

Original Research Article

EFFECT OF EXTENDED CULTURE OF DAY 3 THAWED EMBRYOS ON CLINICAL PREGNANCY RATE

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ABSTRACT

Background: Frozen embryo transfer (FET) has become an integral component of assisted reproductive technology due to advances in vitrification techniques and improved embryo survival. However, the optimal duration of post-thaw embryo culture before transfer remains controversial. Extended culture permits assessment of continued embryonic development and may facilitate better embryo selection. This study was conducted to evaluate the effect of extended culture of Day 3 thawed embryos on clinical pregnancy rate in frozen embryo transfer cycles

Materials and Methods: A prospective observational study was conducted in the Department of Reproductive Medicine and Surgery, NIMS Hospital and EVAA Hospital, Jaipur, over 18 months. A total of 107 women undergoing frozen embryo transfer were included. Patients were allocated into three groups according to thawing and transfer schedule: Group I: Day 3 thawing and Day 3 transfer (n=30). Group II: Day 5 thawing and Day 5 transfer (n=35). Group III: Day 3 thawing followed by extended culture until Day 5 transfer (n=42). Baseline demographic variables, biochemical pregnancy, clinical pregnancy, implantation, miscarriage, multiple pregnancy and ongoing pregnancy rates were compared.

Results: Baseline characteristics including age, BMI, antral follicle count and endometrial thickness were comparable among the three groups. The biochemical pregnancy rate was significantly higher in Group III (71.4%) compared with Group I (50%) and Group II (42.9%) (p=0.03). Clinical pregnancy rate was highest in Group III (64.3%), although statistical significance was not achieved (p=0.06). Implantation rate was significantly higher in Group III (37.1%) than Group I (22.7%) and Group II (14.7%) (p=0.002). No significant differences were observed in miscarriage, multiple pregnancy or ongoing pregnancy rates.

Conclusion: Extended culture of Day 3 thawed embryos before frozen embryo transfer improves implantation and biochemical pregnancy rates and shows a favourable trend toward higher clinical pregnancy rates without adversely affecting ongoing pregnancy or miscarriage rates.

Keywords: Frozen embryo transfer, embryo culture, vitrification, implantation, IVF, blastocyst, embryo selection.

INTRODUCTION

Cryopreservation has revolutionized assisted reproductive technology by allowing efficient utilization of supernumerary embryos while reducing the incidence of ovarian hyperstimulation syndrome and multiple pregnancy. Improvements in

vitrification techniques have resulted in survival rates exceeding 90%, making frozen embryo transfer (FET) an essential component of modern IVF practice.^[1]

Embryo selection after warming remains one of the most important determinants of treatment success. Two principal approaches are currently practiced. The first involves short post-thaw culture (2–4

hours), where embryo survival is assessed by blastomere integrity. The second strategy involves extended culture for approximately 24–48 hours, allowing evaluation of continued cleavage or blastocyst development before transfer.^[2,3]

Extended culture offers the theoretical advantage of selecting embryos with greater developmental competence while allowing naturally arrested embryos to be excluded. Several studies have suggested improved implantation following extended culture, whereas others have demonstrated comparable pregnancy outcomes between immediate and delayed transfer. Consequently, there remains no consensus regarding the optimal duration of post-thaw culture.^[4-8]

The present prospective observational study was therefore undertaken to evaluate whether extended culture of Day 3 thawed embryos improves pregnancy outcomes in frozen embryo transfer cycles.^[9,10]

MATERIALS AND METHODS

Study Design: Prospective observational study.

Study Setting: Department of Reproductive Medicine and Surgery, NIMS Hospital and EVAA Hospital, Jaipur.

Study Duration: July 2024 to December 2025.

Participants: A total of 107 infertile women undergoing frozen embryo transfer were enrolled after satisfying eligibility criteria.

Inclusion Criteria

- Age 21–40 years
- BMI 18–29.9 kg/m²
- Endometrial thickness ≥ 7 mm
- Frozen embryo transfer cycles using good-quality embryos

Exclusion Criteria

- Uterine abnormalities

- Endocrine or autoimmune disorders
- Fresh embryo transfer cycles
- Poor-quality embryos

Controlled Ovarian Stimulation: Patients underwent antagonist protocol stimulation followed by oocyte retrieval 35 hours after trigger administration. Intracytoplasmic sperm injection (ICSI) was performed in all cycles. Fertilized embryos were cultured until Day 3 and vitrified using standard cryopreservation protocols.^[11]

Frozen Embryo Transfer Protocol: Hormone replacement therapy was used for endometrial preparation. Embryos were warmed using sequential thawing media and subsequently transferred according to study group allocation.

Group I: Day 3 thaw → Day 3 transfer

Group II: Day 5 thaw → Day 5 transfer

Group III: Day 3 thaw → extended culture → Day 5 transfer

Outcome Measures

Primary Outcome: Clinical pregnancy rate.

Secondary Outcomes:

- Biochemical pregnancy
- Implantation rate
- Ongoing pregnancy
- Multiple pregnancy
- Miscarriage

Statistical Analysis: Continuous variables were expressed as mean $\pm$ SD and analysed using ANOVA. Categorical variables were analysed using Chi-square test. Statistical significance was considered at $p < 0.05$.

RESULTS

The study included 107 women. Baseline characteristics were comparable among the three groups with respect to age, BMI, antral follicle count and endometrial thickness.

Table 1: Baseline Characteristics

Variable	Group 1	Group 2	Group 3	P value
Age (years)	32.6 $\pm$ 3.5	31.9 $\pm$ 3.6	32.2 $\pm$ 3.2	0.70
BMI	24.96 $\pm$ 2.32	24.59 $\pm$ 1.83	24.84 $\pm$ 2.29	0.76
AFC	12.80 $\pm$ 1.54	12.77 $\pm$ 1.68	12.29 $\pm$ 1.23	0.23
Endometrial thickness	8.53 $\pm$ 0.88	8.54 $\pm$ 2.17	8.36 $\pm$ 0.74	0.83

Biochemical pregnancy rate differed significantly among groups. Clinical pregnancy rate demonstrated a strong positive trend favouring extended culture.

Implantation rate was significantly improved after extended culture.

Table 2: Pregnancy Outcomes

Outcome	Group I	Group II	Group III	P value
Biochemical pregnancy	50%	42.9%	71.4%	0.03
Clinical pregnancy rate	43.3%	40.0%	64.3%	0.06
Implantation rate	22.7%	14.7%	37.1%	0.002
Miscarriage rate	7.7%	14.3%	11.1%	0.86
Ongoing pregnancy	92.3%	78.6%	88.9%	0.52

DISCUSSION

The present study demonstrates that extended culture of Day 3 thawed embryos before transfer

significantly improves implantation rate and biochemical pregnancy while showing a clinically meaningful trend toward improved clinical pregnancy.

Baseline demographic variables were well matched among study groups, minimizing confounding factors. Maternal age, BMI, ovarian reserve and endometrial thickness have all been recognized as major determinants of IVF success; therefore, comparability strengthens the validity of the observed differences.^[12,13]

The implantation rate of 37.1% in the extended culture group was significantly superior to both comparison groups. This supports the concept that embryos capable of continued development following thawing possess greater implantation potential.

Our findings are consistent with studies by Albahlol et al., Li et al., and Wang et al., which reported improved embryo selection following extended culture. Conversely, studies by Agha-Rahimi et al., Jin et al., and Ramji et al. found comparable pregnancy outcomes between short and prolonged culture strategies. Differences in embryo quality, laboratory protocols, and patient selection may explain these discrepancies.^[4-11]

Although clinical pregnancy rate did not achieve statistical significance ($p=0.06$), the absolute improvement from approximately 40% to 64% is clinically relevant and suggests that a larger sample size may have demonstrated statistical significance. Importantly, extended culture was not associated with increased miscarriage or reduced ongoing pregnancy, indicating that prolonged culture does not adversely affect embryo viability.

CONCLUSION

Extended culture of Day 3 thawed embryos before frozen embryo transfer significantly improves implantation and biochemical pregnancy rates. Although improvement in clinical pregnancy did not reach statistical significance, the observed trend suggests a potential clinical benefit. Larger multicentre randomized controlled trials are required to establish the optimal post-thaw culture strategy.^[4-9]

Limitations

- Single-centre study
- Moderate sample size
- No live birth analysis
- No genetic testing of embryos

- Lack of randomization

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