

Clinical application of oocyte vitrification: a systematic review and meta-analysis of randomized controlled trials

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Objective: To perform a systematic review of the literature to identify randomized controlled trials assessing the efficacy of oocyte vitrification in terms of oocyte survival, fertilization, embryo development, and pregnancy rates.

Design: Systematic review and meta-analysis of randomized controlled trials.

Setting: Private university-affiliated IVF center, university-based hospital.

Patient(s): Patients recruited in randomized controlled trials considering oocyte vitrification as one of the experimental arms and slow freezing or fresh oocytes control as the other.

Intervention(s): Vitrification of human oocytes vs. slow freezing or fresh oocytes.

Main Outcome Measure(s): Ongoing pregnancy rate; secondary outcomes were clinical pregnancy rate, implantation rate, embryo development, fertilization rate, and oocyte survival.

Result(s): Five eligible studies were finally included. They involved 4,282 vitrified oocytes, 3,524 fresh oocytes, and 361 slow-frozen oocytes between 2005 and 2009. The rates of ongoing pregnancy, top-quality embryo, embryo cleavage, and fertilization did not differ between the vitrification and the fresh oocyte groups. The oocyte survival rate was higher in vitrified vs. slow-frozen oocytes (odds ratio [OR] 2.46, 95% confidence interval [CI] 1.82–3.32), although heterogeneity between studies was observed. The fertilization rate was higher in vitrified vs. slow-frozen oocytes (OR 1.50, 95% CI 1.07–2.11). Vitrification also resulted in a higher rate top-quality embryo (22.4% vs. 8.0%, OR 3.32, 95% CI 1.37–8.02) and embryo cleavage rate (day 2: 64.6% vs. 47.7%, OR 2.00, 95% CI 1.33–3.00; day 3: 53.0% vs. 33.3%, OR 2.25, 95% CI 1.32–3.85) as compared with slow freezing.

Conclusion(s): Vitrification is an efficient method to preserve oocytes, although more large controlled clinical trials are needed to strengthen this conclusion. (*Fertil Steril*® 2011;96:277–85. ©2011 by American Society for Reproductive Medicine.)

Key Words: Oocyte, vitrification, cryopreservation, slow freezing, fertility preservation, ongoing pregnancy rate, meta-analysis

Clinical applications for oocyte cryopreservation include fertility preservation in cancer patients (1–3), fertility preservation for social reasons (4), ovum donation programs (5–7), minimization of ovarian hyperstimulation syndrome risk, oocyte accumulation in low-responder patients (8), and surplus oocyte storage after controlled ovarian stimulation when embryo cryopreservation is not feasible (9). Oocyte cryopreservation is being increasingly applied in the above-mentioned situations (6–17). Our oocyte donation program alone has so far resulted in more than 20,000 cryostored-donated oocytes. Nevertheless, the practice committee of the American Society for Reproductive Medicine concludes that “Oocyte cryopreservation presently should be considered an experimental technique only to be performed under investigational protocol under the auspices of an IRB” (18). However, this committee opinion was published in June 2008, and 3 years later, at the time of writing this article, there is a growing body of evidence that may help to change this perspective (6–17).

The inefficiency of technologies available in the last 2 decades has greatly limited the implementation of oocyte cryopreservation in clinical practice. Although slow-freezing (SF) protocols for

oocytes have greatly improved (19, 20), the introduction of vitrification has meant a significant advance in assisted reproductive technologies, being able to provide outcomes similar to those achieved with fresh oocytes (5, 6). Moreover, vitrification methodologies give excellent results with both embryos at early stages of development (21–24) and those at blastocyst stages (25–27). Vitrification protocols use very high cooling rates in combination with a high concentration of cryoprotectants. As a result, ice crystal formation is successfully avoided (28). On the other hand, it is not always possible to effectively avoid crystallization using SF protocols. Despite its advantages, the main problem posed by vitrification is the toxic effects inherent in the use of high concentrations of cryoprotectants. This problem has been fully overcome with the development of new vitrification methods that use extreme cooling rates (29) to significantly reduce the concentration of these substances. This is achieved by the use of open systems in which the samples are put into direct contact with liquid nitrogen (LN) during the vitrification process. Conversely, closed systems require the loading of samples into devices that are sealed before the vitrification process with the sole purpose of avoiding direct LN contact. The thermal isolation of samples in this way greatly slows the cooling rate, a great shortcoming in comparison with the rapid freezing that can be achieved with open systems. On the other hand, this intervention avoids the hypothetical risk of cross-contamination with pathogens that may be present in the LN.

Despite the higher efficiency achieved with open systems, the issue of cross-contamination has generated great concern and

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has become a major impediment to the wide use of oocyte vitrification (28). Although there are no documented cases of cross-contamination in the history of assisted reproductive technology cryotransfers, it is vital that efforts be made to increase the safety of our cryopreservation methods. Hence, the design of devices that are able to prevent the risks associated with direct contact with LN during storage, while maintaining the advantages of open systems during the vitrification process, would be highly advisable. The sterilization of LN, either by filtering or by ultraviolet irradiation (30), is also a good alternative, as is the use of vapor storage tanks, which are known to be safer than traditional ones and have proven to be very efficient in terms of preserving sample viability (31).

Accurate assessment of oocyte cryopreservation efficacy is made more difficult owing to the wide variety of techniques and protocols reported in the literature. Nevertheless, increasing reports of successful cryopreservation of metaphase II (MII) oocytes in both oocyte recipients and infertile couples warrant re-examination of whether oocyte vitrification should still be considered an experimental technique (6–17).

The aim of this study was to perform a systematic review of the literature to identify randomized controlled trials that assessed the efficacy of oocyte vitrification in terms of oocyte survival, fertilization, embryo development, and pregnancy rates compared with SF cryopreservation methods and fresh cycles. This review was performed according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement principles (32).

MATERIALS AND METHODS

Types of Studies

The inclusion criteria of the selected studies were defined a priori during the design phase of this systematic review. Only randomized controlled trials using human oocytes and reporting data from at least one of the outcome measures (see below) were selected. Units of randomization included patients, cycles, and oocytes. No language, date, or publication status was imposed.

Types of Participants

Women undergoing ovum pickup and subsequent oocyte cryopreservation, regardless of the indication for cryopreservation, were included.

Types of Interventions

Trials comparing oocytes obtained after vitrification with those coming from SF cryopreservation or with fresh oocytes were included. There were no restrictions derived from the vitrification method, the cryoprotectant used, or the SF protocol applied.

Types of Outcome Measures

The primary outcome considered in this review was the ongoing pregnancy rate, defined as the number patients with at least one fetus with visible heart beating beyond 12 weeks/transfer.

The secondary outcomes considered in this study included clinical pregnancy rate, defined as the number of patients with at least one gestational sac evidenced by sonography/transfer, implantation rate (number of gestational sacs per number of embryos transferred), oocyte survival (number of viable oocytes per number of thawed-warmed oocytes), and fertilization rate (number of zygotes per number of injected oocytes). Embryo development was also considered as an outcome, and it was assessed through two different surrogate variables: the rate of top-quality embryos (number of best-quality

embryos per number of surviving oocytes after cryopreservation) and cleavage rate on days 2 and 3 (number of embryos cleaved per number of surviving oocytes after cryopreservation).

Variables other than pregnancy rates/implantation were expressed per surviving oocyte. The rationale behind this approach was to obtain additional information about the functionality of the surviving eggs. Pregnancy rates were expressed per transfer and implantation rate per embryos, because the number of embryos usually transferred in the oocyte cryopreservation studies was almost always between two and three, and the surplus embryos could be revitrified/frozen (6, 33).

Search Methods for the Identification of Studies to be Included

A literature search was performed in parallel on MEDLINE and SCOPUS. The search strategy is summarized as supplementary information. All references were introduced into the EndNote reference manager, and duplicated publications were identified. Registered clinical trials were searched for in the Cochrane Controlled Clinical Trials Register (CENTRAL) and in Clinicaltrials.gov databases using the terms “oocyte vitrification” and “oocyte cryopreservation.” In addition, we hand-searched content pages of the European Society of Human Reproduction and Embryology and American Society for Reproductive Medicine meeting abstract books from 1990.

Selection of Studies

Two independent reviewers (A.C. and C.D.) assessed the studies for inclusion in our review, in a standardized nonblinded manner, using a list containing the inclusion criteria, interventions, and outcomes to be studied. Disagreements among reviewers were discussed and resolved by consensus between both authors.

Data Collection and Analysis

A data extraction sheet was developed for this study, which included the following variables: name of the study, first author name, country, founding sources and conflicts of interest, type of study, study period, number of patients/oocytes included, method of randomization, unit of randomization, method of allocation concealment, blinding level, inclusion and exclusion criteria, characteristics of the study population, type of intervention and cryopreservation protocol used, ovarian stimulation and ovulation triggering protocols, maturation stage of the oocyte before cryopreservation, and raw data for the outcome measures previously described. All of the authors were contacted for further information.

Assessment of the Risk of Bias in the Included Studies

Taking into account the small number of studies meeting our inclusion criteria, we decided not to perform a stratified analysis or meta-regression to assess the risk of bias, but rather to present a narrative description of the risk of bias and to do a restricted analysis on only the studies at low and unclear risk of bias for the different outcomes. To identify potential bias in the methodologic aspects of randomization, the demographic aspects of the populations included, the extent of loss of follow-up, the different cryopreservation methods used, and the embryology aspects were screened and discussed between the reviewers. A heterogeneity analysis of the included studies was performed using a test based on the Q value for the different outcomes (χ^2 distribution; a probability of $P < .05$ was considered statistically significant). The consistency between studies was measured with the I^2 value, which represents the proportion of

variability in the observed effects due to the heterogeneity between studies. If heterogeneity between studies was evident, the influence of small-study effects was subsequently analyzed by comparing the fixed- and random-effects estimates of the intervention effect. If the estimates were similar, then any small-study effects had little effect on the intervention effect estimate. If the random-effects estimate was more beneficial, authors concluded that the intervention was more effective in the smaller studies and considered them a potential source of bias.

Measures of Treatment Effect

The odds ratio (OR) of ongoing pregnancy was the primary measure of the intervention effect. Other prespecified measures of effect included OR of clinical pregnancy, implantation, fertilization, day-2 and day-3 quality embryos, day-2 cleavage rates, and oocyte survival.

Data Synthesis

Quantitative analysis was confined to the thawed/warmed oocytes. Raw data were extracted from the eligible studies for each defined outcome and pooled using RevMan 5.1 Software (The Nordic Cochrane Centre, The Cochrane Collaboration, 2011). The effects of the intervention in each different study were expressed as OR with a 95% confidence interval (CI) and then combined using the inverse of the variance method for fixed effects.

RESULTS

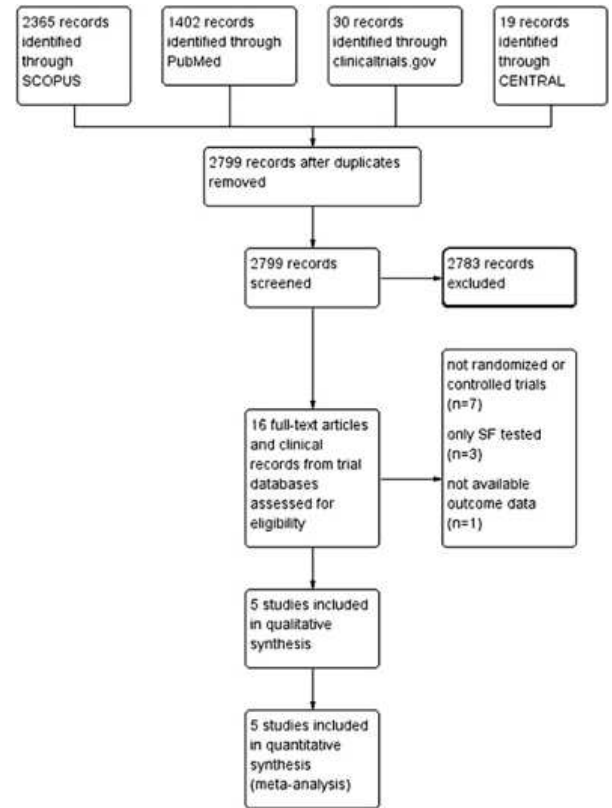
The first search in the different databases yielded 3,816 potential records, 1,017 being duplicate records that were subsequently removed. A secondary screening was performed with the information retrieved from the title and abstract of the PubMed and SCOPUS records and the online protocols of Clinicaltrials.gov and CENTRAL. After that, 16 studies were identified for further full-text analysis. Eleven of these studies were excluded because of their failure to implement randomization, lack of data regarding the prespecified outcome measures, or because the oocyte vitrification was not analyzed as an intervention (Fig. 1).

Included Studies

The five selected studies (5, 6, 10, 33, 34) (Tables 1–3) were unicentric and involved 4,282 vitrified oocytes, 3,524 fresh oocytes, and 361 slow-frozen oocytes, originally studied between 2005 and 2009. Only metaphase II (MII) oocytes fertilized after intracytoplasmic sperm injection were compared in these studies. The basal characteristics of the populations differed between studies, and these are summarized in Table 2. The studies reported by Cobo et al. (5, 6) were based on an oocyte donation program. In another two studies (10, 34) sibling oocytes from the same patient were used, and embryos derived from those oocytes were transferred. In the study reported by Cao et al. (33) none of the resulting embryos were transferred. In two of the studies SF was compared with vitrification (33, 34), whereas the other three compared fresh oocytes with vitrified ones (5, 6, 10). Three vitrification protocols using different cryoprotectant combinations and devices were analyzed: Cryotip (349 oocytes), Cryotop (2,645 oocytes), and Cryoleaf (292 oocytes) (Table 1). The two SF protocols included in this review used a programmable freezer to decrease the temperature as follows: $-2^{\circ}\text{C}/\text{min}$ until -7°C , followed by manual seeding, a further drop in temperature of $-0.3^{\circ}\text{C}/\text{min}$ down to -30°C or -33°C , and subsequent immersion in LN. Results of risk of bias assessment are summarized in Table 3.

FIGURE 1

Flow chart of the study selection process.



Cobo. Meta-analysis of the use of oocyte vitrification. *Fertil Steril* 2011.

Pregnancy Rate (Ongoing and Clinical) and Implantation Rate

Data regarding pregnancy rates were not pooled because embryo recipients were randomized in only one (6) of the four studies (5, 10, 34) that analyzed this outcome. In this study the ongoing pregnancy rate was 49.1% and 48.3% in the vitrification and fresh groups, respectively (OR 1.03, 95% CI 0.73–1.45, $P>.05$). On the basis of an intention-to-treat analysis the pregnancy rates per patient decreased to 43.7% in the vitrification group and to 41.7% in the fresh group (OR 1.08, 95% CI 0.78–1.50, $P>.05$) (6). The clinical pregnancy rate was 55.4% and 55.6% in the vitrification and fresh groups, respectively (OR 0.99, 95% CI 0.70–1.40, $P>.05$). The implantation rates were 40.0% (vitrification) and 41.0% (fresh) (OR 0.96, 95% CI 0.75–1.24, $P>.05$). Only one study (34) compared the clinical pregnancy rate between vitrification and SF (38.3% vs. 21.0%; OR 2.31, 95% CI 0.67–8.12, $P>.05$). In this study, when the pregnancy rates were compared per thawed/warmed oocyte, the vitrification group showed a higher pregnancy rate compared with the SF group (5.2% vs. 1.7%; OR 3.18, 95% CI 1.06–9.52, $P<.05$).

Oocyte Survival

Oocyte survival after warming/thawing was reported as 97.0% (224 of 231) (5), 92.5% (3,039 of 3,286) (6) and 97.0% (120 of 124) (10) in the three studies comparing vitrification vs. fresh oocytes. There was a significant difference regarding postthawing oocyte survival when vitrification was compared with SF (OR 2.46, 95% CI 1.82–3.32;

TABLE 1

Summary of findings and methods used in the studies included.

Parameter	Authors/year (reference)				
	Smith et al. 2010 (34)	Rienzi et al. 2010 (10)	Cao et al. 2009 (33)	Cobo et al. 2008 (5)	Cobo et al. 2010 (6)
Study period	Jan 2005–Apr 2009	Sep 2008–Mar 2009	Not stated	Dec 2006–Nov 2007	Nov 2008–Sep 2009.
Country	Brazil	Italy	China	Spain	Spain
Intervention	Vitrification vs. SF	Vitrification vs. fresh	Vitrification vs. SF	Vitrification vs. fresh	Vitrification vs. fresh
Patients (oocytes) undergoing vitrification	48 (349)	40 (124)	(292)	30 (231)	295 (3,286)
Patients (oocytes) undergoing SF	30 (238)	—	(123)	—	—
Patients (oocytes) undergoing fresh cycles	—	40 (120)	—	30 (219)	289 (3,185)
Ovarian stimulation protocol	rFSH + antagonist	rFSH + antagonist; rFSH + agonist (long protocol)	hMG + agonist (long protocol) ^a	rFSH or hMG + agonist (long protocol)	rFSH or hMG + agonist (long protocol)
Ovulation triggering	rhCG (250 µg)	rhCG (10,000 UI)	???	rhCG (10,000 UI)	rhCG (10,000 UI)
Vitrification protocol	7.5% EG + 7.5% DMSO + 12% SSS. Cryotip	15% EG + 15% DMSO + 0.5 M sucrose. Cryotop	15% EG + 15% PROH + 0.5 M sucrose. Cryoleaf	15% EG + 15% DMSO + 0.5M sucrose. Cryotop	15% EG + 15% DMSO + 0.5 M sucrose. Cryotop
Slow freezing protocol	1.5 M PROH + 0.3 M sucrose + 12% SSS	—	1.5 M PROH + 0.3 sucrose + 30% SSS	—	—
Oocyte assessment	ZP, cytoplasm, oolema	ZP, cytoplasm, oolema	Membrane morphology	ZP, cytoplasm, oolema	ZP, cytoplasm, oolema
Embryo assessment	Number, size, and fragmentation degree of blastomeres	Number, size, and fragmentation degree of blastomeres	Number, size, and fragmentation degree of blastomeres	Number, size, and fragmentation degree of blastomeres	Number, size, and fragmentation degree of blastomeres
Day of ET	3	2	—	3	3
Oocyte survival rate (%)	VIT: 74.5; SF: 65.1	96.8	VIT: 91.8; SF: 61.0	96.9	92.5
Clinical pregnancy rate (%)	VIT: 38.3; SF: 21.0	VIT: 38.5; FRESH: 43.5	—	VIT: 65.2; FRESH: 100	VIT: 55.4; FRESH: 55.6

Note: DMSO = dimethyl sulfoxide; ZP = zona pellucida; VIT = vitrification; EG = ethylene glycol; SSS = synthetic serum substitute; PROH = propanediol.

^a The authors of the original manuscript refer to “a long protocol” and to “down-regulation with a GnRH antagonist,” which is probably a typographic mistake, although we were unable to check this point with the authors.

Cobo. Meta-analysis of the use of oocyte vitrification. *Fertil Steril* 2011.

TABLE 2

Demographic variables of the study populations involved in the included reports.

Parameter	Authors/year (reference)				
	Smith et al. 2010 (34)	Rienzi et al. 2010 (10)	Cao et al. 2009 (34)	Cobo et al. 2008 (5)	Cobo et al. 2010 (6)
Basal characteristics of the patients	Tubal factor Severe male factor Unexplained factor Regular, spontaneous menstrual cycles (25–35 days) Follicular phase serum: FSH \leq 10 IU/L LH \leq 13.5 IU/L E ₂ \leq 60 pg/mL BMI \leq 30 kg/m ² Both normal ovaries Normal uterine cavity Compliant with procedures	Patients requiring ICSI	Patients requiring ICSI	Oocyte donors (oocyte donation program) 18–35 y old Regular spontaneous menstrual cycles (21–35 d) Normal karyotype No previous history of exposure to radiation or hazardous chemical substances Tested negative for STDs Normal results on physical and gynecologic examination	Oocyte donors (oocyte donation program) Same as Cobo et al. 2008 Oocyte recipients (oocyte donation program) Patients requiring oocyte donation 18–49 y old
Inclusion criteria	Patients who had >9 MII oocytes were included Only oocytes from patients who did not get pregnant in the fresh cycle were thawed/warmed	Patients who had >6 normal MII oocytes Only oocytes from patients who did not get pregnant in the fresh cycle were thawed/warmed	Patients who had >15 MII oocytes	Oocyte donors who met the base characteristics of the donation program, who did not meet any of the exclusion criteria	Oocyte donors and recipients who met the base characteristics of the donation program and who did not meet any of the exclusion criteria
Exclusion criteria	Previous history of OHSS Intolerance to any of the agents used in the study Clinically significant conditions/disease(s) Active substance abuse Abnormal gynecologic bleeding of unknown origin PGS	Age >42 y Motile sperm count <500,000/mL Surgically extracted spermatozoa PGS	Severe male factor infertility	Oocyte donors (oocyte donation program) >2 previous miscarriages Endometriosis BMI <18 or >30 kg/m ² <8 or >15 follicles retrieved after ovarian stimulation	Oocyte donors (oocyte donation program) Same as Cobo et al. 2008 Oocyte recipients (oocyte donation program) <3 previous IVF attempts BMI <18 or >30 kg/m ²

Note: ICSI = intracytoplasmic sperm injection; STDs = sexually transmitted diseases; BMI = body mass index; OHSS = ovarian hyperstimulation syndrome; PGS = preimplantational genetic screening.

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TABLE 3

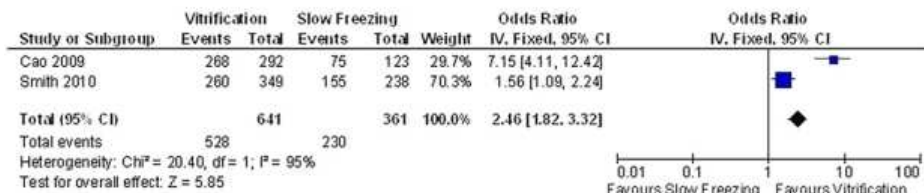
Risk of bias assessment.					
Parameter	Authors/year (reference)				
	Smith et al. 2010 (34)	Rienzi et al. 2010 (10)	Cao et al. 2009 (33)	Cobo et al. 2008 (5)	Cobo et al. 2010 (6)
Study period	Jan 2005–Apr 2009	Sep 2008–Mar 2009	Not stated	Dec 2006–Nov 2007	Nov 2008–Sep 2009
Country	Brazil	Italy	China	Spain	Spain
Intervention	Vitrification vs. SF	Vitrification vs. fresh	Vitrification vs. SF	Vitrification vs. fresh	Vitrification vs. fresh
Design	Two parallel arms	Two parallel arms	Two parallel arms	Two parallel arms	Two parallel arms
Randomization method	Random number generator	Random number generator	Not stated	Random number generator	Random number generator
Unit of randomization	Patient (own-eggs cryopreservation)	Sibling oocyte	Not stated	Sibling oocyte	Patient (recipients of an oocyte donation program)
Allocation concealment	Random number generator	Central randomization	Not stated	Central randomization	Central randomization
Blinding level	Open label	Open label	Open label	Double blinded	Triple blind
Sample size calculation	One sample size was determined by the number of warmings and thawings within a specific time	Noninferiority of vitrification basis (17% max absolute difference of fertilization rate); n = 111 oocytes/group	Not stated	Equivalence test for the proportion of zygotes/MII oocytes (mean 0.75 ± 0.18 /MII oocyte. Equivalence defined as $\pm 5\%$); n = 222 oocytes/group	Superiority of fresh cycles basis (12% min absolute difference of ongoing pregnancy rate); n = 287 patients/group
Conflict of interest	The main author is a member of the Scientific Advisory Board of Medicult	Not stated	None	None	None

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FIGURE 2

Odds ratio of postthawing/warming oocyte survival rate after vitrification and SF. (A) Fixed-effects model. (B) Random-effects model.

A Survival rate of Vitrification vs. Slow freezing. Fixed effects model



B Survival rate of Vitrification vs. Slow freezing. Random effects model



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heterogeneity: $P < .00001$, $I^2 = 95\%$, fixed-effects model, Fig. 2A). This difference was not significant any more when a random effect model was applied (OR 3.29, 95% CI 0.74–14.59; heterogeneity: $P < .00001$, $I^2 = 95\%$, Fig. 2B), although a higher survival rate trend after oocyte vitrification was still evidenced.

Fertilization Rate

Higher fertilization rates were observed when vitrification was compared to SF (OR 1.50, 95% CI 1.07–2.11; heterogeneity: $P = .56$,

$I^2 = 0$, fixed-effects model) (Fig. 3A). On the other hand there were no differences when vitrified oocytes were compared with fresh ones (OR 1.02, 95% CI 0.91–1.13; heterogeneity: $P = .17$, $I^2 = 44\%$, fixed-effects model) (Fig. 3B).

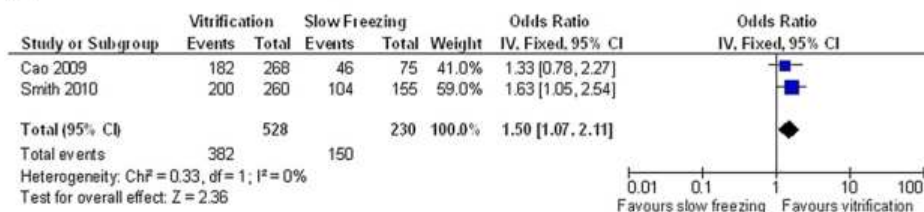
Top-Quality Embryos and Cleavage Rates

The rate of top-quality embryos was similar after fertilization of vitrified/warmed oocytes and fresh oocytes on day 2 (OR 1.01, 95% CI 0.91–1.11; heterogeneity: $P = .74$, $I^2 = 0$, fixed-effects model) and

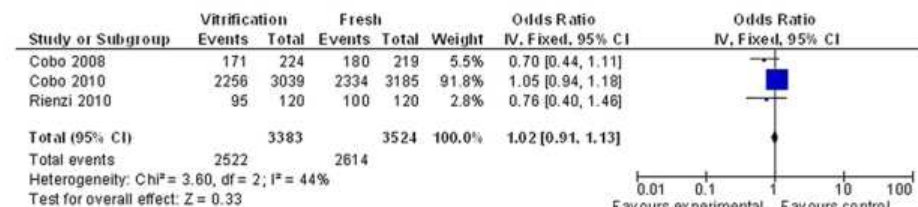
FIGURE 3

Odds ratio of fertilization rate. (A) Vitrification versus SF. (B) Vitrification versus fresh oocytes. Fixed effects-model.

A Vitrification vs. Slow freezing. Fixed effects model



B Vitrification vs. Fresh oocytes. Fixed effects model



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on day 3 (OR 0.91, 95% CI 0.83–1.01; heterogeneity: $P=.10$, $I^2 = 62%$, fixed-effects model). None of the studies comparing SF and vitrification reported data on embryo quality on day 2, and only the study published by Cao et al. (31) reported data on the number of day-3 top-quality embryos between vitrification and SF, showing a higher rate of top-quality embryos in the vitrification group (22.4% vs. 8.0%, OR 3.32, 95% CI 1.37–8.02, $P<.01$).

The day-2 cleavage rate did not differ between embryos derived from vitrified or fresh oocytes (OR 1.00, 95% CI 0.90–1.11; heterogeneity: $P=.06$, $I^2 = 65%$, fixed-effects model) (OR 0.95, 95% CI 0.65–1.38; heterogeneity: $P=.06$, $I^2 = 65%$, random-effects model). Similar results were found for the day-3 cleavage rate: no differences were noted (OR 0.96, 95% CI 0.87–1.06; heterogeneity: $P=.01$, $I^2 = 84%$, fixed-effects model) (OR 0.79, 95% CI 0.48–1.30; heterogeneity: $P=.01$, $I^2 = 84%$, random-effects model). One study compared the day-2 cleavage rate of embryos derived from vitrified and slow-frozen oocytes (30), showing a higher cleavage rate in the former (64.6% vs. 47.7%, OR 2.00, 95% CI 1.33–3.00, $P<.01$). Another study evidenced a higher day-3 cleavage rate in the vitrification group when compared with SF (53.0% vs. 33.3%, OR 2.25, 95% CI 1.32–3.85, $P<.05$).

DISCUSSION

This review supports the hypothesis that the potential of fertilization, embryogenesis, and pregnancy from oocytes derived from vitrification/warming cycles is not significantly different from that of fresh oocytes. It also suggests that oocytes coming from vitrification/warming cycles could result in better survival and fertilization rates than those coming from SF/thawing cycles.

However, several important limitations should be considered with respect to our meta-analysis: [1] only five studies have been included in this review, all of them presenting an evident clinical heterogeneity regarding the inclusion criteria and basal characteristics of the samples (Table 2). [2] The external validity of the study may be limited to good responders because all the included patients had at least six MII oocytes after controlled ovarian stimulation, or they were oocyte donors (Table 2). [3] Statistical heterogeneity between studies was observed for some of the measures studied, especially for the oocyte survival rate. This could be

explained by the two different methods of vitrification used in the trials that reported this outcome (33, 34). Open devices improve oocyte survival when compared with the closed ones. [4] In four (5, 10, 33, 34) of the five studies randomization was not used to allocate embryos derived from cryopreserved oocytes to the recipients. This could introduce a selection bias in the studies that considered clinical variables, such as pregnancy and implantation rates. Furthermore, in the studies by Rienzi et al. (10) and Smith et al. (34) only patients who did not achieve pregnancy during their fresh cycle underwent ET. In one of the studies published by Cobo et al. (5) there were no pre-established criteria for transfer, 23 patients were transferred in the vitrification group, and only 1 was transferred in the fresh group. These were the main reasons why we did not include these outcomes in the meta-analysis. Nevertheless, these selection biases should not affect the variables studied before ET. In one study, the randomization took place after oocyte vitrification (6), although both fresh and vitrified oocytes came from the same donor population (which met strict inclusion criteria), and the oocytes were obtained during the same study period. [5] Only two of the studies were blinded (6); although in theory open-label studies could overestimate the effect of the intervention (vitrification), through the introduction of detection or performance bias in the different studies, it is very unlikely that variables such as fertilization, cleavage, or pregnancy can be affected by detection bias. Nevertheless, variables related to a more detailed morphologic evaluation such as oocyte evaluation or embryo quality could be more sensitively affected by this kind of bias. [6] Sample size calculation was not stated in one of the studies (33), and it was unclear in another (34) (Table 3).

Finally, only one of the five studies included a large sample size, which greatly contributed to validation of the conclusions drawn by these authors (6).

In conclusion, on the basis of the evidence provided by the randomized studies available, vitrification seems to be an efficient method to cryopreserve oocytes. However, more large-scale controlled trials, aimed at evaluating clinical outcomes after oocyte vitrification, will be required to further strengthen this conclusion.

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