Editorial

# "Freeze all" protocol – Has the debate concluded?

## INTRODUCTION

Assisted reproductive technology has progressed over the last few years, both in terms of techniques and results. An important contribution to this has been the evolution of successful cryopreservation techniques leading to improved pregnancy rates with frozen embryo transfer (FET) over the years.[1] With introduction of vitrification, the cryosurvival and pregnancy rates are increased in comparison to slow freezing protocols. Cryopreservation has become an integral part of an assisted reproductive technologies (ART) program and is not just an additional elective procedure. It increases cumulative pregnancy rate and also enables clinicians to go in for single embryo transfer (ET) as the rest of the embryos can be preserved for another transfer. Besides, it is essential in case of preimplantation genetic diagnosis and screening, ovarian hyperstimulation, or where there is a poor endometrial development. However, recently with better results, the policy for "freezing all" has been introduced in some clinics and needs evaluation.

The "freeze all" policy, that is, all embryos being frozen and transferred in the next cycle, was introduced to avoid ovarian hyperstimulation syndrome (OHSS). It allowed the ovulation trigger to be a GnRH agonist to avoid hyperstimulation, which the human chorionic gonadotropin (hCG) trigger would have caused. As implantation and pregnancy rates are lower with GnRH agonist trigger, due to its luteolytic action leading to poor endometrial receptivity, transfer is done in the next cycle. Besides, freezing also avoids appearance of OHSS due to increased β hCG levels if pregnancy occurs in the same cycle.

As "freeze all" policy came in for OHSS, various groups started analyzing data of FET and fresh ET. A systemic review and meta-analysis found frozen cycles to have a better pregnancy rate (odds ratio, OR, 1.32; 95% confidence interval, CI, 1.10–1.59).[2] This and similar studies have brought a trend to freezing embryo in all cycles and transferring in the next cycle, as it is felt that a more physiological environment in an unstimulated cycle would yields better results. Before we can make "freeze all" a norm, it is important to evaluate its immediate and long-term impact on pregnancy and the baby. Currently, the debate on this policy is ongoing, as risks and benefits need evaluation before universal implementation.

## IMPACT OF CONTROLLED OVARIAN STIMULATION (COS) ON PREGNANCY OUTCOME

### Endometrial receptivity

One of the primary causes cited for a better result with FET was an adverse effect on endometrial receptivity by ovarian stimulation.[2] Superovulation may alter the window of implantation and cause advancement in development of endometrium. Premature appearance of endometrial nuclear channels systems, subnuclear vacuoles, pinopodes, and secretory changes indicates advanced endometrial maturation following controlled ovarian hyperstimulation, especially in cases where large number of oocytes are recovered. Advanced endometrial development of more than 3 days leads to impaired implantation.[3,4]

It is thought that the immune environment and NK cell concentration are altered. A difference was found in gene profiling of endometrium in stimulated and unstimulated cycles and there was an alteration in endometrial gene expression advancing endometrium by 2–4 days.[5,6]

Superovulation also causes a high progesterone (P) level at the time of hCG trigger which is responsible for poor implantation rates, as it causes advancement in endometrial development. A study showed a lower pregnancy rate with OR of 0.67 if progesterone >1.1 ng/ml. This was not seen in frozen cycle and donor cycles.[7]

However, it is just not the rise in progesterone which is causing a lower pregnancy rate. A recent study showed a better pregnancy rate with ″freeze all" policy and FET in cases where progesterone levels were less than 1.5 ng/ml on the day of transfer. When fresh ET was compared with FET in this study, the implantation rate was 19.9 and 26.5%; clinical pregnancy rate was 35.9% and 46.4%; and ongoing pregnancy rate was 31.1% and 39.7%.[8] This shows that even where progesterone levels are normal (P levels ≤1.5 ng/ml), endometrial receptivity may have been impaired by COS. This could also be because of other factors affecting

endometrial receptivity and, development of fetus and placenta, leading to poor ongoing pregnancy rates.

## Pregnancy outcomes

### *Low birth weight, preterm labor, and small for gestational age*

Besides, causing a poor implantation rate, it has been suggested that advanced endometrial development may also impact the development of a successfully implanted embryo and may lead to an adverse obstetric outcome. Animal studies have shown that embryos may show abnormal development and placentation.[9] It has been demonstrated that when the same mother conceived both with fresh and FET, low birth weight (LBW) in pregnancy with fresh ET was found. Results were similar in donor cycles between fresh and FET in terms of length of pregnancy and birth weight, showing that it is not the freezing process but the environment caused by COS which is responsible.[10-12] A study showed that singletons born after FET had a lower risk of LBW (adjusted odds ratio, aOR) 0.81, preterm birth (aOR 0.84), and small for gestational age (aOR 0.72), compared with singletons born after fresh invitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI).[13]

### *Perinatal outcome*

There is no randomised controlled trial (RCT) studying effect of FET and fresh transfers on pregnancy and the baby. However, pooled data from various observational studies have been compiled into systemic reviews and meta-analysis. A recent review of 11 studies showed that singleton pregnancies after the transfer of frozen thawed embryos were associated with better perinatal outcomes compared with those after fresh IVF embryos. Antepartum hemorrhage, preterm birth, small for gestational age, LBW, and perinatal mortality were lower in women who received frozen embryos. No difference in congenital anomalies and admission to neonatal intensive care unit was seen in the two groups. Authors concluded that pregnancies with FET had a better perinatal and obstetric outcome than fresh ET.[14]

### *Placental pathology – Antepartum hemorrhage and preeclampsia*

A study showed that the ART pregnancies had more antepartum haemorrhage (APH) 6.7% vs 3.6% for placenta previa (PP) and 2.6 vs 1.1 for abruptio placentae (AP). Higher incidence was seen in fresh cycles vs frozen cycles. This suggests that the events around implantation may be responsible for abnormal placentation.[15] Studies have suggested that superovulation causes an impaired trophoblast differentiation which leads to placental pathology and affects fetal growth leading to small for gestational age fetus.[16] It also causes a higher incidence of placental pathology syndromes like preeclampsia which have been reported in COS.[17] This has been well demonstrated in mouse where superovulation led to small placentas. A histological difference between these placenta and those of nonstimulated mice was found suggesting that areas of nutrient transfer are affected. This difference suggested that environment and hormonal milieu are responsible for early trophoblastic differentiation.[18] Excessive estrogen alters the invasion of extravillous trophoblasts affecting uterine blood vessel development and dynamics.[19] Vascular endothelial growth factor (VEGF) alterations can cause placental insufficiency by leading to abnormal trophoblastic invasion. This is often seen in preeclampsia which has a larger incidence in OHSS where estradiol levels are high.[6]

### *Effect on gamete and embryo development*

The other factor that can affect ongoing pregnancy rates is related to affect on gamete development. Methylation occurs during gamete development and sex-specific methylation patterns need to be maintained. Superovulation can have effect on methylation of oocyte and postimplantation embryo, especially methylation of certain paternally imprinted genes.[20] Loss of methylation has been known to effect fetal growth and placentation. This may also affect the long-term health of the baby.[21]

### *Ectopic pregnancy*

It has been postulated that ovarian stimulation increases the incidence of ectopic pregnancy (EP). Freezing all embryos and transferring in the next cycle should decrease ectopic pregnancy risk. There are studies which show that ectopic pregnancy risk is double in fresh ET vs FET (1.97% vs 1.01%). The EP per clinical pregnancy was fresh vs FET 4.62 % vs 2.22%.[22] High estrogen progesterone levels like in superovulation would influence the incidence of ectopic pregnancy and freezing and transferring in the next cycle would lead to a decreased risk. It shows that adverse effects on endometrium in COS may lead to the increased ectopic pregnancy.

## IMPACT OF CRYOPRESERVATION ON PREGNANCY OUTCOME

### Cryosurvival

An important factor on which results of freezing are dependent is the quality of the freezing program and skill of embryologist which could be subjective and vary. However good the program is, the embryos are still put through the risk of cryopreservation where complete survival is not guaranteed by any ART laboratory.

### Congenital anomalies

One of the concerns of cryopreservation was whether there is an increased risk of congenital malformations which may be due to cryoprotectants or the process of freezing which causes shrinkage of the cell. Various studies have highlighted that the rate of malformations remains the same in fresh and FETs: 4.2% in FET group, 4.5% in fresh ET group, and 3.2% in reference group. There is also no increase of a particular organ system anomaly with FET.[23] While analyzing the data, it is to be remembered that it is important to include all pregnancy terminations for congenital anomalies and not just the live births.

### Long-term health of child

Long-term health status of children born by FET has been analyzed. The number of hospital visits, the risk of admissions, and cause for visit were similar in fresh and FET groups during the 3-year follow-up.[24]

### Large for gestational age (LGA)

FET singleton pregnancy has shown to be at risk of LGA in many of the studies. The LGA was found with sibling cohort where one is fresh and other FET showing that it is the freezing thawing process which is responsible, not maternal factors. In a recent meta-analysis, LGA and macrosomia in FET vs fresh ET were aOR 1.54 (95% CI 1.31–1.81) and aOR 1.64 (95% CI 1.26–2.12), respectively. The corresponding figures for FET vs natural conception singletons were for LGA aOR 1.32 and macrosomia aOR 1.41, respectively.[25] With FET, there is a lower risk of small for gestational age (SGA) and a larger risk of LGA compared to fresh transfer and natural conception. FET also had a higher risk of post-term birth (aOR 1.40, 95% CI 1.27–1.55), compared with singletons born after fresh IVF and ICSI.[13] LGA babies have the additional risk of shoulder dystocia, birth asphyxia, hypoglycemia, still birth, and a higher perinatal morbidity and mortality.

We recognize that with "freeze all," there are certain logistics advantages like decreased requirement for intensive monitoring of hormone levels and decisions based on progesterone level. It has been suggested in a recent study that scheduling of oocyte retrieval can become flexible, as endometrial receptivity is not a concern. They also suggested that stimulation can be started at any phase of the cycle, like luteal phase, and does not need to be scheduled with the period as is done in regimes for fertility preservation in cancer patients.[26] This would ease both the patient and the IVF laboratory with timing of oocyte retrievals. While considering convenience, it is essential to have an FET regime which does not require too many visits but at the same time works as well.

However, the meta-analysis and trials, which were included in evaluating FET cycles, have drawbacks in methodology. Most of the data put in meta-analysis on which decisions are being based is nonrandomized. Only a few RCT are included which are only on hyper responders. Hence, it must be viewed with caution. The research methodology, definitions used, population studied, method of cryopreservation, stage of freezing (cleavage or blastocyst), and preparation of endometrium are not uniform. Confounding factors like maternal age weight, medical problems, and smoking which can affect results have not been considered. There is a bias in these studies as most women who went in for freezing had many good quality embryos which could be frozen. This in itself creates a bias when compared to women undergoing fresh ET which had all grades of embryos. Freezing is only done for embryos with good grading. There is no study with live birth rates which is the ultimate success of an ART cycle. In fact, a very recent study showed no significant differences between biochemical pregnancy rate (23% vs 18.8%), gestational sac, and fetal heart activity (87.2% vs 93.6%) in fresh ET and FET cycles.[27] Hence, there is definite need to have randomized control trials before giving the final verdict to change protocols to a "freeze all" policy.

As "freeze all" policy still remains debatable, it is important to include a cost benefit analysis and patient's convenience into the picture.[27] Patients may find extension of the treatment to the next cycle tiresome and stressful and most patients prefer a shorter time to outcome, that is, pregnancy.

## CONCLUSION

It has been established that abnormal hormonal milieu in ovarian stimulation leads to adverse outcomes in pregnancy and that the perimplantation environment affects implantation and further fetal growth. Physiological conditions in FET may lead to optimal implantation, placentation, and fetal growth. Vitrification has yielded excellent results in terms of post-thaw recovery and pregnancy rates but a higher incidence of LGA babies needs further evaluation. Optimal endometrial preparation regime for FET is still evolving. At present, individualized approach is needed rather than a "freeze all" protocol which considers clinical parameter, embryology outcomes of that cycle, and patients' characteristics. There

may be a sect of patients who would benefit more than others like those with elevated progesterone levels, OHSS, endometrial embryo asynchrony, and those undergoing preimplantation genetic diagnosis and screening. More randomized controlled trials are needed before a verdict for "freeze all" can be passed. The goal in ART is not just to give the patient a pregnancy but give her a healthy and safe pregnancy yielding a live birth.

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## Conflicts of interest

There are no conflicts of interest.

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