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ORIGINAL ARTICLE

Outcome Analysis of Day-3 Frozen Embryo Transfer v/s Fresh Embryo Transfer in Infertility: A Prospective Therapeutic Study in Indian Scenario

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Abstract

Introduction Advanced fertilization techniques like frozen embryo transfer (FET) and assisted reproductive technology have become popular and commonly used methods to treat patients suffering from infertility. Incidences of infertility are on a rise due to increased representation of females in the work place, delay in marriages, stress, and ignorance.

Methods We performed this prospective therapeutic study to compare FET and fresh embryo transfer in the treatment of infertility in terms of conception rate, patient acceptance, complications, and patient's compliance. A prospective screening therapeutic study on 108 patients, from September 2013 to September 2014 in Karnataka, India, randomized the patients into 2 groups ($n = 54$), Group-I treated with day-3 FET while Group-II was treated with fresh embryo transfer, after performing ICSI.

Results In 108 patients, 45 % patients were within 35 years of age, 35 % were in the age group 35–39. Significantly, 22 (40.75 %) patients treated with FET conceived (P = 0.022), whereas 16 (29.63 %) patients treated with fresh embryo transfer conceived ($P = 0.59$).

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Discussion There is limited published literature from the subcontinent, comparing techniques like FET and embryo transfers in the treatment of infertility. Awareness and economic reforms must be formulated in India to facilitate individuals facing infertility problems to conceive.
Conclusion FET has better and significant conception rates compared to fresh embryo transfers. FET shares an advantage of providing good quality embryos for future and subsequent implantations in cases of failure. Patient counseling and motivation play a pivotal role in the success of therapeutic procedure.

Keywords Infertility · Frozen embryo transfer · ART · Family · Reproduction · India

Introduction

Advanced fertilization techniques like frozen embryo transfer (FET) and assisted reproductive technology (ART) have become the popular and commonly used methods to treat patients suffering from infertility. Incidences of infertility are on a rise due to increased representation of females in the work place, delay in marriages, stress, and ignorance. In India, unlike the olden days, due to increased efforts of the government and NGOs to create awareness, many couples report and seek consultation for infertility issues. However due to lack of published literature from the subcontinent, magnitude of the problem and the results of these procedures in treating infertility are unknown.

A cycle of in vitro fertilization can be completed using frozen embryos as well as fresh embryos. Good quality embryos should be frozen quickly so that they can be stored for use in future. Frozen embryos have the advantage of being stored for later use and at multiple times, avoiding repeated ovarian stimulation [1].

Cryopreservation of embryos has been an important supplementary procedure in the treatment of infertility since the advent of FET which has become an important component of ART [2–10]. FET allows controlled embryo transfer thus lowering the risk of multiple pregnancies [11]. Embryo cryopreservation provides additional clinical safety in the presence of ovarian hyper stimulation [12–16].

Following FET, pregnancy outcomes depend on the patient's age, infertility duration, infertility type (primary or secondary), and ocyte fertilization i.e., IVF/ICSI, embryo cryopreservation timings and the endometrial thickness on the day of embryo transfer [17–20].

We performed this prospective therapeutic study to compare FET and fresh embryo transfer in the treatment of infertility in terms of conception rate, patient acceptance, complications, and patient's compliance.

Materials and Methods

In this prospective screening therapeutic study, we prospectively treated females with infertility from September 2013 to September 2014 at our infertility center in Karnataka, India. Patients visiting gynecological outdoor with a diagnosis of infertility were explained, counseled and after obtaining consent from the patient and her husband were included in the study. Out of 1012 patients screened, 108 patients comprised the study population. Patients were randomized into 2 comparable groups, Group-I ($n = 54$) were treated with a day-3 FET while in Group-II ($n = 54$), patients were treated with fresh embryo transfer, after performing intracytoplasmic sperm injection (ICSI). The patients were followed up to assess the study and compare the conception rate (CR) and patient convenience and compliance.

Inclusion Criteria

1. Infertile women who were using self-embryos.
2. Male factor infertility, TESA.
3. Women who developed OHSS (ovarian hyper stimulation) in a previous IVF cycle.
4. Women known to be at high risk of OHSS.
5. All patients with $>$ or =2 stimulated eggs/follicles, with E2 $\geq$ 2000.

Exclusion Criteria

1. Donor embryos.
2. Poor responders with <4 stimulated follicles.
3. Subject with previous history of uterine curettage, endocrine disorders (diabetes mellitus, hypothyroidism).
4. Embryo transfer performed in a natural cycle.

Methodology

Among 1012 infertility patients attending the outpatient department, 108 patients who met the inclusion criteria were selected as the study group, following randomization 54 patients were subjected to a day-3 FET, while the other 54 patients were subjected to a day-3 fresh embryo transfer.

Controlled ovarian stimulation was achieved mainly using the gonadotropin-releasing hormone (GnRH) antagonist for pituitary suppression and recombinant FSH. The patients underwent pituitary desensitization with the use of GnRH antagonist. Immediately after the ovum pick-up, ICSI was performed for all the oocytes. The day-3 embryos were either transferred in the same cycle or were frozen using vitrification technique and transferred in the next cycle [21].

Vitrification involved rapid freezing of the embryo to prevent any ice formation in the embryo cells. Successful

conception depends upon the rapidity of freezing. Embryos are known to survive for many years once they are frozen; currently they may be stored for 10 years [22–24]. Embryos are stored in a special solution in a sterile vial/straw inside a container of liquid nitrogen at a temperature of −196 °C [25]. Labeling was done very carefully, and embryos were thawed after obtaining a written informed consent from both the parents.

Procedure

On Day 2, a transvaginal baseline scan was performed with serum estradiol (E2) and LH levels to assess the hypothalamo–pituitary–ovarian status of the patient. The stimulation protocol followed for all the subjects was the antagonist protocol. From Day 2 of the cycle, gonadotropins (r-FSH 225 mg/day) were administered till Day 6. Patients were reviewed on Day 7 and a transvaginal scan, serum estradiol (E2) levels, and serum LH levels were done for follicular monitoring, assessment of the number of developing follicles, and to diagnose premature LH surge, if any, respectively.

GnRH antagonist (Cetrorelix 0.25 mg/day) was started once the follicles reached 1.4 cm and was continued until the follicles reached 1.7 cm. Once the follicles were 1.7 cm in size, r-HCG (250 micrograms stat) was given, and 34–35 h later, ovum pick-up was done under general anesthesia and antibiotic cover.

In patients who developed less than 6 matured follicles, transfer was performed in the same cycle. In these patients, immediately after ovum pick-up, progesterone (400 mg per vaginal/day) was started, and two day-3 embryos were transferred in the same cycle itself.

In patients who developed more than six matured follicles, we had let go of the ovum pick-up cycle and called the patient on the 2nd day of the next cycle. From the 2nd day of next cycle, the patient was started on estradiol valerate (4 mg/day) to prepare the endometrium for implantation [26–29]. E2 levels and TVS were done after 5 days and were performed periodically once in 3 days until the E2 reached 250 pg/ml and the endometrium was 1 cm or 10 mm [30–32]. At this stage, progesterone treatment was started (400 mg per vaginal/day) for 3 days before the embryo transfer for the final preparation and maturation of the endometrium [30]. The two best frozen embryos on day 3 were thawed and chosen for transfer[33–36]. These Day-3 (8A celled) embryos were transferred on the 4th day of progesterone under ultrasound guidance [37, 38]. The duration of the treatment was defined as the period from embryo storage till ET.

After the ET, the woman was continued on progesterone support (400 mg per vaginal/day) [39–41]. Three weeks later, a UPT was done [42–45]. The cases in which pregnancy test was positive, progesterone support was continued until 12 weeks of pregnancy [39–41]. Thereafter, exogenous progesterone treatment was stopped as placental hormones take over. A confirmatory ultrasound was done at 7 weeks for viable gestational sac [25, 41–44]. B-HCG test was done in addition in cases of doubt.

Randomisation Protocol

Randomisation of 108 patients into two groups ($n = 54$) were performed according to internet-based, computer generated number by a person not involved in treating patients, who coded the numbers and sealed them in envelopes which were then given to the treating physician.

Statistical Analysis

Patient characteristics such as age and duration of infertility were represented as mean, range, and SD. For variables, χ^2 test and P values were calculated. The data were compiled using standard Microsoft Office Tools.

Observations

In the study population, 108 patients, 45 % patients were within 35 years of age, 35 % were in the age group 35–39, and 20 % patients >40 years. 47 % patients had infertility of 7–9 years, followed by 42 % patients with 4–6 years of infertility with minimum of 2 years and maximum of 16 years. 70 % patients had a primary infertility. The patients in Group-1 were in the range of 25–47 with mean 33 years, SD 5. The patients in Group-2 were in the range of 24–45 with mean 31 years, SD 6. The baseline characteristics of 2 groups i.e., mean age, mean duration, and type of infertility are represented in Table 1. Causes of secondary infertility are listed in Table 2.

In Group-I, patients treated with FET, 22 (40.75 %) patients conceived significantly, 12 of them being <35 years, followed by 8 in the group 35–39 years. 32 (59.25 %) patients had a failure ($P = 0.022$) with a χ^2 value of 7.59, Df = 2. The pregnancy rate after FET in women aged <35 years was 63.15 % (12/19), in patients aged 35–39 years was 36.36 % (8/22), and >40 years was 15.3 % (2/13) (see Table 3).

In Group-II, patients treated with fresh embryo transfer, 16 (29.63 %) patients conceived, nine of them being <35 years was 31 % (9/29), followed by six in the group 35–39 years. 38 (70.37 %) patients had a failure ($P = 0.59$) with χ^2 value 1.04, Df = 2. The pregnancy rate in fresh embryo transfers in the age group <35 years was 56.25 %, 35–39 years was 37.5 % (6/16), and in >40 years age group was 11 % (1/9) (see Table 4). Statistical analysis

Table 1 Patient characteristics

S.no.	Variables	Group-1 ($n = 54$) (FET)	Group-2 ($n = 54$) (FET)
1.	Age (Years, mean)	33	31
2.	Duration of Infertility (Years, mean-S.D)	9 ± 2	8 ± 2
3.	Type of Infertility		
	Primary	40	36
	Secondary	14	18

Table 2 Causes of secondary infertility in two groups

Secondary infertility ($n = 32$)	Frozen embryo transfer ($n = 14$) Group-1	Fresh embryo transfer ($n = 18$) Group-2
1. Previous natural conceptions		
Previous 2 IUD	2	–
Previous 1 child	1	3
Previous 1 Ectopic	1	1
Previous 2 Ectopic	1	–
Previous repeated abortions		
(a) <3	1	4
(b) >3	2	2
2. Previous IVF failures		
Previous 1 IVF failures	1	5
Previous 2 IVF failures	2	3
Previous 3 IVF failures	2	–
Previous 4 IVF failures	1	–

Table 3 Group-1: outcome with FET

	Age (years)			$n = 54$	%	P value
	<35	35–39	>40			
Conceived with FET	12	8	2	22	40.75	0.022
Not conceived (IVF failure)	7	14	11	32	59.25	

$\chi^2 = 7.59$; Df = 2; P value = 0.022 significant

showed that young (<35) and old (35–40) mothers had significant differences in pregnancy rates in FET.

Discussion

Our study compared 3-day FET with fresh embryo transfer in patients with infertility ranging from 2 to 16 years. The results reveal a significant difference between the conception rates following FET as compared to a fresh embryo transfer. Patient compliance is better with FET as the procedure stores the ovum for future purpose, minimizing unnecessary ovarian hyper stimulation, and allows the embryo transfer in a normal hormonal milieu, minimizing risks of a failure. FET has less known complications.

Table 4 Group-2: outcome with FET

	Age (years)			$n = 54$	%	P value
	<35	35–39	>40			
Conceived	9	6	1	16	29.63	0.59
Not conceived	20	10	8	38	70.37	

$\chi^2 = 1.04$; Df = 2; P value = 0.59 not significant

Due to lack of published studies from the subcontinent, the outcomes from other centers are less known. The results also suggest that in normal- and high-responder patients, it may be advantageous to cryopreserve all viable embryos and use them in a subsequent FET. Importantly, the data were extracted to allow for an intention-to-treat analysis. Patient counseling and motivation play a pivotal role in the success of the therapeutic procedure; couples who are motivated do better in terms of patient compliance, follow-up, and success rates.

The results favoring FET instead of fresh embryo transfer may be related to the adverse effects of COH on endometrial receptivity, as well as the improved results that can be achieved with current cryopreservation methods [46–48].

Embryo implantation remains an unsolved problem in ART, being responsible for 2/3 failures, whereas the embryo itself is responsible for only 1/3 of the failures [49]. Subtle increases in serum P levels (i.e., premature luteinization) show a positive correlation with FSH levels at the end of the follicular phase in COH [50–57]. With COH, the elevated P may cause advanced endometrial maturation, without affecting embryo quality which may lower implantation rates due to asynchrony between embryo and the endometrium in fresh cycles [58, 59].

Uterine receptivity is better achieved during natural cycles or with hormone replacement therapy with exogenous E_2 and P, compared to stimulated cycles [26, 60, 61]. There is evidence showing that high E_2 levels (>2500 pg/mL) may impair the endometrium maturation and implantation [62, 63]. The cryopreservation of embryos has become a vital procedure in ART. Endometrial priming for FET renders endometrium more receptive than in fresh embryo cycles [64–68]. Whereas a universal priming protocol lacks, vitrification technique has shown a higher embryo survival rate, compared to slow freezing, resulting in significantly higher implantation and pregnancy rates per transfer [69, 70].

In summary, the results of this analysis suggest that there is evidence of moderate quality that the implantation, clinical and ongoing pregnancy rates of ART cycles may be improved by performing FET compared with fresh embryo transfer. These results may be explained by improved embryo-endometrium synchrony achieved with endometrium preparation cycles instead of COH cycles. If embryos are frozen immediately after fertilization, which are still in pronuclear stage, and are being used, then the procedure differs as these embryos are literally only one cell at the point of freezing and there is no way to tell how good their quality will be. They must be thawed and cultured for at least 2–3 days in the lab until they reach a stage where they can be assessed using pre-implantation screening techniques if required. One should ensure that the embryos and the uterus are ready on the same day.

In view of the increasing incidence of infertility, keeping in mind the cost factor associated with these procedures and the failure rates, a huge economic burden may be imparted on individuals, especially the middle and low income group people who cannot sometimes afford this luxury. In India, insurances and government policies for infertility do not exist, depriving many of this privileged facility and providing a hope for parenthood. Thus, awareness and economic reforms must be formulated, especially in India, to facilitate individuals facing infertility problems with treatment options, providing them with a hope to live.

Conclusion

In India, published material and data comparing the techniques like FET and embryo transfers in treatment of infertility are lacking, thus the magnitude of the problem and the results of these procedures in treating infertility is unknown.

FET has better and significant conception rates compared to fresh embryo transfers in cases of infertility. FET shares an advantage of providing good quality embryos for future and subsequent implantations in cases of failure.

Awareness and economic reforms must be formulated, especially in India.

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