

A Retrospective Cohort Study: Frozen-Thawed Embryo Versus Fresh Embryo Transfer In Assisted Reproductive Technology

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ABSTRACT

- **Background:** Aim of study was to compare outcomes between frozen-thawed ET with fresh ET for women undergoing ART. Some evidences suggests that frozen embryo transfers improve live birth rate and reduce complications such as OHSS, as there is a improvements in preservation techniques.
- **Methods:** This was a retrospective cohort study of patients who underwent embryo transfers from Aug 2022 to Sep 2023 at a fertility Centre in Vapi, Gujarat. 350 cycles of embryo transfers (238 fresh and 112 frozen) were included in the study. Outcomes were measured as positive, clinical and ongoing pregnancy rate.
- **Result:** There was no statistically difference between positive pregnancy rate (55.5% versus 60.5%) Odds ratio (OR) 0.77, Clinical pregnancy rate (48.73% versus 57.14% OR 0.53%, 95% CI 0.1-2.5), ongoing clinical pregnancy rate (45.4% versus 51.8% Or 1.4, 95% CI 0.29-6.66 in Fresh and FET cycles respectively.
- **Conclusion:** Although the FET cohort showed higher rates of positive biochemical, clinical and ongoing pregnancies per transfer, these differeces did not achieve statistical significance. Therefore, both the option strategies can be used.
- **Keywords :** A Fresh embryo transfer, Cryo preservation, Frozen thawed embryo transfer, Artificial reproductive technique, Clinical pregnancy rate.

INTRODUCTION

According to WHO, infertility is defined as disease of male or female reproductive system who fail to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse ⁽¹⁾. Globally infertility is a public health problem as the age of marriage is delayed in both men and women compared to previous generation , and due to modified life style changes , the incidence of infertility has increased drastically.

Around 17.5 % of the adult population roughly 1 in 6 world wide experience infertility showing the urgent need to increase access to affordable, high quality fertility care for those in need⁽²⁾

The lifetime prevalence was 17.8% in high income countries and 16.5% in low- and middle-income countries ⁽²⁾

Currently , Assisted reproductive technique has delivered over 8 million babies worldwide ⁽³⁾

The most dreaded complication of IVF cycle is OHSS (Ovarian hyper stimulation syndrome)

Devroey P et al ⁽⁴⁾ in 2011 developed the “freeze all” strategy in order to avoid ovarian hyper-stimulation syndrome.

It is a potentially life threatening condition where in fluid shifts from blood vessels to abdominal cavity. This can result in bloating, a higher risk of thrombosis, liver and kidney ischemia. The

hallmark of OHSS is an increase in the permeability of capillaries, resulting in a fluid shift from intravascular space to extravascular space compartments.

Vascular endothelial growth factor (VEGF) plays a critical role in the pathogenesis of OHSS by increasing vascular permeability

VEGF is secreted by the granulosa cells, and human chorionic gonadotropin (HCG) stimulates its secretion

Severe OHSS is associated with higher levels of VEGF ⁽⁶⁾

The other suggested factors that may act directly or indirectly on the development or severity of OHSS are, insulin like growth factor, transforming growth factor alpha and beta, basic fibroblast growth factor, platelet derived growth factor interleukin – 1B, and interleukin – 6 ⁽⁶⁾⁽⁷⁾

The intra-ovarian renin angiotensin system (RAS) is another pathophysiological mechanism implicated in OHSS. Furthermore, HCG activates the RAS, which is confirmed by the association of high renin activity in the follicular fluid of women with OHSS

Higher levels of VEGF and the RAS seem to play a role in development of OHSS ⁽⁸⁾

The transfer of fresh embryos is the usual practice in most IVF units when such embryos are available . In some cases , all embryos may be cryopreserved without a fresh embryo transfer, most commonly to prevent ovarian hyperstimulation syndrome when excess follicle development has occurred. After ovarian recovery, embryos are thawed and transferred to the uterus after a programmed physiologic cycle of hormone replacement to prepare the endometrium. With fresh-embryo transfer the medication given for ovarian stimulation or the resulting supraphysiological sex steroids may alter the endometrial receptivity ^{9,10} and adversely affect trophoblastic invasion or placentation¹¹.

The study aims to compare the pregnancy rate of fresh embryo transfers following ovarian stimulation with antagonist protocols to those of frozen-thawed embryo transfers into an artificially prepared endometrium after embryo cryopreservation during stimulated cycle.

OBJECTIVE

The primary objective of this retrospective cohort study is to compare the live birth rates between fresh & frozen-thawed embryo transfer

Secondary objective includes comparing outcomes such as

- Biochemical pregnancy
- Clinical pregnancy
- Miscarriage rates
- Ectopic pregnancy and to know incidence of multiple pregnancies like twins/triplets

By analyzing these parameters, the study aims to provide robust evidence to guide clinical practice and enhance patient outcomes in ART

STATEMENT OF PROBLEM

In ART, embryo transfer techniques in a critical component for success rate of IVF cycles.

Fresh embryo transfers involve transferring embryos shortly after fertilization in the same cycle of ovulation induction.

In frozen-thawed embryo transfers, embryos are frozen for future use and thawed before being transferred

There are many studies with mixed varying results for fresh vs frozen-thawed embryo transfer regarding effectiveness and safety of these procedures and the decision-making process for clinicians remain complex and multifaceted.

It is therefore essential to analyze large scale data retrospectively to determine the efficacy and safety of these two methods

HYPOTHESIS

- There is significant difference in the live birth rate of fresh vs frozen-thawed embryo transfers in ART
 - There are low incidences of OHSS in frozen-thawed ET when compared to fresh ET
 - The overall pregnancy rates in biochemical, clinical and ongoing clinical pregnancies may vary between procedures
- The result of the study would contribute to our understanding of the comparative effectiveness & safety of these two approaches, which will help in clinical decision making

RESEARCH METHODOLOGY

Study design – It was a retrospective cohort study

Data collection – We collected the data from a register at a fertility centre from August 2022 to September 2023 in Vapi, Gujarat

Inclusion criteria

- Women from 21- to 49-year-old who underwent ICSI by ovarian stimulation with antagonist protocol with D3 and blastocyst transfers.
- Serum progesterone of less than or equal to 1.5ng/ml on the day of trigger in a fresh cycle and day 12 for frozen ET.
- Endometrium ≥ 8 mm, grade 1 vascularity on the day of transfer.
- Transfer of 1 or 2 good quality embryos only 6-10 cells with $\leq 20\%$ fragmentation.

Exclusion criteria

- Patients with a history of recurrent pregnancy loss
- Uterine pathology- Asherman syndrome, thin endometrium, endometrial polyps and fibroid.
- Patients undergoing ART using embryo donation /gamete /surrogate pregnancy.

Procedure

- Ovarian stimulation was done with recombinant gonadotrophins or highly purified urinary gonadotrophins from day 2 or day 3 of menstrual cycle after confirming the FSH level between 3-10 IU/L and LH level $< 5-7$ IU/ml and serum estradiol level between 20 to 80 pg/ml.
- The dose of gonadotrophins was selected on patients age, BMI, AMH, AFC and modified according to follicular response.

Protocol

Mainly antagonist protocol was used.

Follicular growth monitoring was done using transvaginal sonography.

When follicle reached 14mm, Inj cetrorelix acetate (0.25mg) s.c was added daily till the day of trigger. Once follicles reached 18 to 20mm with adequate number, ovulation was triggered using human chorionic gonadotrophin (hcG) (Inj Ovitrelle 500mcg i.e. 13000 IU) or combination of hcG and Inj Buserelin acetate(0.5mg) s.c endometrial thickness was measured along with serum LH, estradiol and progesterone values on the day of trigger. Ovum pick up was performed 34 to 36 hours after trigger injection.

The process of Embryo transfer

Fresh ET cycle:

Embryo transfer was done if serum progesterone level on day of trigger was <1.5ng/ml. Following the egg retrieval, either a day 3 embryo transfer or day 5 blastocyst transfer was done depending on availability of high-quality embryo. A maximum of 2 embryos were transferred per cycle to optimize the chances of successful implantation. Any excess embryos were cryopreserved using kitazato embryo vitrification kit (cryotop method)

When the endometrial thickness exceeded 8mm, luteal phase support commenced with the administration of micronized natural progesterone supporting twice daily each containing 400mg administered vaginally. This regimen began from the day of oocyte retrieval until 12th week post conception.

Frozen ET cycle:

During frozen embryo transfer cycle, patients underwent FET if they did not achieve pregnancy in prior attempts and surplus embryos were available for cryopreservation.

The ET could not be performed during the initial attempt due to suspected risk of ovarian hyperstimulation, serum progesterone levels greater than 1.5ng/ml, inappropriate endometrium on the day of transfer.

Following ovarian down regulation with daily injections of triptorelin acetate 0.1mg daily starting 4-5days before the date of expected menses for 5 doses, patients were administered estradiol valerate tablet 4mg BD for 5days followed by TDS for next 15days starting from day 2 of menses.

If the endometrial thickness exceeded serum, vaginal progesterone was administered using the same regimen as in fresh ET cycles. Following four days of progesterone regimen, frozen embryos were thawed and transferred.

Luteal phase support with estradiol valerate and micronized vaginal progesterone persisted until 12weeks post conception.

Frozen embryo thawed using kitazato embryo thawing media and transferred.

Upon consideration of the availability of high-quality embryos and a maximum of 2 embryos were transferred per cycle following provision of written informed consent.

Statistical analysis

The statistical analysis of the data was conducted utilizing SPSS (Statistical Package for Social Science version 28.0, Chicago Illinois, USA) software.

Differences between variables in the two groups were assessed using the students t- test, while the chi square test was employed – to compare categorical variables. A p-value less than 0.01 was deemed statistically significant for all analyses.

ANALYSIS

Table 1

There were no notable differences observed in patient’s characteristics between the groups (p<0.05). In Fresh ET group, unexplained infertility was most prevalent cause while in Frozen ET group, it was male factor infertility .

Table 1: Patient characteristics

		Fresh ET	Frozen ET	P-values
1	Types of infertility			
*	Primary	192	94	0.259
*	secondary	56	18	0.259

2	Causes of infertility			
*	Male factor	94	42	0.2
*	Female factor	34	28	
*	Unexplained	110	42	
3	Age of females	30.2 ± 4.4	29.82 ± 4.02	0.475
4	Age of males	33.3 ± 3.17	32.8 ± 4.02	0.652
5	Duration of fertility	4.08 ± 1.44	4.39 ± 1.82	0.22
6	S. AMH	2.52 ± 0.81	2.4 ± 0.72	0.355
7	Antral follicle count (both ovaries)	9.45 ± 1.72	8.93 ± 1.42	0.052

Table 2: Pregnancy rates in fresh and frozen thawed ET

It represents while there were no statistically significant differences between

- positive pregnancy rate (55.5% vs 60.5%)
- clinical pregnancy rate (48.73% vs 57.14%) &
- on going pregnancy rate (45.4% vs 51.8%)

Table 2 Pregnancy rate in fresh and frozen embryo transfers.

	Fresh ET	Frozen ET	Odds ratio	p-value
<u>Biochemical pregnancies</u>	130	68	0.77	0.45
*B-hcg Positive				
*B-hcg Negative	108	44	-	-
<u>Clinical pregnancies</u>	116	64	0.53	0.42
*Gestational sac present				
*Gestational sac absent	14	4	-	-
<u>Ongoing clinical pregnancies</u>	108	58	1.4	0.6
*Fetal cardiac				

activity present				
*Fetal cardiac activity present	8	6	-	-

Table 3: Secondary outcome measures

	Fresh ET	Frozen ET	Odds ratio	p-value
1. Viable pregnancy	108	58	1.4(0.29-6.666)	0.674
2. Miscarriage	8	6	1.4(0.29-6.666)	0.674
3. Ectopic pregnancy	0	0	1.4(0.29-6.666)	0.674
4. Singleton	60	40	0.56(0.23-1.36)	0.234
5. Twin	54	16	0.56(0.23-1.36)	0.234
6. Triplets	14	2	0.56(0.23-1.36)	0.234

Conclusion

Although the frozen ET cohort displayed higher rates of positive biochemical, clinical and ongoing pregnancy per transfer, these differences did not achieve statistical significance. Therefore, both the transfer strategies remain viable option, although there is a tendency favoring towards the freeze all strategy.

Recommendation

- There is scope for additional prospective studies with large sample sizes to validate the findings of this retrospective study and provide more robust evidence.
- The long term follow-up assessments to evaluate not only short-term outcomes such as pregnancy rates but also outcomes such as obstetric complications, neonatal outcomes and child development can be done.

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