ORIGINAL RESEARCH

A Study to evaluate the Implantation and Clinical Pregnancy Rates in Patients undergoing Sequential frozen Day 3 Embryo and Day 5 Blastocyst transfer

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Abstract

Background: Sequential transfer is a technique in which cleavage stage embryo(s) and blastocyst(s) are transferred sequentially in the same cycle. It prevents the potential disadvantage of cycle cancellation due to the nonformation of a blastocyst. The present study is undertaken to evaluate the implantation and clinical pregnancy rates in patients undergoing sequential transfer and to assess the success rates in different categories of patients.

Materials and methods: A retrospective multicentric case series study was done on patients undergoing intracytoplasmic sperm injection (ICSI) cycles at GarbhaGudi IVF centers in Bengaluru. A total of 155 women had undergone frozen embryo sequential transfer of day 3 embryos and day 5 blastocysts. During frozen embryo transfer (ET), conventional hormone replacement therapy (HRT) downregulated HRT, and mild stimulation protocols were used to prepare the endometrium. Adequate luteal phase support was given for 14 days, and pregnancy was confirmed by doing serum human chorionic gonadotropin (hCG).

Results: The majority underwent HRT (76.13%) protocol. The average endometrial thickness on the day of the hCG administration was 8.68 ± 1.13. The clinical pregnancy rate was 67.1 with 73.34% singletons, 22.86% twins, and 3.81% triplets. The study reported 57.14% ongoing pregnancies, 34.28% live births, and only 20% abortions.

Conclusion: Sequential transfer resulted in better clinical pregnancy and birth rates. It also had the advantage of blastocyst transfer without exposing the whole cycle to the risk of cancellation.

Keywords: Clinical pregnancy rate, Embryo transfer, Sequential transfer.

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Introduction

The ET plays an indispensable role in assisted reproductive technology (ART).[1] It mainly depends on the skill of the embryologist and fertility specialist. The study has shown that the first clinical pregnancy was with blastocyst transfer, and subsequently, studies have proven that 80% of the clinical pregnancy rates were due to blastocyst.[2,3] The concept of single ET was from the blastocyst culture, which prevented multiple births. In comparison with the cleavage stage embryo, the blastocyst transfer results in better synchrony with the endometrium, confirms the euploidy status, causes lesser uterine contractility, and has higher implantation potential.[4] The major drawback of relying on blastocyst transfer is the possible situation of cancellation due to embryonic arrest where the embryos fail to proceed to the blastulation.

To avoid this disastrous consequence a "sequential ET" was proposed in which cleavage stage embryos and blastocysts are transferred successively in the same cycle.[5] This approach can have the potential advantages of blastocyst transfer with the insurance against possible cancellation by having a cleavage stage ET.[6] Published data on sequential ET are limited, therefore the present study was undertaken to assess the success rates in different categories of patients.

Materials and Methods

A multicentric retrospective study was conducted at GarbhaGudi IVF centers in Bengaluru from January 2019 to October 2020, which involved patients who underwent sequential ET. The present study was conducted after receiving approval from the Institutional Ethical Committee (GEC/GGIRH19_8/26052020). The study included patients with[1] good endometrium thickness (>8 mm),[2] at least three good quality day 3 frozen embryos,[3] recurrent *in vitro* fertilization (IVF) failures, and[4] failed day 3 ET. Women with endometriosis, immune diseases, recurrent abortion, and embryos created with donor sperm or oocyte, uterine abnormalities such as fibroid uterus, previous uterine surgeries, ovarian cysts, hydrosalpinx, known contraindications or allergies to oral estradiol

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or progesterone therapy, poor oocyte and embryo quality, and difficult or traumatic transfers were excluded. The sample size was calculated considering the expected proportion of 48.5% of the clinical pregnancy rate, 8% absolute precision, and a 95% confidence level,[5] and was found to be 150. Therefore, the present study included data of 155 couples. During the scheduled period, 155 women underwent sequential ET. The present study also included demographic details such as maternal age, basal follicle-stimulating hormone (FSH) concentrations, type of infertility, ovarian stimulation protocols, number of oocytes retrieved, number of previous IVF cycles, and number of embryos transferred. All subjects underwent controlled ovarian stimulation using the antagonist protocol.

Women were stimulated with downregulated HRT and mild stimulation protocols. Women with endometrial thickness >8 mm and good subendometrial blood flow, uterine artery pulsatility index (PI) <3, and resistance index (RI) <0.8 were considered for sequential ET. On day 3, the straws containing three to four embryos were thawed. One cleavage or early compacting embryo was transferred on the 4th day of progesterone using Cook's soft Echo tip ET catheter under ultrasonography. The remaining two or three embryos were cultured for blastocyst in one-step media. On the 6th day of progesterone stimulation, one day 5 blastocyst was transferred to the same patient. Adequate luteal phase support was given for 14 days. On the 15th day, pregnancy was confirmed by a urine pregnancy test and serum beta-hCG.

The implantation of the embryo and clinical pregnancy rates were calculated. Biochemical pregnancy was also recorded. The patients were followed-up till the labor. Data were analyzed using Statistical Package for the Social Sciences software. A *p*-value <0.05 was considered statistically significant.

Results

The present study involved a total of 155 patients. The mean age of the wife and husband was 31.4 ± 4.65 and 36.02 ± 5.07 years, respectively. The proportions of couples with primary and secondary infertility were 66.45 and 33.5%, respectively (Fig. 1). Overall the mean duration was 5.57 ± 3.7 years. The average prolactin, FSH, thyroid-stimulating hormone (TSH), and luteinizing hormone among the subjects were 16.76 ± 8.53, 5.76 ± 2.94, 1.94 ± 1.01, and 4.47 ± 7.19, respectively (Table 1).

The most common etiology of infertility was polycystic ovarian syndrome in 56 (36.1%), followed by a tubal factor in 52 (933.5%) and low reserve among 50 (32.3%) (Fig. 2).

In infertile men, 70% of patients exhibited normozoospermia, 15% showed oligozoospermia, 9% showed azoospermia, and 5% showed severe oligozoospermia (Fig. 3). Normal motility was observed in 53% of normozoospermia, 44% of asthenozoospermia, and 3% of necrozoospermia infertile men (Fig. 4). Furthermore, 69% of infertile men showed teratozoospermia, and the remaining had normal morphology (Fig. 5).

The majority of the infertile women (76.13%) were stimulated with the HRT protocol. The average endometrial thickness on the day of the hCG administration was 8.68 ± 1.13, the mean endometrial PI value on the day of the hCG administration was 2.32 ± 0.62, the mean endometrial RI value on the day of the hCG administration was 0.8 ± 0.24, and the mean number of days of estrogen priming was 16.85 ± 1.73 (Table 2).

Among 155 women, 105 (67.7%) had a clinical pregnancy with 77 (73.34%) singletons, 24 (22.86%) twins, and 4 (3.81%) triplets. At the time of reporting the present study, 60 (57.14%) were ongoing pregnancies, 36 (34.28%) had live births, and 9 (20%) had undergone abortions (Table 3).

Table 1: Demographic and biomarker characteristics of infertile couples undergoing sequential frozen ET (*n* = 155)

Sl no.		*Mean ± standard deviation (SD)*
1	Age of wife in years	31.4 ± 4.7
2	Age of husband in years	36.02 ± 5.07
3	Duration of infertility in years	5.6 ± 3.7
4	Prolactin level (µg/L)	16.8 ± 8.5
5	FSH level (IU/L)	5.8 ± 2.9
6	Anti-mullerian hormone level (ng/L)	3.5 ± 2.7
7	LH level (IU/L)	4.5 ± 7.2
8	TSH level (mIU/L)	1.9 ± 1.01

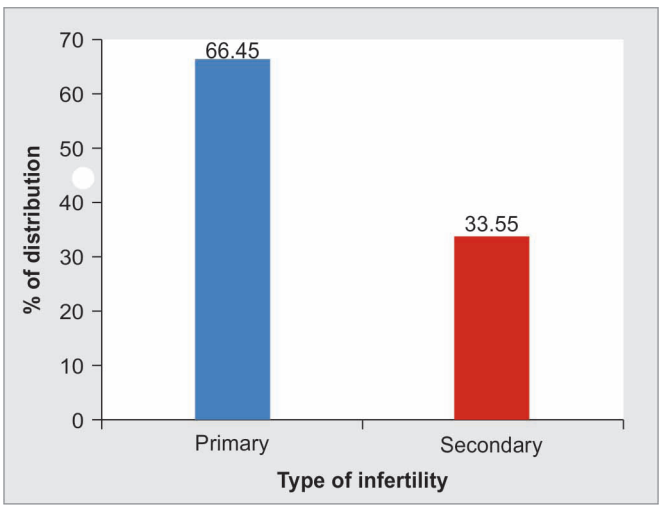

Fig. 1: Infertility type among infertile couples who had sequential frozen ET (*n* = 155)

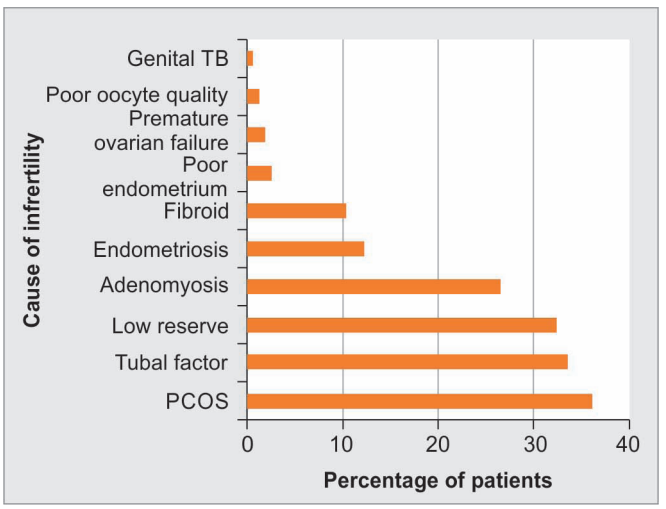

Fig. 2: An analysis of the causes of infertility among infertile women who underwent sequential frozen ET (*n* = 155)

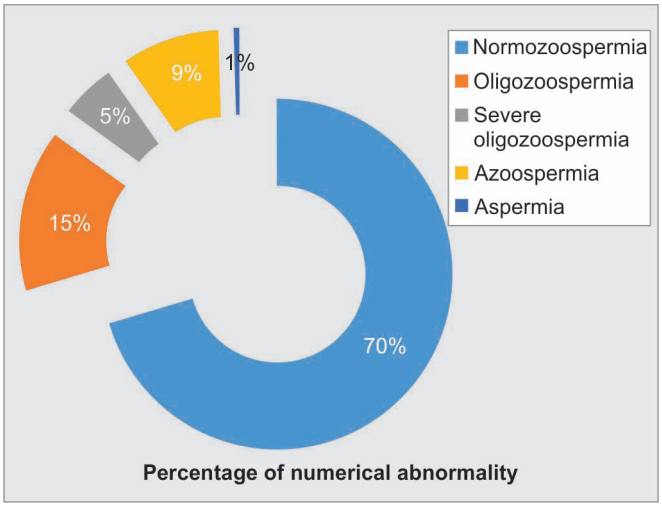

Fig. 3: Semen analysis of infertile men used for ICSI (*n* = 155)

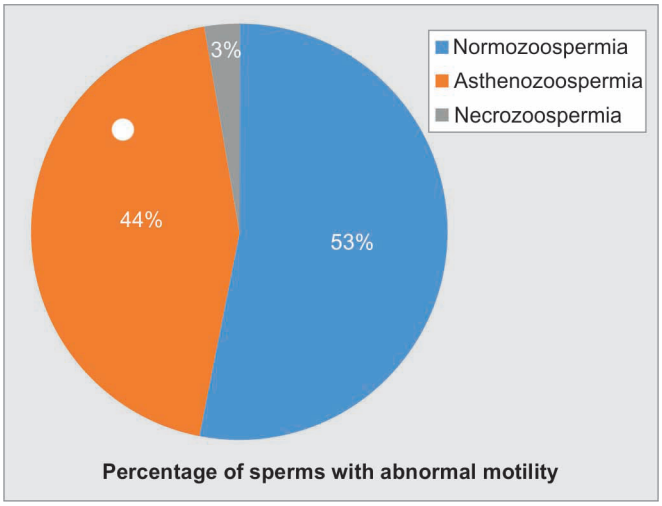

Fig. 4: Sperm motility of infertile men used for ICSI (*n* = 155)

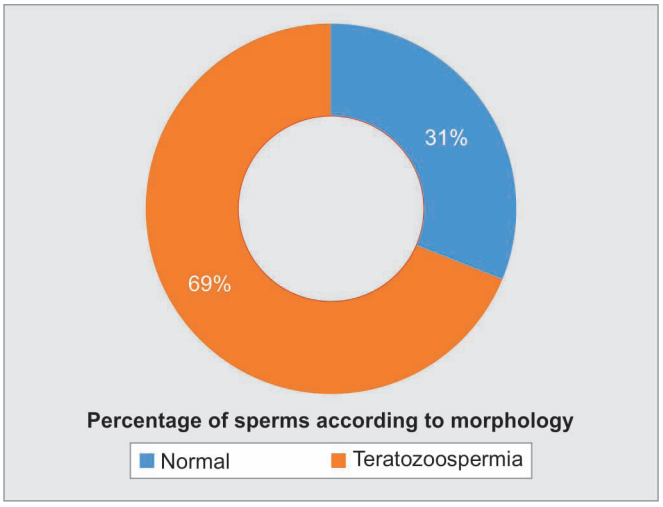

Fig. 5: Sperm morphology of infertile men used for ICSI (*n* = 155)

Table 2: The protocols used to stimulate the infertile women for sequential frozen ET (*n* = 155)

Sl no.	*Protocols*	*Values*
1	Downregulated [*n* (%)]	22 (14.19%)
2	HRT [*n* (%)]	118 (76.13%)
3	Natural/modified natural [*n* (%)]	2 (1.29%)
4	Stimulated [*n* (%)]	13 (8.39%)
5	The endometrial thickness on the day of hCG administration (mean ± SD)	8.68 ± 1.13
6	Endometrial blood flow on the day of hCG administration (mean ± SD)	3.02 ± 0.69
7	Endometrial PI value on the day of hCG administration (mean ± SD)	2.32 ± 0.62
8	Endometrial RI value on the day of hCG administration (mean ± SD)	0.8 ± 0.24
9	No. of days estrogen priming (mean ± SD)	16.85 ± 1.73

Table 3: Pregnancy outcomes of infertile women who underwent sequential frozen ET (*n* = 155)

Sl no.	*Parameter (n %)*	*Summary*
	IVF outcome (n = 155)	
1	Clinical pregnancy	105 (67.7%)
2	Biochemical pregnancy	4 (2.58%)
3	No pregnancy	46 (29.68%)
	Final pregnancy outcome (*n* = 105)	
1	Singleton	77 (73.34%)
2	Triplets	4 (3.81%)
3	Twins	24 (22.86%)
	Live birth status (*n* = 105)	
1	Ongoing pregnancy	60(57.14%)
2	Live birth	36 (34.28%)
3	Abortion	9 (20%)

Discussion

Sequential transfer has emerged as the preferred type of ET, nullifying the disadvantages of the day 3 and day 5 transfers and resulting in increased success rates. The proposed logic behind sequential transfer is to get the "best of day 3 and 5 transfers" or to find a middle ground between having high pregnancy rates of blastocyst transfer and avoiding the potential disadvantage of cycle cancellation due to the nonformation of a blastocyst. Furthermore, for sequential ET, we have hypothesized that during the first day 3 ET, the embryos may induce greater endometrial receptivity, thereby producing a better endometrial environment for the second ET on day 5.[7–11]

Our study reported a clinical pregnancy rate of 67.7% and an ongoing pregnancy rate of 57.14% among women undergoing sequential transfer. Reported pregnancy rates with sequential transfer were quite varied across the studies. Clinical trials reported that the clinical pregnancy rate ranged between 25 and 67% among women who underwent sequential transfer.[11,12–15] Phillips et al. discovered that sequential transfers on day 3 followed by a transfer on day 5 increased overall pregnancy rates to 37.5%.[16]

In the current study, out of the total number of women conceived, 23.08% had twin pregnancies, and 3.85% had triplets. Similarly, Fang et al. reported that, despite the higher number of transferred embryos, the incidence of multiple pregnancies was not different between sequential transfer and day 5 groups.[8] However,

Nadkarni et al. reported the maximum pregnancy and highest implantation rates with the highest number of multiple pregnancies (67.34%) observed in the sequential ET group.[6,17,18] The current study reported the success rate following sequential transfer with the critical limitation of not having a comparative group, preventing us from drawing any conclusions regarding the relative superiority. Another fundamental limitation is the relatively smaller sample size, which was insufficient to perform any subgroup analysis.

Conclusions and Clinical Significance

Despite the limitations, the current study has reported a high success rate with sequential transfer among Indian couples undergoing IVF/ICSI with different etiologies. This reaffirms the view that sequential transfer may result in superior clinical pregnancy rates. Further large-scale comparative studies or randomized controlled trials are required to validate the results of the present study. Sequential transfer can be a useful modality for an ART program and deserves more attention in clinical practice.

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