

DOI: <https://dx.doi.org/10.18203/2320-1770.ijrcog20233638>

Original Research Article

## Comparison between sequential transfer vs. day 3 and day 5 frozen embryo transfer in IVF patients

Rohan Palshetkar<sup>1</sup>, Mayuri More<sup>1\*</sup>, Nandita Palshetkar<sup>2</sup>, Hrishikesh Pai<sup>2</sup>,  
Rishma Pai<sup>2</sup>, Arnav Pai<sup>2</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, D. Y. Patil Medical College, Navi Mumbai, Maharashtra, India

<sup>2</sup>Bloom IVF, Mumbai, Maharashtra, India

**Received:** 09 October 2023

**Revised:** 06 November 2023

**Accepted:** 07 November 2023

**\*Correspondence:**

Dr. Mayuri More,

E-mail: mayurimore1512@gmail.com

**Copyright:** © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

### ABSTRACT

**Background:** Sequential embryo transfer is when both cleavage-stage embryo is transferred on day 3 and blastocyst is transferred on day 5, sequentially in the same cycle. This has been suggested for increasing embryo implantation rate. Sequential transfer gives benefit of both day 3 as well as day 5 transfer in the same cycle, giving better outcome in patients suffering infertility. This study compares the implantation rates in sequential transfer vs Day 3 and day 5 transfers.

**Methods:** This multi-centric study is a retrospective study conducted over a period of one year at D. Y. Patil Fertility Centre, Navi Mumbai. Total of 432 transfers were conducted in patients, out of which 262 were Day 3 or cleavage stage embryo transfer, 109 were Day 5 or blastocyst embryo transfer and 61 were sequential embryo transfer.

**Results:** Day 3 transfer group had the clinical pregnancy rate of 52.67%, whereas day 5 transfer group had 60.55% of clinical pregnancy positive cases. Sequential embryo transfer had implantation rate of 60.66%, which was slightly higher than day 5 (60.55%) and day 3 (52.67%) implantation rates.

**Conclusions:** Sequential transfer has marginally increased rate of implantation and clinical pregnancy when compared to day 5 and day 3 transfers.

**Keywords:** Blastocyst transfer, Implantation rate, Sequential transfer, ART, Embryo transfer

### INTRODUCTION

Assisted reproductive technology (ART) is the most important choice for a couple with subfertility. Stimulation protocols and laboratory techniques have developed massively over the last three decades, resulting in better quantity and quality of embryos and ultimately the success of the process. Implantation of the embryo is the end product of a series of complex process which require high potential embryos, good endometrial receptive and ultimately effective interaction between the embryo and the endometrium.<sup>1,2</sup> One of the most crucial steps during

ART cycle is transfer of embryo from the laboratory to the uterus. Conventionally, cleavage-stage embryos were transferred on day 3; however, over the past decade there has been a switch to transfer day 5 blastocysts. Blastocyst stage transfer mimics a physiologically right time of natural implantation and also increases synchrony between the development of the embryo and endometrium. Use of blastocyst transfers in ART practices has been reinforced by current evidence. According to the studies, the clinical pregnancy and live birth rates were higher in women opting for blastocyst transfers when compared to those undergoing cleavage-stage transfers.<sup>3</sup> However, there might be decrease in quantity of viable embryos for

embryo transfer or cryopreservation because of the prolonged time for culture to the blastocyst stage. Hence, to avoid cycle cancellation, both cleavage stage embryos on day 3 and blastocyst on day 5 are transferred in sequential embryo transfer. However, IVF-ET doesn't have an ideal outcome as the implantation rate remains 25-40%.<sup>3</sup>

Repeated implantation failure (RIF) refers to failure of implantation in three consecutive attempts in which 1-2 embryos of good quality are transferred.<sup>4</sup> The factors affecting implantation generally are uterine anomalies, age, immune, thrombophilia, genetics, embryo, male, IVF protocol factors and so on. With the recent developments in techniques to culture, embryo can be grown to the blastocyst stage *in vitro* and then transferred on day 5. When compared to cleavage stage embryos, prolonging embryo culture to blastocysts provides selection of higher quality embryos for transfer, and promotes synchronization between the embryo and endometrium, inturn making implantation rate (IR) and clinical pregnancy rate (CPR) of blastocyst transfer higher. Although, it comes with a drawback of possible cancellation of transfer due to failure of progression of embryo to the blastocyst stage for patients with fewer good-quality embryos.<sup>5</sup>

The regulation of embryonic genome is activated after Day 3 (Cleavage stage). But, the grading and selection is done on the basis of morphology alone.<sup>6</sup> Prolonging the time of culture and letting the embryos grow till Day 5 (Blastocyst stage) gives way to natural selection leading to better quality and higher implantation potential of embryos but, at the risk of cycle cancellation and hence, is not suitable for patients with fewer embryos.<sup>7,8</sup> Sequential transfer was introduced to overcome the draw backs and theoretically provide patient the benefit of both the procedures simultaneously.<sup>2</sup>

## METHODS

This study is a retrospective study of the data collected over 1 years comparing the implantation rates of sequential transfer with day 3 and day 5 transfer in patients.

### Patient selection

We conducted a single-center, retrospective study at D. Y. Patil Fertility Centre, Navi Mumbai, India over a period of one year from 1 April 2022 to 31 March 2023. Total of 432 frozen embryo transfers were conducted in patients, out of which 262 were Day 3 (cleavage stage) embryo transfer, 109 were Day 5 (blastocyst embryo) transfer and 61 were sequential embryo transfer. Patients with primary infertility and previous one implantation failure were offered day 3 and day 5 transfers randomly. Whereas, patients with RIF (more than 2 previous implantation failures) were offered sequential transfer.

### Embryo selection and transfer

Embryo transfer was done with only good quality embryos. Two good quality embryos and blastocyst were transferred in day 3 as well as in day 5 group respectively. One day 3 embryo and one blastocyst were transferred in the sequential group.

20 µl of media was used with a soft transfer catheter under ultrasound guidance for the embryo transfers. In the this study, we transferred two embryos in each group, since two embryos are needed in the sequential media group. Luteal phase support was given with vaginal progesterone, 200 mg twice daily or injectable progesterone 100 mg intramuscular, which was started three days prior to frozen embryo transfer day in day 3 group, five days prior to the transfer day in day 5 group and continued for 12 weeks of gestation, if pregnancy was confirmed.

### Outcome measurement

The primary outcome measure were implantation rate and clinical pregnancy rate. Serum Beta HCG test was performed 14 days after embryo transfer and repeated on the 16<sup>th</sup> day for confirmation and doubling. Ultrasound examination was performed at week 6-7 of gestation (about 4 weeks after transfer) to assess number of fetal sac and heartbeat. Implantation rate was defined as the number of gestational sacs seen on the ultrasound, divided by the total number embryos/blastocyst transferred. It was calculated for all patients undergoing the embryo transfer and not only the patients who were pregnant. Clinical pregnancy was defined as the presence of a fetal heartbeat on ultrasound examination at 6-7 weeks of pregnancy. Spontaneous miscarriage was defined as a clinical pregnancy loss before 20 weeks of gestational age.

### Statistical analysis

Data was entered into Microsoft Excel and statistical analysis was carried out in SPSS software version 17.0. Age, type of fertility, cause of infertility and number of IVF attempts were converted into categories and reported as percentages. Qualitative variables like type and cause of infertility, D3, D5 and sequential transfer, pregnancy outcomes, number of IVF attempts were presented as proportions. Bar diagrams were used for graphical representation of data. The association between the age categories, type of infertility, cause of infertility, d3, D5 and sequential transfer with live birth was done. A p value of less than 0.05 was considered as statistically significant.

## RESULTS

The basic demographic characteristics included age of the study population, type of infertility, cause of infertility, number of IVF attempts and levels of serum anti Mullerian Hormone (AMH) and number of antral follicular counts, which is tabulated in below (Table 1). Mean age ( $\pm$ SD) for day 5 transfer group (33.99 $\pm$ 6.64 years) for slighter higher than mean age ( $\pm$ SD) for day 3 transfer group (31.76 $\pm$ 5.50 years), whereas mean age ( $\pm$ SD) for sequential transfer

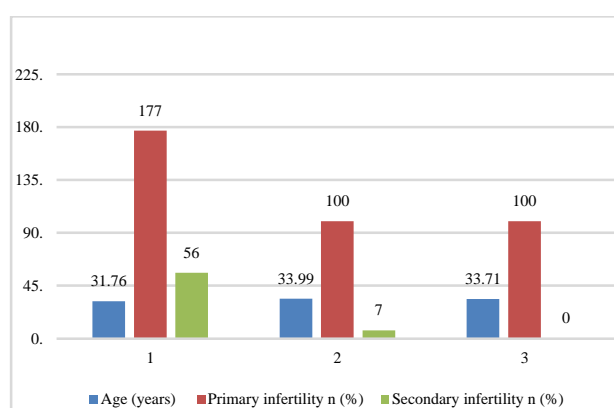
group was 33.71±5.31 years. In the study period, the center conducted total of 432 frozen embryo transfers. 121 patients out of 262 patients who underwent day 3 transfer

were tested negative, leading to an implantation rate of 53.81 % in the Day 3 transfer group.

**Table 1: Demographic data of the three study groups.**

Parameters	Day 3	Day 5	Sequential	P value	
<b>Age (years) (Mean±SD)</b>	31.76±5.50	33.99±6.64	33.71±5.31	NA	
<b>Type of infertility, N (%)</b>					
Primary infertility	177 (68.08)	22 (20.37)	14 (35.90)	NA	
Secondary infertility	56 (21.54)	7 (6.48)	0 (0)		
<b>Cause of infertility, N (%)</b>					
Endometriosis	15 (5.73)	5 (4.59)	8 (13.11)	0.0007	
PCOS	81 (30.92)	15 (13.76)	11 (18.03)		
Thin endometrium	3 (1.15)	2 (1.83)	1 (1.64)		
Fibroids	19 (7.25)	14 (12.84)	10 (16.39)		
Hypothyroidism	7 (2.67)	5 (4.59)	4 (6.56)		
Ovarian cysts	42 (16.03)	11 (10.09)	5 (8.20)		
Tubal blockage	19 (7.25)	6 (5.50)	4 (6.56)		
Oligospermia	11 (4.20)	10 (9.17)	8 (13.11)		
Teratozoospermia	9 (3.44)	7 (6.42)	2 (3.28)		
Azoospermia	26 (9.92)	19 (17.53)	4 (6.56)		
Unexplained infertility	30 (11.45)	15 (13.76)	4 (6.56)		
Total	262 (100)	109 (100)	61 (100)		
<b>Number of IVF attempts, N (%)</b>					
First attempt	214 (81.68)	86 (78.90)	0 (0)		0.43
Second Attempt	48 (18.32)	23 (21.10)	0 (0)		
Third Attempt	0 (0)	0 (0)	36 (59.02)		
Fourth attempt	0 (0)	0 (0)	15 (24.59)		
Fifth attempt	0 (0)	0 (0)	10 (16.39)		

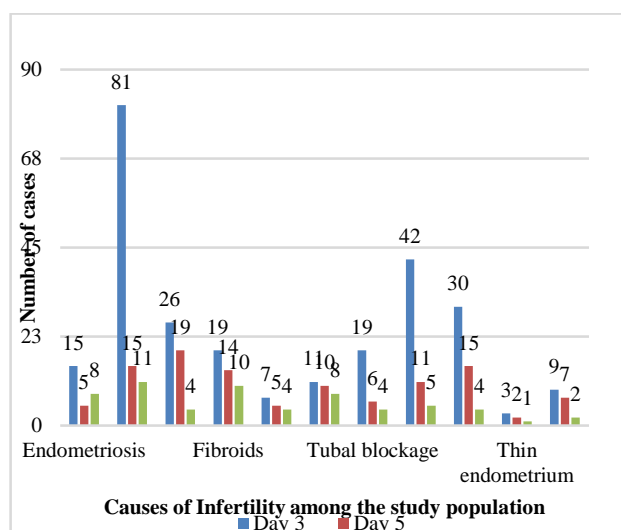
In the Day 5 transfer group, 43 patients out of the 109 patients tested negative, leading to an implantation rate of 60.55%. Below mentioned (Figure 1-3) graphically represents the basic demographics involving study population, list of causes of infertility among the study population and number of IVF attempts among the study population.



**Figure 1: Basic demographics involving study population.**

As per the below (Table 2), the sequential transfer group consisting of 61 patients had 23 patients who tested negative, leading to implantation rate of 60.66%, giving a

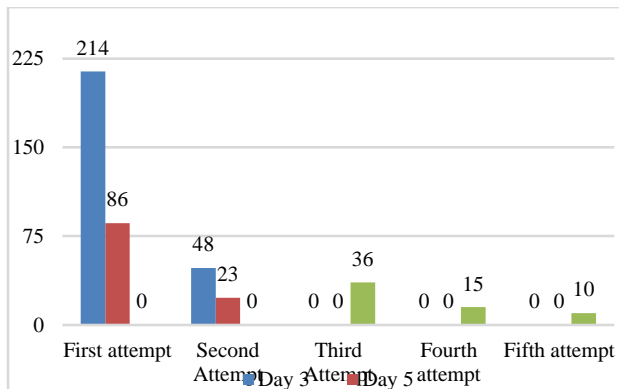
marginally better implantation rate in the sequential transfer group.



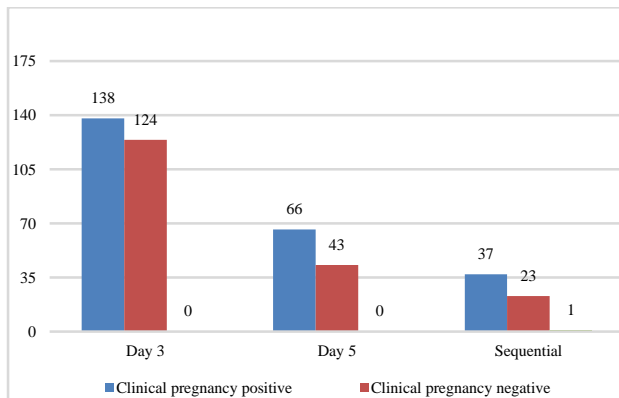
**Figure 2: List of causes of infertility among the study population.**

A single case of ectopic pregnancy positive was recorded in sequential transfer group (1.64%). Day 3 transfer group recorded the highest number (47.33%) of clinical pregnancy negative cases whereas day 5 transfer group recorded the second highest number (60.55%) of clinical

pregnancy positive cases. Below (Figure 4) graphically represents the Clinical outcomes of the three study groups.



**Figure 3: The number of IVF attempts among the study population.**



**Figure 4: The clinical outcomes of the three study groups.**

**Table 2: Comparison of outcomes for the 3 groups.**

Outcomes, N (%)	Day 3 transfer	Day 5 transfer	Sequential transfer	P value
<b>Clinical pregnancy positive</b>	138 (52.67)	66 (60.55)	37 (60.66)	0.01
<b>Clinical pregnancy negative</b>	124 (47.33)	43 (39.45)	23 (37.70)	
<b>Ectopic pregnancy positive</b>	0	0	1 (1.64)	

**DISCUSSION**

Basis of implantation depends on two important factors: the seed (healthy embryo) and the soil (good endometrium). The interactions between these two lead to successful implantation and placentation.<sup>4</sup> Previously, Day 3 (Cleavage stage) transfer was considered the gold standard. This was due to difficulties in maintaining the embryo in the laboratory for more than forty-eight hours and also because the uterus was considered a natural

incubator for the embryos. There also was fear of cycle cancellation and loss of embryo due to failure of progression of the embryo. This leads to negative emotional, financial and legal impact on both the patient and IVF centre.<sup>9</sup> According to a Cochrane meta-analysis, the cumulative pregnancy rates were lower in blastocyst transfer when compared to cleavage stage transfer due to the reduced number of frozen embryos available.<sup>10</sup> Developments in the culture techniques and use of sequential media has now made the growth of embryo to Day 5 (Blastocyst stage) easier.<sup>11</sup> This has resulted in availability of more number of blastocyst, leading subsequently to increased rates of implantation compared to cleavage stage.<sup>12</sup> Blastocyst transfer provides availability of more natural environment for implantation and closer resemblance to a natural cycle.<sup>13</sup> They also provide euploid status when compared to cleavage stage embryos.<sup>14</sup> Therefore, blastocyst transfer not only gives higher quality embryos but, also provides better endometrial receptivity and is capable of achieving the “implantation window”.<sup>4</sup> In certain cases, where couples face recurrent implantation failure, “sequential” transfer in the same cycle is proposed. This gives advantage of both Day 3 as well as Day 5 transfer in the same cycle and much less chance of cycle cancellations.<sup>15</sup> Some studies have indicated that an absence of endometrial receptivity causes failure in embryo implantation in two-thirds of cases. The endometrium is receptive to the embryo for a specific period recognized as the window of implantation (WOI). A displacement of the WOI during the mid-luteal phase is reported in 25 to 30% of patients with RIF by some researchers. That is where a sequential transfer plays a role. It may overcome the problem with choosing the right moment for embryo transfer. The chance of hitting the WOI may also increase in sequential transfer, which is only open for 2 to 4 days.<sup>16,17</sup> Although, the previous studies show debatable data about sequential transfer.<sup>18-23</sup> Older studies show better pregnancy rates with sequential transfer but, recent studies show non-significant difference in pregnancy rates between single transfer and sequential transfer.

**Limitations**

The main limitation of this study was the failure to evaluate live birth rate.

**CONCLUSION**

To conclude, in women with RIF, sequential transfer has a higher implantation rate and clinical pregnancy rate when compared to day 3 and day 5 transfer, however it was not statistically significant. It provides the benefits of both procedures to the patients and at the same time nullifying the possibility of cycle cancellation that can occur in only Day 5 transfers. Sequential embryo transfer could be considered as an alternative approach for improving IVF outcomes in RIF patients. However more studies need to be conducted to analyze this procedure further.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

## REFERENCES

1. Fukui A, Funamizu A, Yokota M. Uterine and circulating natural killer cells and their roles in women with recurrent pregnancy loss, implantation failure and preeclampsia. *J Reprod Immunol.* 2011;90(1):105-10.
2. Simon A, Laufer N. Assessment and treatment of repeated implantation failure (RIF). *J Assist Reprod Genet.* 2012;29(11):1227-39.
3. Graham ME, Jelin A, Hoon AH, Wilms Floet AM, Levey E, Graham EM. Assisted reproductive technology: Short-and long-term outcomes. *Develop Med Child Neurol.* 2023;65(1):38-49.
4. Simon A, Laufer N. Assessment and treatment of repeated implantation failure (RIF) *J Assist Reprod Genet.* 2012;29:1227-39.
5. Awonuga AO, Camp OG, Abu-Soud HM, Rappolee DA, Puscheck EE, Diamond MP. Determinants of Embryo Implantation: Roles of the Endometrium and Embryo in Implantation Success. *Reprod Sci.* 2023; 29:1.
6. Lu X, Liu S, Dong X. Research progress of two-step sequential embryo transfer strategy in in vitro fertilization-embryo transfer cycles. *Chin J Reprod Contracept.* 2018;38(4):333-6.
7. Guerif F, Bidault R, Gasnier O. Efficacy of blastocyst transfer after implantation failure. *Reprod Biomed Online.* 2004;9(6):630-6.
8. Levitas E, Lunenfeld E, Har-Vardi I. Blastocyst-stage embryo transfer in patients who failed to conceive in three or more day 2-3 embryo transfer cycles: a prospective, randomized study. *Fertil Steril.* 2004; 81(3):567-71.
9. Marek D, Langley M, Gardner DK, Confer N, Doody KM, Doody KJ. Introduction of blastocyst culture and transfer for all patients in an in vitro fertilization program. *Fertil Steril.* 1999;72:1035-40.
10. Glujovsky D, Farquhar C, Retamar AM, Sedo CR, Blake D. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. *Cochrane Database Syst Rev.* 2016;6:CD002118.
11. Gardner DK, Vella P, Lane M, Wagley L, Schlenker T, Schoolcraft WB. Culture and transfer of human blastocysts increases implantation rates and reduces the need for multiple embryo transfers. *Fertil Steril.* 1998;69(1):84-8.
12. Nadkarni PK, Nadkarni KM, Singh PP, Singh P, Nadkarni A, Kalyani KR, et al. A comparative study of pregnancy outcome of sequential versus day 3 versus only blastocyst (day 6) transfer at a single IVF center over one year. *Int J Reprod Contracept Obstet Gynecol.* 2015;4:2032-5.
13. Haitham T, Ali A, Ahmed H, El-Sayed D, Rania A, Mona R, et al. Comparing sequential vs day 3 vs day 5 embryo transfers in cases with recurrent implantation failure: randomized controlled trial. *JBRA Assist Reprod.* 2021;25(2):185-92.
14. Dalal R, Mishra A, Pai HD, Palshetkar N. A prospective trial comparing sequential day 3/day 5 transfer with cleavage stage transfer and blastocyst stage transfer. *IVF Lite.* 2015;2:30-6.
15. Goto S, Takebayashi K, Shiotani M, Fujiwara M, Hirose M, Noda Y. Effectiveness of 2-step (consecutive) embryo transfer. Comparison with cleavage-stage transfer. *J Reprod Med.* 2003;48:370-4.
16. Craciunas L, Gallos I, Chu J, Bourne T, Quenby S, Brosens JJ, et al. Conventional and modern markers of endometrial receptivity: a systematic review and meta-analysis. *Human reproduction update.* 2019;25(2):202-23.
17. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. *Fertil Steril.* 2011;96(2):344-8.
18. Phillips SJ, Dean NL, Buckett WM, Tan SL. Consecutive transfer of day 3 embryos and of day 5-6 blastocysts increases overall pregnancy rates associated with blastocyst culture. *J Assist Reprod Genet.* 2003;20:461-4.
19. Levron J, Shulman A, Bider D, Seidman D, Levin T, Dor J. A prospective randomized study comparing day 3 with blastocyst- stage embryo transfer. *Fertil Steril.* 2002;77:1300-1.
20. Blake DA, Farquhar CM, Johnson N, Proctor M. Cleavage stage versus blastocyst stage embryo transfer in assisted conception. *Cochrane Database Syst Rev.* 2007;4:CD002118.
21. Abramovici H, Dirnfeld M, Weisman Z, Sorokin Y, Lissak A, Rofe A, et al. Pregnancies following the interval double-transfer technique in an in vitro fertilization- embryo transfer program. *J In Vitro Fert Embryo Transf.* 1988;5:175-6.
22. Al-Hasani S, van der Ven H, Diedrich K, Reinecke A, Hartje H, Krebs D. Effect of sequential embryo transfer on pregnancy following in vitro fertilization. *Geburtshilfe Frauenheilkd.* 1990;50:640-3.
23. Ashkenazi J, Yoeli R, Orvieto R, Shalev J, Ben-Rafael Z, Bar-Hava I. Double (consecutive) transfer of early embryos and blastocysts: aims and results. *Fertil Steril.* 2000;74:936-40.

**Cite this article as:** Palshetkar R, More M, Palshetkar N, Pai H, Pai R, Pai A. Comparison between sequential transfer vs. day 3 and day 5 frozen embryo transfer in IVF patients. *Int J Reprod Contracept Obstet Gynecol* 2023;12:3583-7.