, Shabani F. The pregnancy outcomes of day 2 versus day 3 embryo transfer: a cross-sectional study. *Iran J Fertil Steril* 2007;1(2):47-54.
21. Ertzeid G, Dale PO, Tanbo T, Storeng R, Kjekshus E, Abyholm T. Clinical outcome of day 2 versus day 3 embryo transfer using serum-free culture media: a prospective randomized study. *J Assist Reprod Genet* 1999 Nov;16(10):529-534.
22. Baruffi RL, Mauri AL, Petersen C, Felipe V, Franco Júnior JG. Day 2 vs. day 3 embryo transfer after intracytoplasmic sperm injection. A prospective, randomized study. *The J Reprod Med* 2003 Aug;48(8):631-634.
23. Edwards RG, Fishel SB, Cohen J, Fehilly CB, Purdy JM, Slater JM, Steptoe PC, Webster JM. Factors influencing the success of in vitro fertilization for alleviating human infertility. *J In Vitro Fertil Embryo Transf* 1984 Mar;1(1):3-23.
24. Suganya J, Kujur SB, Selvaraj K, Suruli MS, Haripriya G, Samuel CR. Chromosomal abnormalities in infertile men from Southern India. *J Clin Diagn Res* 2015 Jul;9(7):GC05-GC10.
25. Rienzi L, Ubaldi F, Iacobelli M, Ferrero S, Minasi MG, Martinez F, Tesarik J, Greco E. Day 3 embryo transfer with combined evaluation at the pronuclear and cleavage stages compares favourably with day 5 blastocyst transfer. *Hum Reprod* 2002 Jul;17(7):1852-1855.
26. Jones GM, Trounson AO, Gardner DK, Kausche A, Lolatgis N, Wood C. Evolution of a culture protocol for successful blastocyst development and pregnancy. *Hum Reprod* 1998 Jan;13(1):169-177.
27. Cahill DJ, Wardle PG. Management of infertility. *BMJ* 2002 Jul;325(7354):28-32.
28. Shahine LK, Milki AA, Westphal LM, Baker VL, Behr B, Lathi RB. Day 2 versus day 3 embryo transfer in poor responders: a prospective randomized trial. *Fertil Steril* 2011 Jan;95(1):330-332.