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Original Research Article

Fresh versus frozen embryo transfer: a retrospective cohort study

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ABSTRACT

Background: Elective frozen embryo transfer (FET), has recently increased significantly with improvements in cryopreservation techniques. Observational studies and randomized controlled trials suggested that the endometrium in stimulated cycles is not optimally prepared for implantation; risk of ovarian hyperstimulation syndrome reduced and pregnancy rates increased following FET and perinatal outcomes are less affected after FET. However, the evidence is not unequivocal and recent randomised control trials challenge the use of elective FET for the general IVF population. Pregnancy rates were analysed in a cohort of patients undergoing embryo transfers.

Methods: This was a retrospective cohort study of patients who underwent embryo transfers from April 2018 to March 2019 at study centre in Surat. 175 cycles of embryo transfers (119 fresh and 56 frozen) were included in the study. Outcomes measured were positive pregnancy, clinical pregnancy and ongoing clinical pregnancy rates achieved in the IVF-ET cycles.

Results: There were no statistically significant differences between positive pregnancy rate (54.6% versus 60.7%, Odds ratio (OR) 0.78; 95% Confidence Interval (CI) 0.41-1.49), clinical pregnancy rate (48.73% versus 57.14%, OR 0.52; 95% CI 0.1- 2.64) and ongoing clinical pregnancy rate (45.38% versus 51.78% OR 1.4; 95% CI 0.29 - 6.67) in fresh ET and FET cycles, respectively, $p < 0.05$ was considered statistically significant for all measures.

Conclusions: Despite the observed higher rates of positive biochemical, clinical and ongoing clinical pregnancy per transfer in the FET cohort, these did not reach statistical significance. Thus, both transfer strategies are reasonable options, although there is a trend favouring the freeze-all strategy.

Keywords: Cryopreservation, Clinical pregnancy rate, Frozen embryo transfer, Fresh embryo transfer, Vitrification

INTRODUCTION

Conventional in-vitro fertilization (IVF) protocols involve the transfer of a fresh embryo in utero during the same cycle in which the oocytes are retrieved and freezing extra embryos for future use. A novel approach to improving IVF outcomes has recently emerged in which all embryos generated from an oocyte retrieval cycle are electively frozen and transferred in a subsequent

cycle. This 'freeze-all' approach, initially developed as a strategy for minimising risk of ovarian hyperstimulation syndrome (OHSS) in high risk patients, also addresses concerns raised that the drugs used for ovarian stimulation during IVF may have a negative impact on the endometrial receptivity of some patients.¹⁻⁴ In theory, therefore, waiting until a later 'natural' cycle for embryo transfer (ET) should improve outcomes in these patients; however, evidence supporting this claim is limited.⁵ The

objective of the study was to compare the pregnancy rates in fresh embryo transfers after ovarian stimulation using antagonist protocol and frozen-thawed embryo transfers (FETs) in artificially prepared endometrium after cryopreservation of embryos in stimulated cycle.

METHODS

Retrospective cohort study of patients who underwent embryo transfers from April 2018 to March 2019 at Nadkarni's 21st Century Hospital and IVF Centre, Surat. 175 cycles of embryo transfers (119 fresh and 56 frozen) were included in the study.

Inclusion criteria

- Women less than 38 years of age, who had undergone intracytoplasmic sperm injection (ICSI) treatment with controlled ovarian stimulation (COS) using antagonist protocol
- Serum progesterone level of less than or equal to 1.3 ng/ml on the day of trigger in fresh cycle or on day 10-12 of frozen cycle
- Endometrial thickness more than 8 mm, grade I endometrial vascularity on the day of embryo transfer
- Transfer of not more than 3 embryos per cycle (Grade 1 / 2 / 1 and 2).

Exclusion criteria

- Patients undergoing artificial reproductive treatment using gamete/embryo donation/surrogacy
- Fresh cycles where more than 20 oocytes were retrieved, terminal estradiol levels above 2500 pg/ml and cycles complicated by ovarian hyperstimulation syndrome
- History of Asherman syndrome/thin endometrium.

Procedure

Data was collected from the IVF and embryo transfer registers at our centre and analysed.

- Ovarian stimulation: Recombinant FSH/ highly purified urinary FSH/menotropins injections were administered, starting from day 3 of the menstrual cycle after confirming day 2 FSH and LH < 5-7 m IU/ml and serum oestradiol < 50-70 pg/ml. Gonadotropin dose used for ovarian stimulation was based on patient's BMI, AMH, AFC and modified as per the follicular response.

Serial follicular growth monitoring was done using transvaginal ultrasound and injection cetrotrelis acetate (0.25mg) s.c. was added daily from the day when the lead follicle reached 14 mm diameter till the day of trigger. Once there were adequate number of follicles of 18-20 mm diameter, ovulation was triggered using choriogonadotropin alfa (Inj. Ovitrelle®, 500mcg i.e.

13,000 IU) or a combination of 6500 IU choriogonadotropin alfa and Inj. Buserelin acetate (0.5mg) s.c. Endometrial thickness was measured by transvaginal ultrasound and serum LH, oestradiol and progesterone values were determined on the day of trigger. Ovum pick up was performed 34-36 hours after the trigger injection.

Embryo transfer (ET) process

In fresh cycles: Embryo transfer was done if serum progesterone level on the day of trigger was \leq 1.3 ng/ml. Day 3 ET or day 5, blastocyst transfer was done post-ovum pick up i.e. single ET or on day 3 followed by day 5 i.e. sequential ET, based on the availability of good quality embryos and after discussion with patient and written, informed consent. Not more than a total of 3 embryos (grade 1 / 2 / 1 and 2) were transferred per cycle. Surplus embryos, if any, were vitrified with consent using Kitazato® embryo vitrification kit (cryotop method).

If the endometrial thickness was greater than 8 mm, micronized natural progesterone suppositories, at a daily dose of 400 mg, twice, vaginally was started for luteal phase support, beginning on the day of oocyte retrieval until 12 weeks after conception.

In frozen ET cycles: Patients in the study underwent FET if pregnancy was not achieved in previous attempt (in fresh/frozen cycle) and surplus embryos were available for freezing.

When embryo transfer could not be performed during the first attempt because of risk of ovarian hyperstimulation was suspected, serum progesterone level greater than 1.3 ng/ml, endometrium was not appropriate on the day of embryo transfer.

After ovarian down-regulation with inj. Triptorelin acetate (0.1mg) daily starting 4 - 5 days before date of expected menses, for 5 doses, patients were administered estradiol valerate tablets (4 mg) BD for 5 days followed by TDS for next 15 days, starting from day 2 of menses

Vaginal progesterone administered following same regimen as in fresh ET, if endometrial thickness was greater than 8 mm (day 10 - 12 of cycle). After 4 days of the progesterone regimen, frozen embryos were thawed and transferred. The luteal phase support with estradiol valerate and micronized vaginal progesterone continued until 12 weeks after conception.

Frozen embryos were thawed using Kitazato® embryo thawing media and transferred. Either day 3 embryo or day 5 (blastocyst) i.e. single ET or day 3 frozen-thawed embryo followed by blastocyst i.e. sequential ET was done, based on the availability of good quality embryos and after discussion with patient and written, informed consent. Not more than a total of 3 embryos (grade 1 / 2 / 1 and 2) were transferred per cycle.

Table 1: Consensus scoring system for cleavage-stage embryos (in addition to cell number).

Grade	Rating	Description
1	Good	<10% fragmentation
		Stage-specific cell size
		No multi-nucleation
2	Fair	10-25% fragmentation
		Stage-specific cell size for majority of cells
		No evidence of multinucleation
3	Poor	Severe fragmentation (>25%) cell-size not specific
		Evidence of multinucleation

Table 2: Scoring system for blastocysts.

	Grade	Description	
Stage of development	1	Early blastocyst with blastocoele cavity less than half the volume of embryo	
	2	Blastocoele cavity equal to or more than half the volume of the embryo	
	3	A full blastocyst with blastocoele cavity completely filling the embryo	
	4	Expanded blastocyst with blastocoele cavity larger than the volume of the embryo and thinning of the zona (shell)	
	5	Hatching blastocyst with trophoctoderm starting to herniate through the zona	
	6	Hatched blastocyst with complete escape of blastocyst from the zona	
	Grade	Rating	Description
Inner cell mass	1	Good	Prominent, many cells tightly packed together
	2	Fair	Many cells, loosely grouped together
	3	Poor	Difficult to discern, very few cells
Trophecto-derm	1	Good	Many cells forming a cohesive epithelium
	2	Fair	Few cells forming a loose epithelium
	3	Poor	Very few cells

In both fresh and frozen cycles

Embryo transfer done under abdominal ultrasound guidance with full urinary bladder using Cook® Sydney IVF Embryo Transfer Catheter Set, by the same provider

and the same transfer technique was scrupulously maintained in all patients included in the study. Embryos graded as 1 and /or 2 were transferred.

Pregnancy was diagnosed by increasing concentration of serum β-hCG which was first measured after 15 days post-ET and subsequent demonstration of intrauterine gestational sac on transvaginal ultrasound (TVUS) examination.

Statistical analysis

Statistical analysis of data was done using the SPSS (Statistical Package for the Social Sciences, version 18.0, SPSS Inc, Chicago, Illinois, USA). Differences among variables of the two groups were analyzed using Student’s t- test. Chi- square test was applied to compare categorical variables. p<0.05 was considered statistically significant for all measures.

Outcomes

Primary outcomes measured were

- Positive (biochemical) pregnancy rate: Confirmation of a successful implantation by detecting an increased serum β-hCG concentration (>50 IU/ml) after 15 days post-embryo transfer, was considered as positive biochemical pregnancy
- Clinical pregnancy rate: Clinical pregnancy was defined by a presence of a gestational sac on TVUS
- Ongoing pregnancy rate: Ongoing pregnancy was defined by the presence of an intrauterine gestational sac with foetal heart activity on ultrasound at 12 weeks of pregnancy.

Secondary outcomes measured were-miscarriage rate, ectopic pregnancy rate, singleton and multiple pregnancy rate. Miscarriage was considered as any clinical pregnancy that did not achieve ongoing pregnancy status.

RESULTS

Patients’ characteristics in the two groups are listed in Table 3. There were no significant differences regarding patients’ characteristics between the groups (p < 0.05). The most common cause of infertility was unexplained in the fresh ET group and in frozen ET group it was male factor and unexplained.

Table 4 presents the cycle characteristics. The average number of embryos transferred per cycle in the fresh ET group was 2.56±0.496 and in frozen ET group was 2.5±0.534, and there was no significant difference between the two groups (p <0.05).

Table 5 presents the different outcomes in the two groups. There were no statistically significant differences between positive pregnancy rate, i.e. positive β-hCG (54.6% versus 60.7%), clinical pregnancy rate (48.73%

versus 57.14%) and ongoing clinical pregnancy rate (45.38% versus 51.78%) in fresh ET and FET cycles, respectively. $p < 0.05$ considered statistically significant.

However, positive β -hCG, clinical and ongoing pregnancy rates were higher in the frozen ET group.

Table 3: Patients' characteristics.

	Fresh ET	Frozen ET	P-value
Age of females (years) ^{a,b}	30.23±3.45	29.83±3.02	0.475
Age of males (years) ^{a,b}	33.03±3.17	32.8±3.02	0.652
Duration of infertility (years) ^{a,b}	4.08±1.44	4.39±1.74	0.222
S. AMH (ng/ml) ^{a,b}	2.52±0.81	2.4±0.73	0.355
Antral Follicle Count ^{a,b} (both ovaries)	9.45±1.72	8.93±1.42	0.053
Type of infertility: ^c			
Primary	91	47	0.259
Secondary	28	09	
Cause of infertility: ^c			
Male factor	47	21	0.204
Female factor	17	14	
Unexplained	55	21	

a- Data are presented as mean ± standard deviation.

b- Data were assessed using t- test.

c- Data were assessed using Chi-square (χ^2) test.

Table 4: Cycle characteristics.

Cycle characteristics	Fresh ET	Frozen ET	P-value
Number of embryos transferred ^{a,b}	2.56±0.496	2.5±0.534	0.448
Grades of embryos transferred ^c	(Number of cycles)		
1	32	21	0.138
2	9	7	
1 and 2	78	28	
Endometrial thickness at ET (mm) ^{a,b}	10.8±1.774	10.5±1.798	0.370
Type of transfer ^c	(Number of cycles)		
Single ET	23	12	0.745
Sequential ET	96	44	

a- Data are presented as mean ± standard deviation.

b- Data were assessed using t- test.

c- Data were assessed using Chi-square test.

Statistically significant differences were not observed in the viable pregnancy (45.37% v/s 51.78%) and

miscarriage rates (3.36% v/s 5.35%) nor singleton (55.6% v/s 69%) and multiple (44.4% v/s 31%) pregnancy rates in the fresh ET and FET groups.

Table 5: Pregnancy rates in fresh and frozen ET groups.

	Fresh cycle ET (n=119)	Frozen ET (n=56)	Odds ratio (95% CI)	p-value ^c
(Number of cycles)				
Biochemical pregnancy				
β -hCG positive	65	34	0.78 (0.41-1.49)	0.45
β -hCG negative	54	22		
Clinical pregnancy				
Gestational sac present	58	32	0.52 (0.1 - 2.64)	0.42
Gestational sac absent	7	2		
Ongoing clinical pregnancy				
Foetal cardiac activity present	54	29	1.4 (0.29-6.67)	0.674
Foetal cardiac activity absent	4	3		

c-Data were assessed using Chi-square test.

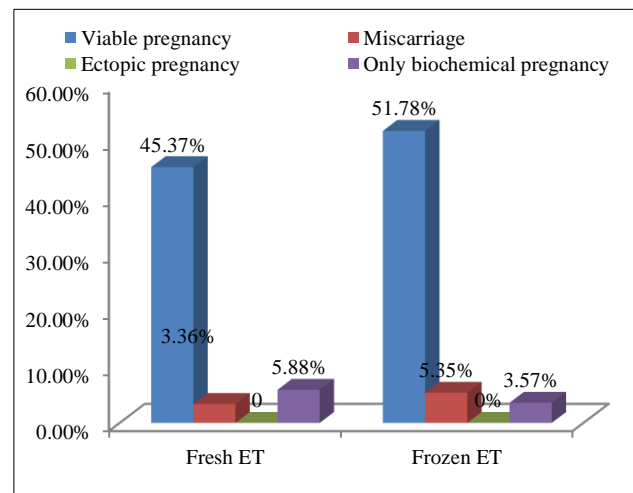


Figure 1: Viable, only biochemical, ectopic pregnancy and miscarriage rates in fresh and frozen ET groups.

Figure 1 presents the pregnancy rates in the two ET groups. The viable pregnancy rate, miscarriage rate and only biochemical pregnancy rate were 45.37%, 3.36% and 5.88%, respectively in the fresh ET group and 51.78%, 5.35% and 3.57%, respectively in the frozen ET group. There was no ectopic pregnancy in either group.

Table 6: Secondary outcome measures.

	Fresh ET	Frozen ET	Odds ratio (95% CI)	p-value ^c
Viable pregnancy	54	29		
Miscarriage	4	3	1.4 (0.29 - 6.67)	0.674
Ectopic pregnancy	0	0		
Singleton	30	20		
Twins	17	8	0.56 (0.23-1.36)	0.234
Triplets	7	1		

DISCUSSION

Successful cryopreservation of human embryos was first reported in 1983 by Trounson and Mohr with multicellular embryos that had been slow-cooled using dimethyl sulphoxide (DMSO).⁶ Subsequent modifications of the technique, introducing 1,2-propanediol and sucrose as cryoprotectants and slow-cooling to -30°C prior to plunging into liquid nitrogen, resulted in the introduction of cryopreservation as a standard method offered by virtually every IVF program world-wide.⁷ Slow freezing is known as equilibrium freezing due to the exchange of fluids between the extra- and intracellular spaces and results in safe freezing without serious osmotic and deformation effects to cells. This technique is accepted to be a safe procedure because of the use of relatively low concentration of cryoprotectants and slow-cooling to -30°C prior to plunging into liquid nitrogen, resulted in the introduction of cryopreservation as a standard method offered by virtually every full-service IVF program world-wide.⁷ However, as low concentrations of cryoprotectants may be insufficient for avoiding ice crystal formation within the cells, the slow freezing is more time-consuming and requires an expensive programmable freezing machine; most of the embryologists are not satisfied with this technique and try to find other cryopreservation protocols such as vitrification.⁸⁻¹¹

Verification technique was first reported by Rall and Fahy in 1985 for the cryopreservation of mammalian embryos, with a later attempt for human cleavage-stage embryo and followed by a successful delivery in 1990.^{12,13} Vitrification is a non-equilibrium method and is a radical approach in which ice crystal formation is totally eliminated. Nevertheless, it requires an extremely high cooling rate along-side much higher concentrations of cryoprotectants when compared with slow freezing.¹⁴ Human embryo vitrification has been attempted with a variety of vessels such as electron microscope grids, open pulled and hemi-straws, the Flexipet, the Cryotop and the CryoLoop. Until now, vitrification has been widely used for the cryopreservation of human oocytes, in vitro matured oocytes, pronuclear stage, cleavage stage or blastocyst-stage. Many studies have shown that vitrification, in contrast to slow freezing, is an efficient

method for cryopreservation of cleavage-stage embryos and blastocysts; with providing higher survival rates and minimal deleterious effects on post warming embryo morphology it can improve clinical outcomes.^{15,16}

Aflatoonian et al, reported that biochemical pregnancy rate was 27% (54/200) in the FET group and 22.1% (122/500) in the fresh ET group and biochemical pregnancy rate was comparable between FET and fresh ET.¹⁷ Another study reported significantly higher implantation, ongoing and clinical pregnancy rates in FET group.¹⁸ A retrospective case-control study by Pei-Yun Ku et al. at a hospital in Taipei, Taiwan, compared the clinical outcomes between fresh and vitrified-thawed day-5 blastocyst transfers with the same morphologic quality of embryos.¹⁹ It included 118 cycles of fresh blastocyst transfer and 59 cycles of vitrified-thawed blastocyst transfer. They found that clinical pregnancy rate, implantation rate and ongoing pregnancy rate did not differ significantly between fresh and freeze-thawed blastocyst transfer groups.

In a retrospective analysis of 1341 IVF-ET cycles, including 1169 fresh ET cycles and 172 FET cycles as the first embryo transfer in the controlled ovarian hyperstimulation cycle, Song T et al. found that the clinical pregnancy rate was significantly higher in frozen ET than fresh ET group.²⁰ In an analysis of 43,5765 cycles from the National ART Surveillance System- the only CDC approved data reporting system for ART procedures in the USA, Keenan et al. found that women above the age of 37 years have superior pregnancy rates with frozen compared with fresh embryo transfers.²¹ Nayar et al, in a randomised controlled trial, concluded that, clinical pregnancy rates in normo-responders is significantly higher in the elective cryopreservation group compared to the fresh embryo transfer group.²² In a prospective cohort study of 126 PCOS patients, by Nayar et al, clinical pregnancy and live birth rates were slightly higher in the FET group compared to fresh ET, though not statistically significant. The miscarriage rate in both the groups were similar.²³ Belva et al, reported that pregnancy rates were significantly higher in the FET group than fresh ET group.²⁴ In this retrospective cross-sectional study of 1014 ICSI-ET cycles (426 fresh ET and 588 FET) in a hospital at Iran, Basirat et al concluded that there were no significant differences between biochemical and clinical pregnancy rate, in fresh ET and FET cycles.²⁵

Some of the mechanisms and reasons behind increased success rate of and patient benefit from FETs are:

- Theory about 'embryo cryo-treatment': According to some authors, the thawing of embryos has a mitigating effect on some epigenetic aberrations as a result of the IVF/ICSI procedure.^{27,28} Freezing/thawing is a way to activate the endogenous survival and repair responses in pre-implantation embryos. Embryo thawing induces a

stress. Controlled stress is not necessarily detrimental, because it generates a phenomenon that is counteracted by several known biological responses aimed to repair mitochondrial damage of membrane and protein misfolding. The term for favourable biological responses to low exposures to stress is called 'hormesis'. The process of freezing and thawing of embryos reduces the levels of reactive oxygen species and mitochondrial DNA mutations.²⁸ Results are more "healthy" embryos with higher implantation potential and a positive influence on placentation and early embryo development in women of advanced maternal age.

- FET is thought to give better results as compared to fresh transfer probably due to disturbed receptivity due to elevated steroidal levels in stimulated cycles.²⁹ Laboratory-based studies demonstrate morphological and molecular changes to the endometrium and reduced responsiveness of the endometrium to hCG, resulting from controlled ovarian stimulation.¹⁻⁴ FET allows ovaries to recover from ovarian stimulation and also allows time for the exposed endometrium to shed.
- FETs overcome the negative effect that elevated progesterone levels have on pregnancy outcomes.
- Risk of ovarian hyperstimulation syndrome is reduced.
- Frozen embryos provide infertile patients with additional cycle opportunities. Also these patients may prefer to generate more embryos for cryopreservation in an attempt to limit the number of stimulated cycles needed to achieve their family goals.
- FET cycles are cheaper than fresh cycles as expenses of both medication and treatment are less than in a fresh cycle. Costs of treatment are reduced since there are fewer monitoring visits and there is no need for oocyte retrieval, microinjection or embryo culture. Various studies endorse the cost-effectiveness of freeze-all cycles when compared to fresh embryo transfers.^{30,31}
- An FET cycle is easier for patients because they need not undergo oocyte retrieval or anesthesia.
- Frozen embryos allow for genetic testing: Preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS). In appropriately selected candidates, this testing may result in improved live birth rates.
- FET has paved the way toward single embryo transfer (SET), thereby decreasing the risk of multiple gestation pregnancy and associated health risks.^{32,33}

In the study, 4 out of 58 clinical pregnancies in the fresh ET group and 3 out of 32 clinical pregnancies in the FET group resulted in miscarriage (a rate of 3.36% and 5.35% respectively). Pittenger et al. found that clinical pregnancies stemming from FET are 1.7 times more likely to result in spontaneous abortions than that following fresh embryo transfers.³⁴

In the study, there was no ectopic pregnancy (EP) in either group. Saidah et al found that there was a lower rate of EP with frozen embryos in comparison to fresh embryos, although this did not demonstrate statistical significance.³⁵ However, Huang et al found that frozen-thawed ET cycles were associated with a statistically significantly lower risk of EP compared to fresh cycles.³⁶

CONCLUSION

The results showed that there were no statistically significant differences in the biochemical, clinical, ongoing clinical pregnancy rates and miscarriage rates between comparable fresh and frozen embryo transfer groups, though each of these rates were higher in the frozen embryo transfer group. Literature demonstrates reduced endometrial receptivity in controlled ovarian stimulation cycles and supports the clinical observations that FET reduces the risk of OHSS and improves outcomes for both the mother and baby. These factors coupled with better optimisation of fertility treatment costs and patient-feasibility make FETs a prudent strategy to improve reproductive outcomes in patients undergoing IVF-ICSI treatment.

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