

Is Frozen Embryo Transfer Cycle Associated with a Significantly Lower Risk of Ectopic Pregnancy in Infertile Women Compared with Fresh Embryo Transfer? Systematic Review and Meta-Analysis

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Abstract

Background: Ectopic pregnancy remains a major cause of maternal morbidity and mortality in the first trimester of pregnancy. The condition is more likely in patients who conceive through assisted reproductive technologies (ART), but its risk within that population may vary according to the infertility factor or the ART procedure type.

Aims: The aim of this review was to determine whether frozen embryo transfer confers a lower risk of ectopic pregnancy compared with fresh embryo transfer.

Methods: A literature search of MEDLINE, EMBASE and LILACS was performed using the key words 'ectopic pregnancy', 'frozen' and 'embryo transfer'. Five studies were selected for the meta-analysis, comprising 9109 clinical pregnancies that resulted from IVF/ICSI. Data were compiled and analysed using Peto's method to determine odds ratio (OR).

Results: A total of 6578 pregnancies were evaluated after fresh embryo transfer and 2531 pregnancies after frozen embryo transfer. We found that frozen embryo transfer was associated with a significantly lower risk of ectopic pregnancy (OR 0.38, 95% confidence interval 0.27 - 0.56; $p = 0.47$).

Conclusions: The combined data presented in this meta-analysis show a lower risk of ectopic pregnancy following frozen embryo transfer.

Keywords: Frozen Embryo Transfer; Ectopic Pregnancy; ART

Background

Ectopic pregnancy (EP) describes a pregnancy where the developing embryo implants outside the endometrial lining of the uterus. EP most commonly occurs in a fallopian tube, but can also be found in the cervix, ovaries or abdomen [1]. Importantly, despite advances in early diagnosis, EP remains a major cause of maternal morbidity and mortality in the first trimester of pregnancy [2].

Tubal EP occurs in 2% to 5% of pregnancies resulting from assisted reproductive technologies (ART) [3]. The occurrence of EP during ART has been explained by multiple hypotheses, such as altered tubal morphology, controlled ovarian stimulation, the stage of embryo development, the number of embryos transferred, and fresh vs frozen embryo transfer [1]. Because EP represents a failure of endometrial

implantation, differences in the hormonal or biochemical environment within the uterus at the time of embryo transfer and implantation may account for the higher rate of EP reported with the use of ART [2].

During *in vitro* fertilization (IVF) treatments the uterine milieu differs between fresh and frozen-thawed embryo transfer cycles [2]. Frozen embryos may be transferred either in a natural cycle or with the use of hormonal supplementation that suppresses development of multiple ovarian follicles. Thus, frozen embryo transfer occurs in a uterine environment that more closely resembles that of spontaneous conception [2]. In contrast, fresh embryo transfer occurs within a supraphysiologic hormonal environment resulting in increased uterine contractions as a result of ovarian stimulation, and dysfunction of the uterine musculature due to high progesterone levels [4]. Interestingly, the use of embryos with high implantation potential could have a protective role against EP [3]. Recent studies have shown that embryo transfer at the blastocyst stage is associated with a lower or similar risk compared with transfers at the cleavage stage [5]. Because ectopic pregnancies are a significant complication of IVF, it is important to better understand specific factors in ART that may influence their occurrence.

Objectives

The purpose of our systematic review and meta-analysis was to assess whether the risk of ectopic pregnancy following frozen embryo transfer was significantly lower than that for fresh embryo transfer.

Methods of the Review

Criteria for selecting studies for this review

Types of studies

The search was open to all published retrospective case-control or cohort studies and prospective randomised controlled trials, with at least one arm dedicated to compare ectopic pregnancy in frozen embryo transfer cycle with fresh embryo transfer. Only studies in English or Spanish were included.

Types of participants

Infertile women, defined as those who attempted one year or more of regular unprotected intercourse without the possibility to conceive, who underwent ART.

Types of interventions

Trials including fresh and frozen embryo transfer comparing the risk of ectopic pregnancy between both methods.

Types of outcome measures

Primary outcome: Risk of ectopic pregnancy in fresh versus frozen embryo transfer. EP was defined as a pregnancy with an extra-uterine gestational sac observed on an ultrasound scan or by laparoscopy or pregnancy of unknown location that required treatment.

Secondary outcomes: Risk of ectopic pregnancy in day-3 thawed embryo transfer versus blastocyst thawed embryo transfer.

Exclusion criteria

Age limit, cause of infertility and number of embryos transferred were not considered as exclusion criteria, but differences between the two groups - frozen and fresh embryo transfer cycle - were not accepted in the studies included in this review.

Trials that defined clinical pregnancy only as the identification of an embryo with positive cardiac activity (and not as the presence of a gestational sac with or without foetal heartbeat), by a transvaginal ultrasound, were excluded unless they described the total number of pregnancies achieved per arm.

Search strategy for identification of studies

A literature search of MEDLINE, EMBASE and LILACS was performed using the key words (“Pregnancy, Ectopic”[Mesh]) AND “Embryo Transfer”[Mesh] AND frozen).

The search was performed for eligible studies from 2000 up to 2016.

Once articles were selected by titles and abstracts, these studies were reviewed extensively to determine their appropriateness for inclusion in this meta-analysis.

We also manually searched the reference list of relevant articles looking for potential additional studies to include.

Data Collection and Analysis

We checked the abstracts of studies generated by the search strategy for relevance, and acquired full reports of any study that might be suitable for the review. Two authors extracted data and checked them independently (JES and CM). Where there was uncertainty regarding eligibility, a third reviewer (RP) also assessed the study and a decision reached through discussion.

Extracted data were transferred to a standard form that included reference, study type, main outcome measure, study population, clinical pregnancy conceived from fresh and frozen embryo transfers, number of ectopic pregnancy in both of them, and comments.

Studies were evaluated on the basis of the quality of the randomization procedure and allocation concealment as described in the Cochrane Handbook (Higgins 2011).

The meta-analysis was performed using STATA (STATA Corp, EEUU). The heterogeneity between studies was evaluated with Chi-squared test. Fixed effects model was used for the meta-analysis, and Peto’s method for statistical analysis was used to obtained the odds ratio (OR) to measure the association between frozen embryo transfer (ET) and EP, with confidence intervals of 95% (95% CI). The results are graphically expressed as a forest plot.

Results

In the first literature search, 29 studies were identified as potentially relevant for retrieval only by title. After screening by abstracts, 15 were excluded and 14 articles were selected to be reviewed extensively to determine their inclusion in this meta-analysis. Finally, 5 studies were included in the systematic review (Table 1).

Reference	Study Type	Main Outcome Measure	Study Population	Results
Shapiro B., <i>et al.</i>	Retrospective Cohort	To compare the rate of EP in autologous frozen ET to that after fresh ET	580 clinical pregnancies in frozen ET and 909 in fresh ET	A total of 2 (0.34%) ectopic pregnancies resulted from frozen ET and 37 (4.07%) from fresh ET
Ozgun K., <i>et al.</i>	Retrospective Cohort	To investigate which of the two blastocyst-transfer strategies (fresh ET or frozen ET) would provide the best possible outcomes in terms of peri-implantation and perinatal outcomes.	140 clinical pregnancies in frozen ET and 323 in fresh ET	A total of 0 (0%) ectopic pregnancies resulted from frozen ET and 5 (1.54%) from fresh ET
Sunny H., <i>et al.</i>	Retrospective Cohort	To assess the incidence of EP resulting from frozen blastocyst transfer compared with fresh blastocyst transfer in their IVF program over an 8-year period.	180 clinical pregnancies in frozen ET and 564 in fresh ET	A total of 5 (2.77%) ectopic pregnancies resulted from frozen ET and 10 (1.77%) from fresh ET
Keegan D., <i>et al.</i>	Retrospective Cohort	To assess the rate of EP and heterotopic pregnancy in fresh and frozen transfers in IVF cycles conducted from 1988 to 2003.	285 clinical pregnancies in frozen ET and 2788 in fresh ET	A total of 2 (0.70%) ectopic pregnancies resulted from frozen ET and 24 (0.86%) from fresh ET
Fang C., <i>et al.</i>	Retrospective Case-Control	To determine if there is a difference in the ectopic pregnancy rate with fresh versus frozen-thawed transfers in patients undergoing IVF-ET	1346 clinical pregnancies in frozen ET and 1994 in fresh ET	A total of 9 (0.66%) ectopic pregnancies resulted from frozen ET and 45 (2.25%) from fresh ET

Table 1: Description of clinical characteristics of the included studies.

Description of studies and methodological quality of included studies

Shapiro, *et al.* [3] performed a retrospective cohort study comparing 580 clinical pregnancies from frozen ET and 909 from fresh ET derived over an 8-year study period. They defined EP as a pregnancy accompanied by sonographic visualization of an extra-uterine gestational sac (including heterotopic gestations and treated persistent pregnancy of unknown location). From the frozen ET group, 2 (0.34%) had EP; from the fresh ET group, 37 (4.07%) had EP.

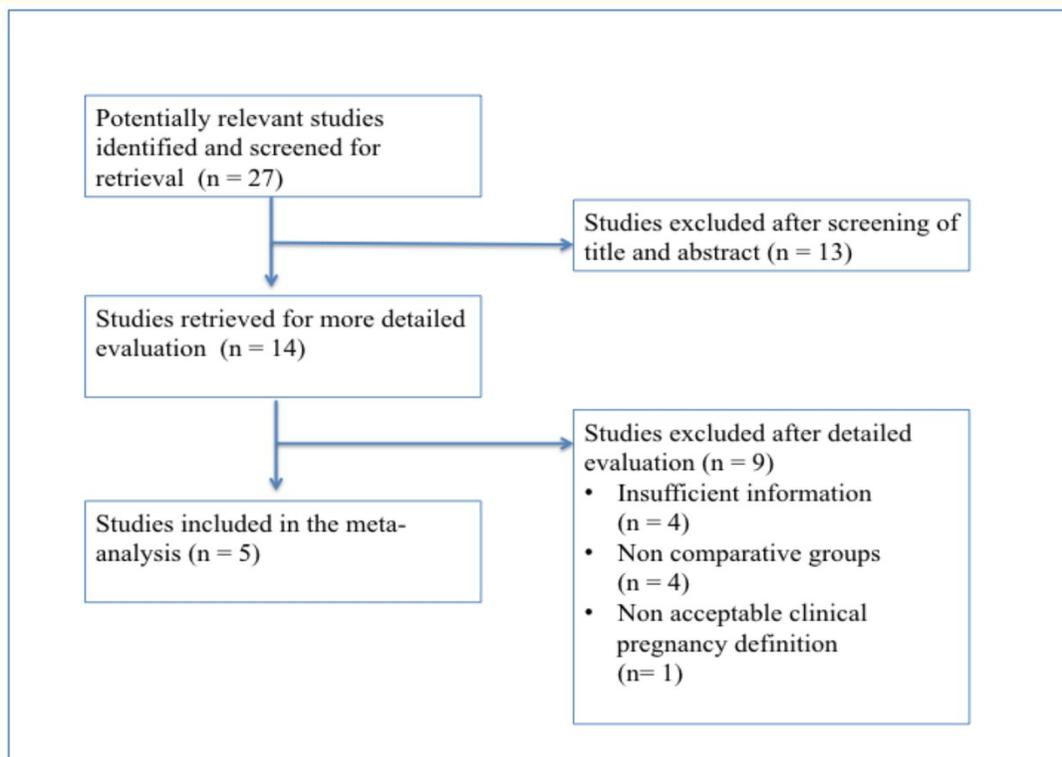


Figure 1: Flowchart to show the selection process of studies with regard to ectopic pregnancy risk after frozen vs. fresh ET.

Ozgur, *et al.* [6] compared the implantation and perinatal outcomes of fresh double blastocyst ET with vitrified-warmed double blastocyst frozen ET. They defined EP as a pregnancy with an extra-uterine gestational sac observed by ultrasound scan or by laparoscopy. A total of 463 clinical pregnancies were achieved, of which 0 (0%) EP resulted following frozen ET and 5 (1.54%) following fresh ET.

Sunny, *et al.* [7] reviewed all clinical pregnancies from fresh and frozen blastocyst transfers conceived in their IVF program between January 1998 and December 2005. They diagnosed EP by ultrasound or by laparoscopic visualization of a gestational sac in the fallopian tube, or by the absence of an intrauterine gestational sac and rising B-hCG levels after the failure of suction dilatation and curettage to reveal products of conception. A total of 744 clinical pregnancies were analysed: 5 (2.77%) EP resulted following frozen ET vs. 10 (1.77%) following fresh ET.

Keegan, *et al.* [8] defined EP as the presence of an extra-uterine location. They analysed 2688 clinical pregnancies from fresh ET and 285 from frozen ET, from which 2 (0.70%) EP resulted following frozen ET and 24 (0.86%) following fresh ET.

Fang, *et al.* [5] presented a retrospective case-control trial that included patients who became pregnant (intra-uterine and ectopic pregnancies) after receiving IVF in their reproductive clinic between June 2010 and November 2013. In a total of 3340 clinical pregnancies, they noted 9 EP (0.66%) following frozen ET and 45 (2.45%) following fresh ET.

In two studies patients underwent ovarian stimulation with gonadotropins (FSH, hMG or both) and pituitary suppression with GnRH antagonist [3,6]. In two others, the controlled ovarian hyperstimulation (COS) protocol consisted of GnRH agonist down-regulation followed by FSH/hMG [5,7]. The final study had no description of the COS protocol [8].

In this review, out of the five selected studies, 1 had its point estimate in favour of fresh embryo transfer, while in three of five the studies, the confidence interval laid in the area favouring this ART. Therefore we do not consider that there is a serious concern in terms of publication bias.

Individually, 2 of 5 studies showed a significantly lower risk of ectopic pregnancy following frozen ET compared with fresh ET [3,5]. In our subsequent meta-analysis, we evaluated a total of 6578 pregnancies after fresh ET and 2531 pregnancies after frozen ET. The heterogeneity of studies was moderate ($I^2 = 58.4\%$; $p = 0.047$). For the primary outcome of ectopic pregnancy, we found that frozen ET was associated with a significantly lower risk of EP compared to fresh ET (OR 0.38, 95% CI 0.27 - 0.56; $p = 0.47$) (Figure 2).

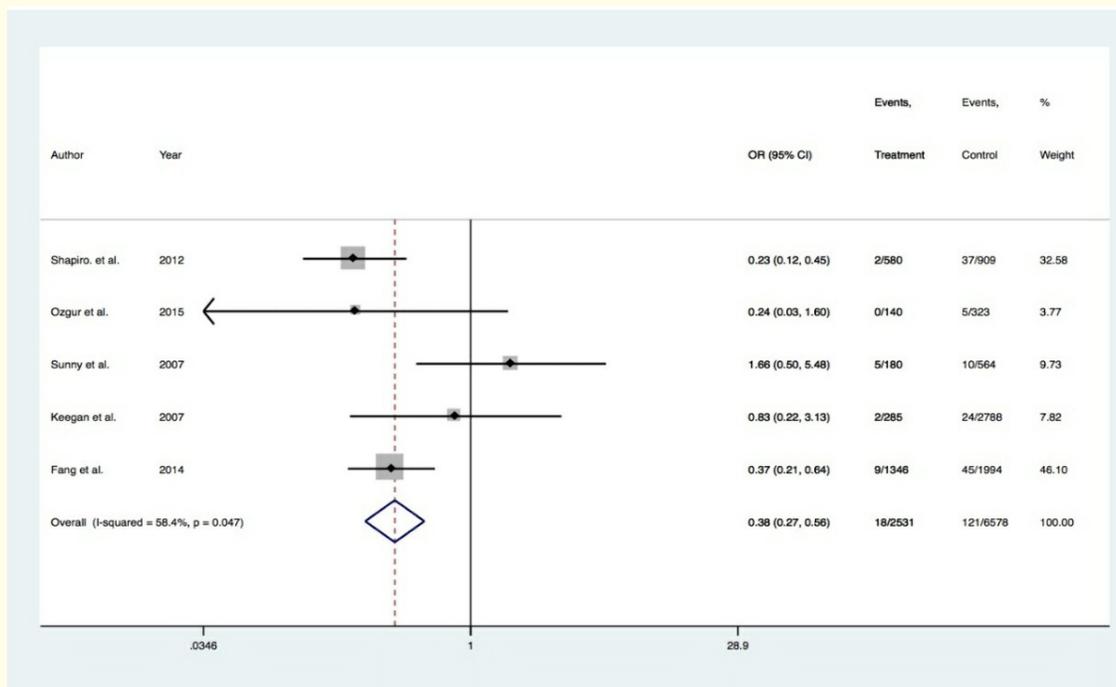


Figure 2: Frozen embryo transfer is associated to a lower odd of ectopic pregnancy. Forest Plot of Meta-analysis.

For the secondary outcome, we assessed the risk between blastocyst embryo transfer and day-3 frozen embryo transfer. Data were available in 2 studies. In one of them [8], no specific numerical information was given to demonstrate their conclusions that there was no statistical difference between the two groups. In the other study, Fang, *et al.* [5] suggested that day-3 embryo transfer may tend to increase the ectopic pregnancy rate (OR 5.3; CI 1.4 - 19,998; $p = 0.013$).

Discussion

The first pregnancy reported after IVF-ET, in 1976, was a tubal pregnancy [9]. Despite major advances in ART since that first effort, ectopic pregnancies remain a significant complication of IVF. To better understand specific factors in ART that may affect the rate of ectopic pregnancy, we compared the risk of EP following frozen embryo transfer versus fresh embryo transfer. Our meta-analysis reveals a statistically significantly lower risk of ectopic pregnancy after frozen versus fresh embryo transfer.

Several explanations may confer a degree of plausibility to this finding. For example, peri-implantation conditions may play a role in the differences observed between fresh ET and frozen ET. The combination of embryos with high implantation potential and a more receptive uterine environment, eventually unimpaired by ovarian stimulation, is conducive to lower risk of EP. Indeed, a higher rate of tubal dysfunction secondary to a hormonal effect and the negative effects of ovarian stimulation on the endometrium may lead to preferential implantation into the fallopian tube, owing to conflicting tubal and endometrial signals [10]. However, other studies suggest that alterations of estrogen and progesterone levels may not be the primary reasons for the increased incidence of ectopic pregnancy during ART [5]. Technical variables, such as assisted hatching, higher transfer volume, and deep fundal transfer, have been reported as factors potentially associated with EP [11]. Unfortunately, these technical aspects cannot be adequately assessed as a variable, because not all transfers are performed under ultrasound guidance. Therefore, it may be prudent to use ultrasound guidance with emphasis on the distance away from the fundus.

For our secondary outcome related to timing of transfer and EP, sufficient data were not available in the selected studies to properly analyze outcomes.

Some advantages of this review are the large population included and the exclusion of studies with confounding factors. A limitation of the study is that it included only retrospective trials (because such studies focused on the outcome and not because prospective studies were excluded). In addition, the reviews that analyse 'ectopic pregnancy in fresh vs. frozen ET' had methodological weaknesses, principally in demographics, which made it difficult for us to include a greater number of trials in our meta-analysis. Further, it could limit us to ensure an optimum homogeneity between selected studies.

Authors' Conclusions

In conclusion, ectopic pregnancy remains a major cause of maternal morbidity and mortality in the first trimester of pregnancy, and the rates following ART are even higher than in spontaneous conception. For these reasons, it is vital to fully comprehend the pathology and try to prevent it in the future. This study shows that frozen embryo transfer compared with fresh embryo transfer significantly reduces the risk of ectopic pregnancy. Ideally, further studies would be required to investigate the sources and the mechanisms involved in ART.

Disclaimer

The views expressed in this article belong to the authors and do not necessarily reflect the official position of the institution.

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Conflicts of Interest

We report no conflicts of interest.

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