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Vitrification and conventional freezing methods in sperm cryopreservation: A systematic review and meta-analysis

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ABSTRACT

Objective: The objective is to systematically review and synthesize the literature on the efficacy with two different cryopreservation methods used for human spermatozoa and evaluate whether vitrification protocol and quality of sperm influence effect estimates.

Design: The following electronic databases were searched up to September 2017: Pubmed, Embase and Web of Science. The search strategy used the following the relevant medical subject heading (MeSH) terms, keywords, and word variants for: sperm parameters, conventional freezing, and vitrification. Queries were limited to those involving humans. Randomized controlled trials (RCTs) that published in English languages were considered eligible. Studies and references were included if they reported total motility, progressive motility, morphology, or DNA fragmentation index (DFI) for vitrified or conventional cryopreserved human spermatozoa. Patients recruited in RCTs considering sperm vitrification as one of the experimental arms and conventional freezing (including slow freezing or vapor fast freezing) sperm control as the other. Studies that had high risks of allocation concealment were excluded when performing sensitivity analysis. We specified 2 subgroup variables, including vitrification protocol and quality of spermatozoa cryopreserved, to investigate sources of heterogeneity. A meta-analysis was performed using a random effects ($I^2 > 50\%$) or fixed effects ($I^2 < 50\%$) model to calculate weighted mean differences (MD) and 95% CI.

Result(s): The search yielded a total of 2428 articles and 13 RCTs were included for analysis. They involved 486 vitrified and 486 conventional cryopreserved sperm samples. Four sperm parameters were reported as mean differences and based on adjusted estimates in all included studies. Meta-analysis of these studies showed significantly higher total motility [weighted mean differences (WMD) 6.98; 95% confidence interval (CI) 2.94; 11.02; $P < 0.0001$] and progressive motility [WMD 4.59; 95% CI 0.78; 8.39; $P = 0.02$] of past-thawed sperm following vitrification compared with conventional freezing methods. However, DNA fragmentation index (DFI) [WMD -1.18; 95% CI -2.81; 0.45; $P = 0.16$] and morphology [WMD 0.11; 95% CI -0.42; 0.63; $P = 0.69$] of past-thawed sperm are similar between two freezing groups. Subgroup analysis shown that the vitrification protocol and quality of spermatozoa are potential risk factors for the efficacy of vitrification. Higher past-thawed sperm parameters following the cryoprotectants-free (CPAs-free) vitrification were observed, as well as a lower past-thawed sperm parameters with the cryoprotectants-presence (CPAs-presence) vitrification, which could reflect the CPAs related cytotoxicity. Meanwhile, vitrification had higher ability in preservation of high quality of spermatozoa compared with vitrification of low quality spermatozoa.

Conclusion(s): According to the results of present meta-analysis, vitrification is superior to conventional freezing methods in preservation of spermatozoa, regarding total and progressive motility. However, the efficacy of vitrification is influence by using different vitrification protocol and cryopreservation of different quality spermatozoa. It is must emphasized that the results of present meta-analysis is limited by the small number of studies of variable vitrification protocol. Further well conducted studies are required to confirm the efficacy of vitrification in cryopreservation of spermatozoa, in addition, allow the examination of the two cryopreservation methods in terms of pregnancy achievement and determination of the role of clinical variable on efficacy of vitrification.

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Introduction

As an efficient method, sperm cryopreservation is an essential aspect of assisted reproductive technique (ART) and male fertility preservation. The number of patients with azoospermia and oligospermia avoid repeated biopsies or aspirations through this technique [1]. Cancer patients also preserve their fertility by this technique before undergoing chemotherapy or radiation. There are several methods which differ from each other in terms of freezing rates, composition of cryoprotectants (CPAs), dilution rates, carrier tools and thawing protocol. Two principal approaches for sperm cryopreservation have been adopted: conventional freezing and vitrification.

There are two conventional freezing methods used in sperm cryopreservation: slow freezing and vapor fast freezing. Slow-freezing consists of 2∼3 steps and usually costs 2∼4 h to complete [2]. Firstly, spermatozoa accumulating by ejaculation or other techniques keep at room temperature for 10 min. Secondly, samples are gradually frozen from 20 °C to 5 °C at a rate of 0.5–1 °C/min and then cooled from 5 °C to −80 °C at rate of 1–10 °C/min. Finally, samples are plunged into liquid nitrogen [3]. Vapor fast freezing is based on a direct contacting of samples to liquid nitrogen vapors for 10–15 minutes [4]. Conventional freezing methods involve the use of CPAs to minimize osmotic damage and to avoid intracellular and extracellular ice crystal formation, but can be cytotoxic [5]. There are two categories of CPAs, one is permeable CPAs including dimethyl sulfoxide, glycerol, glycol, ethylene and methanol, the other is nonpermeable CPAs including albumins, dextrans and egg yolk citrate [6]. It is well known that permeable CPAs stabilize the plasma membrane and nonpermeable CPAs minimize the intracellular ice formation. However, in most cases, nonpermeable play a supporting role that augmented the effectiveness of permeable CPAs [7].

Vitrification is a process to solidify liquid into an amorphous or glassy state [8]. This approach was first proposed in 1937, but at that time, the cooling rate could not meet the vitrification requirement [9]. Currently, vitrification is the most commonly used technique in the preservation of oocytes and embryos, which required addition of high concentration of permeable CPAs [10]. However, this technique cannot successfully preserve mammalian spermatozoa because spermatozoa have higher osmotic fragility than other reproductive tissues [11]. Until 2002, a new vitrification protocol named cryoprotectant-free vitrification (CPA-free) was introduced by Nawroth et al, which differed from former vitrification protocols in absence of high concentrations of permeable CPAs [12]. In the last 16 years, several types of pre-cooling techniques, carrier tools, thawing methods have been introduced to improve the efficacy of vitrification protocol [13]. However, studies that evaluated the efficacy of sperm vitrification showed rather conflicting results.

To the best of our knowledge, this is the first meta-analysis to compare the safety and efficacy of two different freezing techniques used in sperm cryopreservation. The objective of this study was to perform a meta-analysis of the randomized controlled trials that evaluated the efficacy of sperm vitrification in terms of total motility, progressive motility, morphology and DFI compared with conventional freezing methods.

Methods

Types of studies

The inclusion criteria of the selected studies were defined a priori during the design phase of this systematic review. Due to the inherent methodological limitations and low overall quality of observational studies, only randomized controlled trials (RCTs) using human spermatozoa and reporting the sperm quality parameters, including total motility, progressive motility, morphology, or DNA fragmentation index (DFI), were selected. Studies published in English language were selected. No date or publication status was imposed.

Types of participants

Human undergoing sperm cryopreservation, regardless of the indication for cryopreservation, were included.

Types of interventions

Trials comparing sperm obtained after vitrification with those coming from conventional freezing methods (including slow freezing and vapor fast freezing) were included. Patients recruited in RCTs considering sperm vitrification as one of the experimental arms and conventional freezing (including slow freezing or vapor fast freezing) sperm control as the other. Studies that eventually store samples into liquid nitrogen vapor or refrigerator were excluded. No restrictions of the cryoprotectant used, or the conventional freezing protocol were established.

Types of outcome measures

Four sperm parameters considered in this review were the total motility, progressive motility, morphology and DFI.

Literature searches

This study was performed according the checklist provided in the 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses' (PRISMA) statement for writing systematic reviews and meta-analyses. The following electronic databases were searched up to September 2017: Pubmed, Embase and Web of Science. The search strategy used the following the relevant medical subject heading (MeSH) terms, keywords, and word variants for: human, spermatozoa, cryopreservation, freezing, and vitrification. The example of full research of the search strategy by using PubMed was reported in Supplementary Table I. We also examined the reference lists of all known primary studies and review articles to research additional references.

Selection of study

Firstly, the titles and abstracts of electronic studies were scrutinized by two authors independently (Y.X.L. and Y.C.L.) and full manuscript which likely met the predefined selection criteria were obtained. Secondly, final inclusion or exclusion decisions were made after examination of the full manuscripts. Two author independently examined full manuscripts of potentially eligible studies, with disagreements resolved by discussion.

Data extraction and quality assessment

Data were extracted independently by two authors (Y.X.L. and M.Q.L.). For each included study, first author's family name, year of publication, study location, number of patients, types of patients/donors, freezing protocol, the composition of CPAs used in freezing and thawing process, thawing protocol and outcome data (total motility, progressive motility, morphology and DNA fragmentation index (DFI)), were extracted. All sperm parameters were reported as mean differences. Duo to standard study design (i.e. each sperm samples were divided into two equal parts, one part were cryopreserved by using vitrification and other part were cryopreserved by using conventional freezing methods), we have reason to

believe that four sperm parameters in all studies were based on adjusted estimates. Authors of the primary articles were contacted for any missing or unclear information.

The Cochrane Handbook was implemented for quality assessment of RCT. Items assessed included random sequence generation, allocation concealment, blinding, incomplete outcome data, selective reporting and other bias [14]. The risks of bias was classified as high, unclear and low.

Statistics analysis

Weighted mean differences (MD), which were identified with 95% confidence intervals (95% CI), were used to analysis the all included sperm parameters in the different comparison groups. Heterogeneity was assessed through I^2 statistic (an I^2 of<50%, 50.0–75.0% and >75.0% indicating low, moderate and high heterogeneity, respectively) [15]. A fixed-effects model was used when I^2<50% and random-effects model was used when I^2>50.0%. We conducted all data analyses through STATA software 12 (Stata Corp, College Station, TX, USA) and Review manage 5.2 (Cochrane collaboration, Oxford, UK). Publication bias was evaluated by performing a funnel plot and using Egger's test [16].

We conducted sensitivity analysis in order to eliminate the influence of inherent methodological limitations of included studies. Sensitivity analyses restricted eligibility to studies that had no high risks of allocation concealment. In order to assess the efficacy of vitrification, we conducted subgroup analyses based on vitrification protocol and the quality of spermatozoa. CPAs-presence vitrification was characterized by the use of the same concentration of CPAs as conventional freezing methods. CPAs-free vitrification was characterized by the absence of permeable CPAs or use of low concentration of permeable CPAs. High quality spermatozoa defined as concentration > 20 million/ml. Samples collected from healthy donors or normozoospermic patients also defined as high quality spermatozoa.

Results

The research strategy yielded a total of 2428 articles, of which 27 studies were initially identified for full reading as they did fulfill the selection criteria (Fig. 1). Of the remaining 27 studies, 14 studies were excluded after reading the full manuscripts. Reasons for not including the other studies were: (i) insufficient date for analysis (n = 3) [17–19]; (ii) no conventional freezing group (n = 5) [20–24]; (iii) no vitrification group (n = 6) [25–30]. The final inclusion was thirteen studies that met the selection criteria for our meta-analysis. The main characteristics of the included studies and quality assessment are presented in Tables 1 and 2.

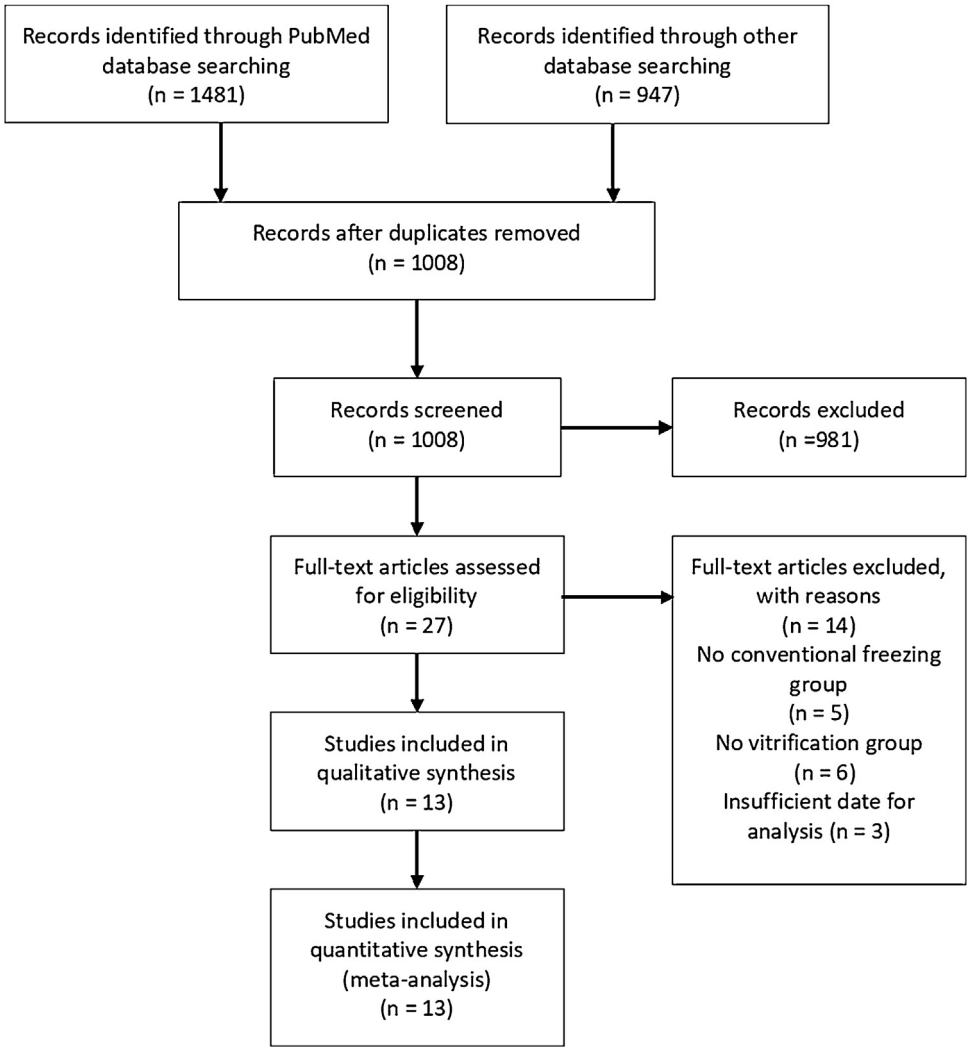

Fig. 1. Flow diagram of study selection for the meta-analysis.

Table 1
Characteristics of the included studies. Vitrification versus conventional freezing methods.

Author(year)	location	Include criteria	Conventional freezing method				vitrification			
			Freezing protocol	medium	thawing protocol	number	Freezing protocol	medium	thawing protocol	number
Aizpurua et al. [35]	Spain	[Unknown][c]	4 °C 30 min LN2 vapor for 30 min	Sperm Cryoprotect™ II with glycerol	37 °C 4min, PureSperm®wash	18	37 °C 5 min	[vitrification medium][a,b]	37 °C 4min	18
Tongdee et al. [32]	Thailand	>20million/ml >35% motility	25 °C ~ 5 °C −1 °C/min 5 °C ~ -85 °C −10 °C/min	SpermFreeze (Commercial medium)	RM 15-20 min	39	Cane 10 min,	SpermFreeze (Commercial medium)	RM 15-20 min	39
Mohamed, [40]	Germany	>15million/ml >32% motility >4% morphology	RM 10 min 8 cm above 30 min	Commercial Medium	37 °C ice complete melted	16 17	37 °C 5 min 5% CO2	[HTM medium][b]	37 °C 5 min 5% CO2	16 17
Agharahimi et al. [35]	Iran	>50% motility >30% morphology	3cm above 30min	CPA (Sperm Freeze Solution)	37 °C 10 min CPA HSA	30	– –	[Ham's F10 medium sucrose][b] CPA (Sperm Freeze Solution)	37 °C under 5% CO2	30
Zhu et al. [39]	China	>20million/ml >50% motility >10% morphology	10 cm above 30min	GEYC	37 °C ice complete melted	58	RM 1min	[Sucrose][b]	42 °C 1min 37 °C ice complete melted	58
Moskovtsev, Lulat, and Librach [6]	Canada	[Unknown][c]	Vapor freezing (Unclear)	Commercial Medium	–	11	–	[G-IVF medium +0.25 M sucrose + LSPS][b]	37 °C 10sec	11
(Isachenko et al. [21]	Germany	>20million/ml >35% motility >3% morphology	10 cm above 30min	TYB(12% glycerol+20% egg yolk)	37 °C 20sec	68	RM 5 min	[HTF+HSA +0.25 M sucrose][b]	37 °C 20sec	68
Vutyavanich et al. [31]	Thailand	>20million/ml >50% motility	20 °C~−5 °C −1 °C/min −5 °C~−80 °C −10 °C/min	SpermFreeze (Commercial medium)	25 °C-28 °C washed with 4 mL of EBSS	30	4 °C 10 min into a hole	[HAS 100 mmol of trehalose 10%glycerol][b]	25 °C-28 °C washed with 4 mL of EBSS	30
Satirapod et al. [41]	Thailand	[Unknown][c]	RM 10 min 10 cm above 10 min	SpermFreeze (Commercial medium)	RM 15-20min	70	RM 10 min	(Commercial medium)	RM 15-20min	70
Vutyavanich, Piromlertamorn, and Nunta [34]	Thailand	>20million/ml >50% motility	RM10 min 20 °C ~ -5 °C 1 °C per min −5 °C~ −80 °C 10 °C per min	SpermFreeze (Commercial medium)	25°-28° [Thawing medium][c]	11	4 °C 10 min into a hole	[HAS 100 mmol of trehalose 10%glycerol][b]	25°-28° [Thawing medium][c]	11
Chang et al. [33]	Korea	[Unknown][c]	biological freezer	TYB-buffered	–	30	plunging into LN2	TYB-buffered	–	30
Nawroth et al. [12]	Italy	>20million/ml >35% motility >15% morphology	22 °C ~4 °C 5 °C/min 4 °C ~ -30 °C 10 °C/min; −30 °C~ 140 °C 20 °C/min	Test-egg-yolk-buffered-glycerol	37 °C 50 sec	30	direct plunge into liquid nitrogen	[Free][b]	37 °C.5% CO2 5 ~ 10 min.	30
(Saritha and Bongso [37]	Singapore	[Unknown][c] < 20 million/ml	RM 10 min 4 °C 1 hour 15 cm	15% glycerol, 0.4% HSA	25 °C 30-40 min	15 12	RM 10 min	15% glycerol, 0.4% HSA	25 °C 30-40 min	15 12

All samples plunge into liquid nitrogen and eventually were stored in liquid nitrogen. G-IVF medium: Bicarbonate buffered medium contained human serum albumin; RM: room temperature.
[a] Cryoprotectant toxicity test : No negative effect.
[b] Defined as cryoprotectant-free vitrification.
[c] Collected from healthy donors or normozoospermic patients.

Funnel plot analysis for the sperm parameters that included more than 10 studies (only total motility available) was moderately symmetrical (Fig. 2). The results of Egger's test were nonsignificant, therefore providing little evidences for publication bias (Total motility: P = 0.09).

Study characteristics

All of the thirteen included studies were randomized controlled trials [6,12,31–41]. Of the included studies, three studies in this review were found to have a high risk of allocation concealment (Table 2).

Table 2
Risk of bias of included trials.

Study	Random sequence Allocation	Allocation concealment	Blinding	Incomplete outcome data	Selective reporting	Unequal co-intervention
Aizpurua et al. [35]	Low	Low	Unclear	Low	Low	Low
Tongdee et al. [32]	Low	[High][b]	Unclear	Low	Low	Low
Mohamed [40]	Low	Low	Unclear	Low	Low	[Unclear][a]
Zhu et al. [39]	Low	[High][b]	Unclear	Low	Low	[Unclear][a]
Agharahimi et al. [35]	Low	Low	Unclear	Low	Low	Low
Moskovtsevet al. [6]	Low	Low	Unclear	Low	Low	[Unclear][a]
Vutyavanich et al. [31]	Low	Low	Unclear	Low	Low	Low
Isachenko et al. [21]	Low	Low	Unclear	Low	Low	[Unclear][a]
Satirapod et al. [41]	Low	[High][b]	Unclear	Low	Low	Low
Vutyavanich, Piromlertamorn, and Nunta [34]	Low	Low	Unclear	Low	Low	[Unclear][a]
Chang et al. [33]	Low	[Unclear][c]	Unclear	Low	Low	Low
Nawroth et al. [12]	Low	Low	Unclear	Low	Low	[Unclear][a]
Saritha and Bongso [37]	Low	Low	Unclear	Low	Low	Low

[a] Incubation time, temperature and CPAs were different between two freezing protocols.
[b] Carrier tools used in two freezing protocols were different.
[c] Carrier tools used in two freezing protocols were unclear.

Although all studies in this comparison reported the parameters of sperm that underwent cryopreservation, they varied in terms of the vitrification protocol and the quality of spermatozoa cryopreserved. Nine studies in this meta-analysis used the CPA-free vitrification method for cryopreservation [6,12,31,34–36,38–40], four studies used the CPA-presence vitrification method for cryopreservation [32,33,37,41] and one study used both CPAs-free and CPAs-presence vitrification [35]. Eleven studies cryopreserved normozoospermic sperm or high quality sperm (definition see above) and two studies cryopreserved low quality sperm.

Outcomes

The pooled results are reported in Table 3 and Figs. 3–6. As shown, the total motility [WMD 6.98; 95% CI 2.94; 11.02; P = 0.0007; I^2 = 91%; Fig. 3] and progressive motility [WMD 4.59; 95% CI 0.78; 8.39; P = 0.02; I^2 = 78%; Fig. 4] of past-thawed spermatozoa were significantly higher after vitrification as compared with conventional freezing methods. Conversely, morphology [WMD 0.11; 95% CI -0.42; 0.63; P = 0.69; I^2 = 40%; Fig. 5] and DFI [WMD -1.18; 95% CI -2.81; 0.45; P = 0.16; I^2 = 38%; Fig. 6] of past-thawed spermatozoa were no significant difference after vitrification as compared with conventional freezing methods. In sensitivity analysis, we observed that morphology [WMD 1.72; 95% CI 0.27; 3.16; P = 0.02; I^2 = 23%; Table 3] of past-thawed spermatozoa resulting from vitrification was higher than those from conventional freezing methods.

The results of subgroup analysis were reported in Table 4. Subgroup analysis based on the vitrification protocol shown that past-thawed spermatozoa resulting from CPAs-free vitrification had higher total motility [WMD 12.51; 95% CI 8.63; 16.40; P < 0.00001; I^2 = 82%] and progressive motility [WMD 5.54; 95%

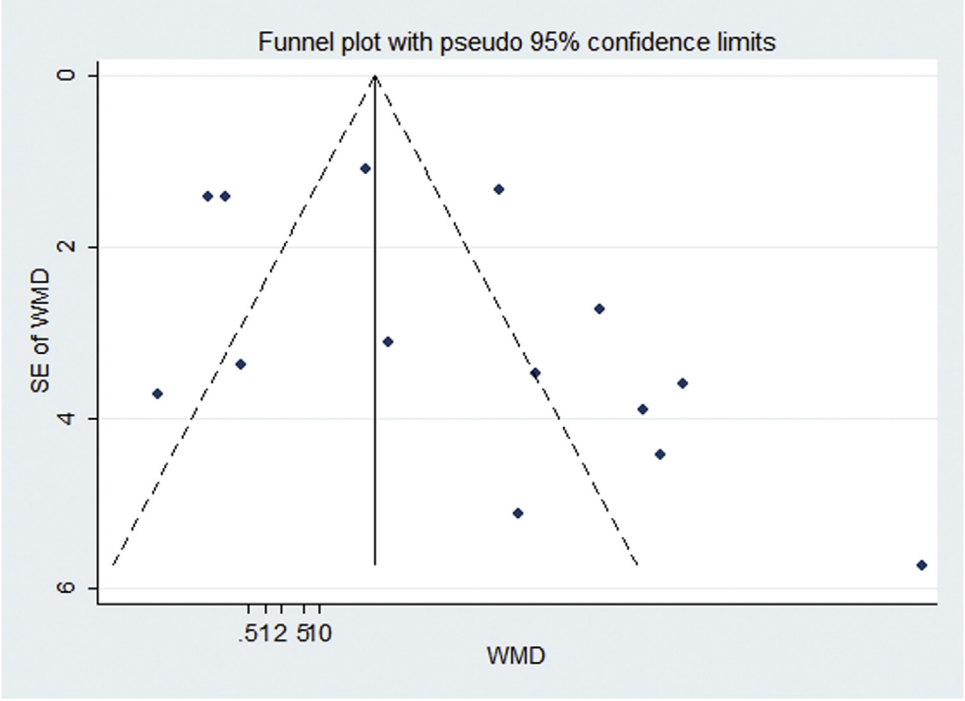

Fig. 2. Funnel plot for publication bias for the studies included in this meta-analysis: total motility.

Table 3
Pooled results for sperm parameters following vitrification versus conventional freezing.

	All studies				Sensitivity analysis[a]				
	Studies/samples	WMD(95%CI)[b]	I^2	P-value	Studies/samples	WMD(95%CI)	I^2	P-value	Interpretation
Total motility	10/834	6.98 [2.94, 11.02]	91	0.0007*	8/644	8.70 [3.96, 13.43]	91	0.0003*	vitrification is better
Progressive motility	5/360	4.59 [0.78, 8.39]	77	0.02*	4/244	2.77 [0.80, 4.73]	0	0.006*	vitrification is better
morphology	9/680	0.11 [-0.42, 0.63]	40	0.69	7/462	1.72 [0.27, 3.16]	23	0.02*	vitrification is better
DFI	4/398	−1.18 [-2.81, 0.45][c]	38	0.16	3/180	−0.06 [-1.95, 1.84]	0	0.95	NS

[a] Sensitivity analysis excluding studies judged to be at high risk of allocation concealment.
[b] A WMD > 0 suggest a better recovery of this outcome in vitrification compared with conventional freezing methods.
[c] A WMD < 0 suggest a lower DNA damage in vitrification compared with conventional freezing methods.
* Significant differences.

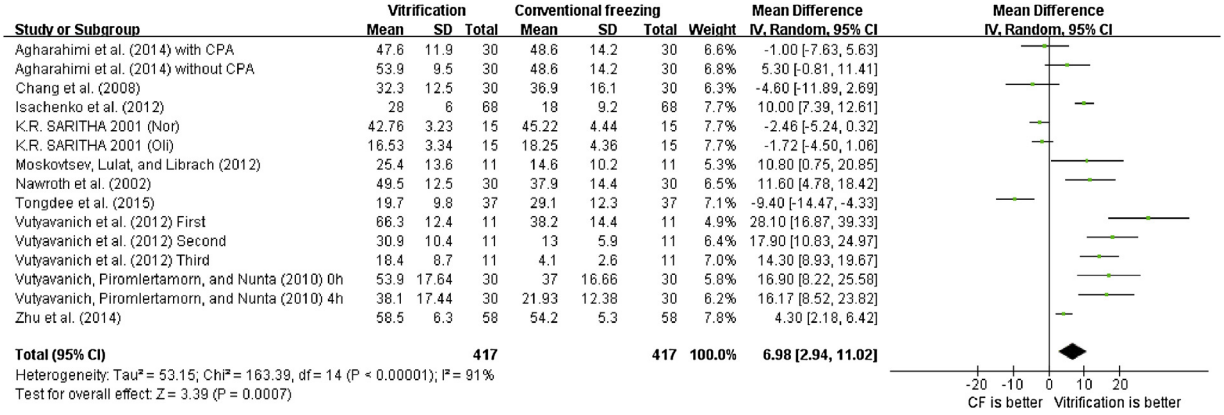

Fig. 3. Comparison of conventional freezing methods versus vitrification: total motility.

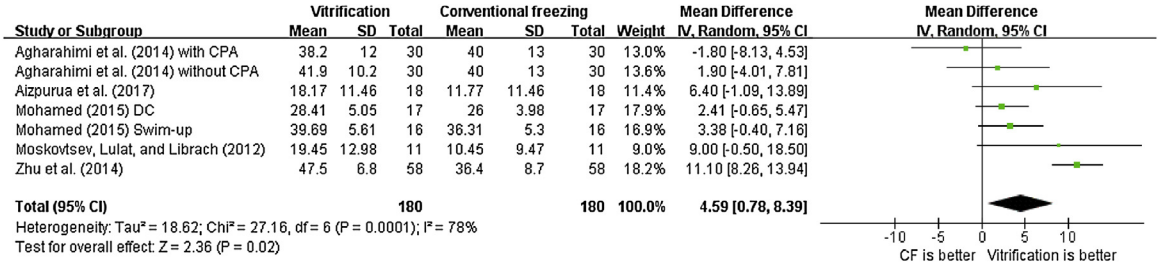

Fig. 4. Comparison of conventional freezing methods versus vitrification: progressive motility.

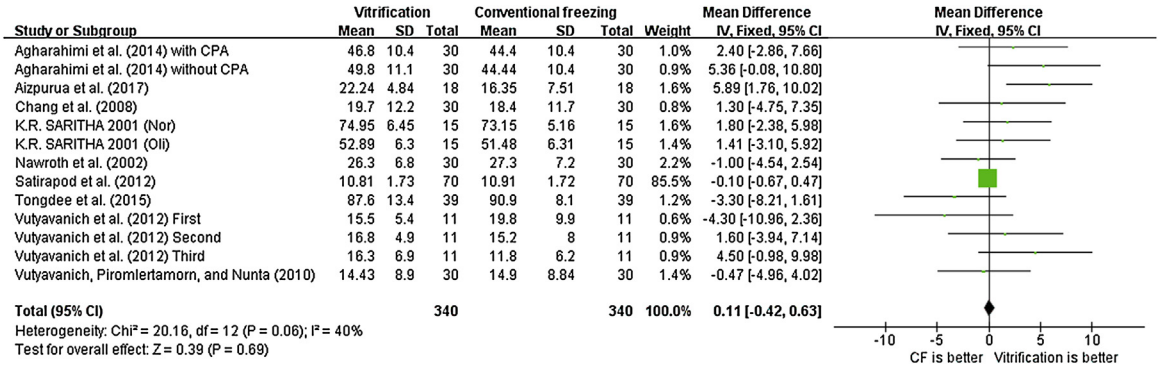

Fig. 5. Comparison of conventional freezing methods versus vitrification: morphology.

CI 1.64; 9.43; P = 0.005; I^2 = 77%] than conventional freezing, whereas the total motility of spermatozoa after CPAs-presence vitrification was significant lower than conventional freezing [WMD -3.47; 95% CI -6.13; -0.81; P = 0.01; I^2 = 48%].

Subgroup analysis based on quality of sperm cryopreserved shown cryopreservation of high quality sperm by using vitrification had higher past-thawed total motility [WMD 8.73; 95% CI 3.88; 13.58; P = 0.0004; I^2 = 92%], progressive motility [WMD

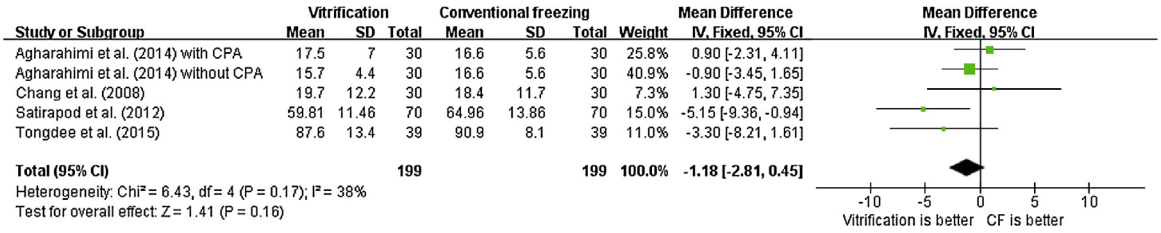

Fig. 6. Comparison of conventional freezing methods versus vitrification: DFI.

Table 4
Subgroup analysis of cryopreservation of spermatozoa according to the vitrification protocol and quality of spermatozoa cryopreserved.

	Subgroup	Sperm parameters	Studies/samples	WMD (95% CI)[a]	P-value	I^2
Vitrification protocol	CPAs-free	TM	7/476	12.51(8.63;16.40)	<0.00001*	82
		PM	5/300	5.54(1.64;9.43)	0.005*	77
		Morphology	5/238	1.77(-0.95;4.48)	0.20	54
		DFI	1/30	−0.90(-3.45;1.65)[b]	0.49	–
	CPAs-presence	TM	4/224	−3.47(-6.13; -0.81)	0.01*	48
		PM	1/60	−1.80(-8.13;4.53)	0.58	–
		Morphology	5/364	−0.04(-0.60;0.51)	0.87	0
		DFI	3/260	−1.59(-4.83;1.64)[b]	0.34	64
The quality of spermatozoa	[High quality][c]	TM	9/684	8.73 (3.88; 13.58)	0.0004*	92
		PM	3/174	10.40 (7.84; 12.96)	<0.00001*	0
		Morphology	8/530	0.01 (-0.52; 0.55)	0.96	42
		DFI	3/2278	−3.13 (-5.96; -0.30)[b]	0.03*	32
	Low quality	TM	2/150	0.34 (-3.86; 4.53)	0.88	53
		PM	2/186	2.19 (0.10; 4.27)	0.04*	0
		Morphology	2/150	2.83 (-0.07; 5.73)	0.06	0
		DFI	1/120	−0.20 (-2.20; 1.79)[b]	0.84	0

TM: Total motility; PM: Progressive motility; DFI: DNA fragmentation index.
CPAs-free vitrification: absence of permeable CPAs or use of low concentration of permeable CPAs.
CPAs-presence vitrification: use of the same concentration of CPAs as conventional freezing methods.
[a] A WMD > 0 suggest a better recovery of this outcome in vitrification compared with conventional freezing methods.
[b] A WMD < 0 suggest a lower DNA damage in vitrification compared with conventional freezing methods.
[c] High quality spermatozoa defined as concentration > 20 million/ml. Samples collected from healthy donors or normozoospermic patients also included in high quality group.
* Significant difference.

10.40; 95% CI 7.84; 12.96; P < 0.00001; I^2 = 0%] and lower DFI [WMD -3.13; 95% CI -5.96; -0.30; P = 0.03; I^2 = 32%] than those cryopreserved by using conventional freezing methods. In contract, cryopreservation of low quality sperm by using vitrification only had higher past-thawed progressive motility [WMD 2.19; 95% CI 0.10; 4.27; P = 0.04; I^2 = 0%] than those cryopreserved by using conventional freezing methods.

Discussion

Our meta-analysis is the first attempt to evaluate and compare the efficacy with two different cryopreservation methods used for human spermatozoa. The principal results of this meta-analysis suggests that spermatozoa coming from vitrification cycles could result in better total and progressive motility than those coming from conventional freezing cycles. This study also reveals that vitrification protocol and cryopreservation of different quality sperm at vitrification/thawing cycles influence the efficacy of vitrification.

It must be emphasized that the overall risks of cryopreservation of spermatozoa are presented with substantial clinical heterogeneity. The causes of this high heterogeneity are as follows. First, component of CPAs in these studies varied, for both vitrification and conventional freezing, and thus pooling of sperm parameters data might not be entirely appropriate. Second, this high heterogeneity could be explained by the inherent characteristics of motility. Motility is the most sensitive parameter for spermatozoa and it is easily affected by the change of extracellular environment [1,42]. Other factors that were likely to affect the past-thawed motility include: carrier tools [13]; thawing protocols [43] and pre-freezing techniques [44]. Furthermore, inclusion criterion of spermatozoa also accounts for heterogeneity [27].

Vitrification has gained popularity in last decade and its extensive application has improved safety and efficacy of embryo, oocyte and blastocyst cryopreservation [10]. However, embryo vitrification technique with high concentrations of permeable CPAs cannot be successfully used in sperm vitrification due to unique properties of spermatozoa [45]. Spermatozoa are osmotically fragile, which cannot undertake the lethal effect of osmotic shock. Meanwhile, high concentration of permeable CPAs is cytotoxic [46]. Compared with embryo vitrification, CPAs-free vitrification used far lower concentrations of permeable CPAs and it suggested that nonpermeable CPAs may play a leading role in sperm vitrification.

Vitrification has several advantages over conventional freezing. Relatively simple process is one of the most remarkable advantages of this technique. It takes much less time to perform because no additional treatment is required during cryopreservation [38]. However, extensively fast cooling rate is essential for vitrification because methodological advantages of this technique can be achieved by cooling at hundreds of thousands of °C/min [47]. Therefore, one challenge in improving vitrification is that large volumes of sperm cannot be successfully vitrified. Meanwhile, traditional carrier tools which have relatively thick walls cannot meet the vitrification requirement. In contrast, vitrification carriers are characterized by having very thin walls or completely

open like the cryoloop. Varied carriers of spermatozoa vitrification have been described previously [21]. But the benefit of different carriers remain controversial.

However, several important limitation should to be considered. First, the principal results of the study are based on a small number of randomized controlled trials (n = 12), suggesting that it is great need of further studies with large sample size to confirm the efficacy of vitrification in cryopreservation of spermatozoa. Another limitation of the current review is that we were not able to estimate the pregnancy outcomes after use of past-thawed spermatozoa. Although several studies reported that pregnancy outcomes are highly dependent on the DNA integrity of sperm [48], only limited literature has focused on the role of vitrification in the DFI, therefore, this issue remains unresolved.

In the current study, sperm cryopreservation by conventional freezing and vitrification were compared. Results of the present meta-analysis indicate that sperm vitrification is superior to conventional freezing based on direct comparison of total motility and progressive motility. However, the efficacy of vitrification is influence by using different vitrification protocol and cryopreservation of different quality spermatozoa. These variable should be considered when assessing the quality of past-thawed spermatozoa after vitrification. Due to the small number of studies with substantial clinical heterogeneity included in this meta-analysis, further well conducted studies are required to confirm the efficacy of vitrification in cryopreservation of spermatozoa, in addition, allow the examination of the two cryopreservation methods in terms of pregnancy achievement and determination of the role of clinical variable on efficacy of vitrification.

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Conflict of interest

The authors declare that no competing interests exist.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ejogrb.2018.11.028.

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