Comparison of intracytoplasmic sperm injection outcome with fresh versus frozen-thawed testicular sperm in men with nonobstructive azoospermia: a systematic review and meta-analysis

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Abstract

Purpose The purpose of the study is to explore testicular sperm cryopreservation in patients with nonobstructive azoospermia (NOA) whether affect the outcome of subsequent intracytoplasmic sperm injection (ICSI).

Methods A systematic review and meta-analysis was conducted by searching the MEDLINE and EMBASE databases for relevant published studies in English language (1997–2017). Studies were eligible if they included the comparison of using fresh and frozen-thawed testicular sperm followed by ICSI. Two reviewers independently performed data extraction, quality assessment and assessed the risk of bias. The overall summary risk estimated the number of events. A meta-analysis was conducted using a random effects or fixed effects model analysis according to the test of heterogeneity.

Results A total of 17 studies with 1,261 ICSI cycles were identified. Analysis of the present data showed no difference in the fertilization outcome when comparing fresh versus frozen-thawed spermatozoa (RR = 1.02, 95% CI 0.86–1.09). Similarly, no difference in CR (RR = 1.01, 95% CI 0.96–1.05), good embryo rate (RR = 1.01, 95% CI 0.95–1.09), and IR (RR = 0.93, 95% CI 0.66–1.30) was observed if the spermatozoa was fresh or frozen-thawed. Finally, no difference in CPR or LBR was noted when using fresh or frozen-thawed cycles were analyzed separately (RR = 1.03, 95% CI 0.86–1.24; RR 1.11, 95% CI 0.88–1.41, respectively).

Conclusions In men with NOA, the ICSI outcome is not affected by whether the retrieved testicular sperm is fresh or frozen. Sperm cryopreservation ought to be considered in every surgical sperm retrieval case, which remain feasible even in patients with few testicular sperm retrieved.

Keywords Nonobstructive azoospermia · Testicular sperm extraction · Cryopreservation · Intracytoplasmic sperm injection

Introduction

The prevalence of azoospermia was about 1% in the male population, and around 10–15% in infertile men [1]. Nonobstructive

azoospermia (NOA) is one of the most challenging subsets, which is caused by spermatogenic dysfunction. Surgical sperm retrieval, such as testicular sperm extraction (TESE) and microdissection testicular sperm extraction (microTESE), combined with intracytoplasmic sperm injection (ICSI) was an effective treatment for men with NOA, and these men can now father their own biologic children [2].

The sperm recovery rate (SRR) of TESE or microTESE varies around 16.7–63% in well-defined NOA populations [3]. If pregnancy is not succeeded, repeat testicular biopsy is required for the next cycle in men with NOA. However, repeated TESE could not achieve 100% recovery rate [4]. Thus, sperm cryopreservation is necessary to avoid repeated testicular biopsies for each ICSI cycle [5], and successful clinical

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pregnancy has been reported using frozen-thawed testicular tissue and testicular spermatozoa [5, 6].

Majority of studies in literature confirmed no significant difference in the fertilization rate (FR), implantation rate (IR), and clinical pregnancy rate (CPR) between the use of fresh and frozen-thawed testicular sperm. A meta-analysis published in 2013 also confirmed no statistical difference between the groups when assessed for FR and CPR in men with azoospermia caused due to spermatogenic dysfunction.

However, the argument between the use of fresh or frozen-thawed spermatozoa in men with NOA needs further research. Among the latest publications with larger sample sizes, Park et al. [7] observed statistical differences in the pregnancy rate and IR between the groups. Comparison between the cycles with fresh and frozen-thawed spermatozoa revealed higher FR, good embryo rate, and CPR in patients with nonmosaic Klinefelter syndrome [8], resulting in NOA.

To review and update the issue regarding the use of fresh or frozen-thawed testicular sperms in patients with NOA followed by ICSI outcome, we performed a systemic review and meta-analysis. All available parameters including FR, cleavage rate (CR), embryo quality, IR, CPR, and live birth rate (LBR) are taken into account to study.

Materials and methods

Search strategy

This meta-analysis was performed in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Applicable papers that addressed the question of interest were exhaustively searched in the literature through MEDLINE and EMBASE databases. The primary search items used were “azoospermia,” “nonobstructive,” “ICSI,” “frozen,” “cryopreservation,” and “freeze” until December 2017. The references of the identified articles were also searched. Our search was limited to human studies and articles published in English.

Eligibility criteria

This meta-analysis included studies evaluating the outcome of ICSI with the use of fresh and frozen-thawed testicular sperm. The inclusion criteria of the studies were as follows: men with NOA, surgically retrieved testicular sperm, comparison of the use of fresh and frozen-thawed testicular sperm followed by evaluation of ICSI, FR data, embryo assessment data, IR data, CPR data and LBR data. We excluded studies that combined obstructive and NOA data, did not provide both fresh and cryopreserved data and did not choose ICSI for further assisted reproduction. Two independent authors assessed all the titles and abstracts retrieved from the search.

Data extraction and quality assessment

Two reviewers independently performed data extraction and quality assessment. Any disagreements between the two reviewers were discussed and settled. The following parameters including study population, publication year, geographic location, study design; number of ICSI cycles, FR, CR, embryo quality, IR, CPR, and LBR were summarized for the purpose of the present review. Two pronuclei (PN) FR were considered as total FR in some included studies where the total FR data was missing. Both grade A and grade B embryos were considered as good embryos. CPR and LBR are calculated using the number of clinical pregnancies and deliveries as per ICSI cycles, respectively. The quality of trials included was assessed using Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence and Newcastle-Ottawa quality assessment scale.

Statistical analysis

Statistical analysis of the data was performed using Stata 14.0 (Statacorp). Fixed-model analysis was primarily applied to create summary risk estimates and 95% confidence intervals (CIs) to test the effect of different parameters: FR, CR, good embryo rate, IR, CPR and LBR. An egger's regression analysis [9] was performed, and each outcome was tested for heterogeneity with the use of Q test and I^2 . Random-model analysis was performed when heterogeneity existed, with significant Q test ($P < 0.05$ or $I^2 > 50\%$).

Results

Through inherent research, only retrospective analyses studies were found. Seventeen papers that met our criteria were identified and included a total of 1261 ICSI cycles. The characteristics of the retrieved trials, including parameters on study quality, are listed in Tables 1 and 2. The study flow was summarized in Fig. 1. Egger regression analysis suggested the absence of publication bias ($P = .892$; Fig. 2).

Among the studies that included the fertility outcomes after ICSI analysis, 16 trials provided the information on FR. Tests of heterogeneity showed significant results ($P = 0.000$) with $I^2 = 63.1\%$, indicating that the studies are heterogeneous. So, a random-model analysis was used. Overall, a total of 10,051 matured oocytes were collected. In the 575 fresh ICSI cycles, a total of 5174 oocytes were injected and 2934 oocytes showed 2PN formation (FR, 56.7%). In 609 frozen-thawed ICSI cycles, a total of 4877 oocytes were injected and 2779 oocytes demonstrated normal 2PN (57.0%). The FR did not show significant differences between fresh versus frozen-thawed testicular biopsy sperm for ICSI (RR = 1.02, 95% CI 0.86–1.09; Fig. 3).

Table 1 Characteristics of included studies in the meta-analysis

Study	Country	Study period	Study design	LE	Study quality*	ICSI cycles
Schachter-safrai et al., 2017	Israel	1999–2011	Retrospective	3	7	70
Park et al., 2015	Korea		Retrospective	3	7	110
Madureira et al., 2014	Portugal	1994–2011	Retrospective	3	7	37
Karacan et al., 2013	Iraq	2006–2012	Retrospective	3	7	209
Raheem et al., 2013	UK	2001–2010	Retrospective	3	7	77
Tavukcuoglu et al., 2013	Germany	2010–2012	Retrospective	3	7	82
Kalsi et al., 2010	UK	1993–2008	Retrospective	3	7	48
Akarsu et al., 2009	Turkey		Retrospective	3	5	6
Konc et al., 2008	Hungary		Retrospective	3	7	157
Wu et al., 2005	USA	2001–2003	Retrospective	3	7	30
Hauser et al., 2005	Israel	1997–2004	Retrospective	3	7	26
Verheyen et al., 2004	Belgium	1998–2002	Retrospective	3	6	86
Friedler et al., 2002	Israel	1995–2001	Retrospective	3	7	128
Sousa et al., 2002	Portugal		Retrospective	3	6	87
Habermann et al., 2000	America		Retrospective	3	5	12
Ben-Yosef et al., 1999	Israel	1995–1998	Retrospective	3	6	57
Friedler et al., 1997	Israel	1995–1996	Retrospective	3	6	39

LE, level of evidence

*Using Newcastle-Ottawa Scale (score from 0 to 9)

Nine studies evaluated the quality of transferred embryos using CR and grading, respectively. The I^2 in clinical trials assessing CR was 67.4% ($P = .002$), and good embryo rate was 23.9% in 7 out of 9 studies ($P = .247$), and so a random-model and fix-model analyses were used, respectively. A total of 2462 embryos were observed. Among them, 1215 embryos (CR, 92.9%) from fresh ICSI cycles and 1057 embryos (91.5%) from frozen-thawed ICSI cycles were cleaved, respectively. A total of 601 embryos (good embryo rate, 72.6%) from fresh ICSI cycles and 496 embryos (69.8%) from frozen-thawed ICSI cycles were classified as good embryos (grade A and grade B). The overall summary risk estimates showed no statistical difference between the use of fresh versus frozen-thawed testicular sperm in CR (RR = 1.01, 95% CI 0.96–1.05; Fig. 4a) or embryo morphology (RR = 1.01, 95% CI 0.95–1.09; Fig. 4b).

IR outcome was assessed in 12 of the included studies. The summarized evidence accounted for a total of 3769 embryos transfer cycles, and the test of heterogeneity yielded an insignificant result ($I^2 = 55.5\%$, $P = .010$), allowing the use of fixed-model analysis. The number of implanted embryos in this cohort was 170 (IR, 12.0%) from fresh ICSI cycles and 247 (10.5%) from frozen-thawed ICSI cycles. No difference in IR was noted when fresh or frozen-thawed ICSI cycles were analyzed (RR = 0.93, 95% CI 0.66–1.30; Fig. 4c).

All the included studies reported data on clinical pregnancies, and 15 of the included studies met the criteria for LBR outcome. Test of heterogeneity demonstrated no significance by Q test ($P = .473$, $I^2 = 0.0\%$; $P = .774$, $I^2 = 0.0\%$, respectively), and so a fixed-model analysis was used. A total of 178

embryos (CPR, 29.4%) in fresh ICSI cycles and 185 embryos (28.2%) in frozen-thawed ICSI cycles resulted in clinical pregnancies and the reported LBR in the two groups were 23.5 and 20.5%, respectively. Meta-analysis showed no difference in either CPR/cycle (RR = 1.03, 95% CI 0.86–1.24; Fig. 5a) or LBR/cycle (RR = 1.11, 95% CI 0.88–1.41; Fig. 5b) between using fresh and frozen-thawed testicular sperm.

Discussion

To our knowledge, this study is the first study to systematically review and meta-analyze all the available papers regarding ICSI outcomes in men with NOA. Corresponding to the previous meta-analysis study [10], our data suggested no statistical difference between the use of fresh versus frozen-thawed testicular sperm when assessing FR and CPR in those couples who accept ICSI. Besides, no difference in CR, good embryo rate, IR or LBR was noted when the fresh or frozen-thawed cycles were analyzed separately.

MicroTESE is first described in 1999, and is considered as the gold standard for sperm retrieval among patients with NOA [11, 12]. Three testicular histologies are known to cause NOA, and were as follows: hypospermatogenesis (HS), which carries the highest sperm retrieval rates (SRR) of 73–100%, maturation arrest (MA) has an SRR of 27–86%, and Sertoli Cell Only (SCO) syndrome, which is the most severe form, and has a SRR of 22.5–41% [13]. There are no definitive markers of spermatogenesis that have been widely accepted,

Table 2 Comparison of ICSI cycles using with fresh versus frozen-thawed surgical retrieved testicular sperm

Study	Sperm origin	ICSI cycles	Fertilization rate (%)	Cleavage rate (%)	Good embryo rate (%)	Implantation rate (%)	Clinical pregnancies rate (%)	Live birth rate (%)
Schachter-safrai et al., 2017	Fresh	22	110/211 (52.1%)			3/41 (7.3%)	4/22 (18.2%)	1/22 (4.5%)
	Frozen-thawed	48	214/437 (49.0%)			12/98 (12.2%)	9/48 (18.8%)	9/48 (18.8%)
Park et al., 2015	Fresh	61	436/731 (59.6%)	262/277 (94.6%)	169/262 (64.5%)	9/41 (22.0%)*	9/61 (14.8%)	5/61 (8.2%)
	Frozen-thawed	49	371/542 (68.5%)	249/263 (94.7%)	165/249 (66.3%)	24/42 (57.1%)	16/49 (32.7%)	2/49 (4.1%)
Madureira et al., 2014	Fresh	20	93/155 (60.0%)*	90/93 (96.8%)	68/90 (75.6%)*	14/41 (34.1%)	12/20 (60.0%)*	10/20 (50.0%)
	Frozen-thawed	17	57/132 (43.2%)	56/57 (98.2%)	31/56 (55.4%)	5/29 (17.2%)	4/17 (23.5%)	4/17 (23.5%)
Karacan et al., 2013	Fresh	99	626/931 (67.2%)			24/188 (12.8%)	29/99 (29.3%)	27/99 (27.3%)
	Frozen-thawed	110	599/922 (65.0%)			31/231 (13.4%)	28/110 (25.5%)	25/110 (22.7%)
Raheem et al., 2013	Fresh	31	56%		88%		9/31 (29.1%)	6/31 (19.4%)
	Frozen-thawed	46	45.7%		85%		16/46 (34.8%)	9/46 (19.6%)
Tavukcuoglu et al., 2013	Fresh	43	178/397 (44.8%)		25/43 (58.1%)		19/43 (44.2%)	16/43 (37.2%)
	Frozen-thawed	39	169/361 (46.8%)		20/39 (51.3%)		17/39 (43.6%)	12/39 (30.8%)
Kalsi et al., 2010	Fresh	41	215/423 (50.8)				15/41 (36.6%)*	13/41 (31.7%)
	Frozen-thawed	7	45/71 (63.4%)				4/7 (57.1%)	4/7 (57.1%)
Akarsu et al., 2009	Fresh	4	37/64 (57.8%)	35/37 (94.6%)			3/4 (75.0%)	3/4 (75.0%)
	Frozen-thawed	2	6/31 (19.4%)	4/6 (66.7%)			0/2 (0%)	0/2 (0%)
Konc et al., 2008	Fresh	64	68/107 (63.6%)			64/592 (10.8%)	20/64 (31.3%)	10/64 (15.6%)
	Frozen-thawed	93	99/149 (66.4%)			93/1400 (6.6%)	22/93 (23.7%)	14/93 (18.3%)
Wu et al., 2005	Fresh	6	38/51 (74.5%)		52.9%	3/19 (15.8%)*	2/6 (33.3%)*	2/6 (33.3%)*
	Frozen-thawed	24	202/307 (65.8%)		53.8%	21/84 (25.0%)	15/24 (62.5%)	10/24 (41.7%)
Hauser et al., 2005	Fresh	13	61/146 (41.8%)	52/61 (85.2%)	32/52 (61.5%)	4/38 (10.5%)	2/13 (15.4%)	2/13 (15.4%)
	Frozen-thawed	13	45/128 (35.2%)	45/45 (100%)	30/45 (66.7%)	2/34 (5.9%)	2/13 (15.4%)	2/13 (15.4%)
Verheyen et al., 2004	Fresh	44	232/400 (58.0%)			8/105 (7.6%)	7/44 (15.9%)	
	Frozen-thawed	42	189/319 (59.2%)			6/81 (7.4%)	6/42 (20.5%)	
Friedler et al., 2002	Fresh	65	426/836 (51.0%)	383/426 (89.9%)		26/205 (12.7%)	19/65 (27.1%)	16/65 (24.6%)
	Frozen-thawed	63	360/706 (51.0%)	317/360 (88.1%)		29/166 (17.4%)	19/63 (25.8%)	15/63 (23.8%)
Sousa et al., 2002	Fresh	50	212/311 (68.2%)	204/212 (96.2%)	177/204 (86.8%)		17/50 (34.0%)	
	Frozen-thawed	37	120/200 (60.0%)	101/120 (84.2%)	85/101 (84.2%)		9/37 (24.3%)	
Habermann et al., 2000	Fresh	3	1/31 (3.2%)	12/16 (75.0%)		1/8 (12.5%)	1/3 (33.3%)	1/3 (33.3%)
	Frozen-thawed	9	68/122 (55.7%)	64/68 (94.1%)		7/29 (24.1%)	6/9 (66.7%)	3/9 (33.3%)
Ben-Yosef et al., 1999	Fresh	15	64/119 (53.8%)	62/64 (96.9%)	41/62 (66.1%)	6/48 (12.5%)	4/15 (26.7%)	3/15 (20.0%)
	Frozen-thawed	42	181/326 (55.5%)	173/181 (94.4%)	129/173 (74.6%)	13/121 (10.7%)	9/42 (21.4%)	7/42 (16.7%)
Friedler et al., 1997	Fresh	25	122/261 (60.7%)	115/122 (94.3%)	89/115 (77.4%)	8/91 (8.8%)	6/25 (24.0%)	5/23 (21.7%)
	Frozen-thawed	14	54/124 (43.5%)	48/54 (88.9%)	36/48 (77.1%)	4/37 (10.8%)	3/14 (21.4%)	1/11 (9.1%)

*Statistical significance ($P < .05$) for fresh versus frozen-thawed

Fig. 1 Flowchart of included studies

and unfortunately the gold standard method could not ensure sperm retrieval in every NOA case. However, testicular biopsy was considered as the strongest predictor of positive sperm retrieval previously. Repeated TESE ensured a high SRR when the first sperm recovery procedure has been successful [4]. Nevertheless, a repeated TESE application for each ICSI cycle is not deemed to be economical.

Addition of spermatozoa cryopreservation for future ICSI cycles could avoid both unnecessary female stimulation and repeated testicular biopsies for each ICSI cycle [5]. The inheritance of surgical sperm retrieval creates enormous pressure for

patients and andrologists, and in turn the sperm cryopreservation reduces the stress. Other advantages of sperm cryopreservation include reduced cost and adequate time to ensure the availability of sperm before IVF processes [13]. Besides, several couples decline to provide additional information for relatives because of privacy concerns, and undergo surgery asynchronously. This subsequently makes the couples propitious in taking care of each other. Since spermatozoa cryopreservation has so many advantages, and could therefore increase the probability of conception in couples with NOA [13].

Does the spermatozoa cryopreservation affect the outcome of ICSI? The feasibility of cryopreservation of testicular spermatozoa was first reported in 1996 [14], and cryopreservation of testicular tissue was published 1 year later [6]. The first published report regarding the comparison between the outcome of ICSI using fresh and cryopreserved testicular spermatozoa of the same NOA patients in the 9 couples revealed no differences in terms of FR, CR and CPR [15]. Until now, among men with NOA, majority of the included studies confirmed no significant differences in FR, and CPR between the use of fresh and frozen-thawed testicular sperm. A previous meta-analysis study had also confirmed that there was no statistical difference among the groups when assessing FR and CPR in men with azoospermia due to spermatogenic dysfunction. However, recent papers with larger sample size demonstrated statistical differences in pregnancy and implantation rate between the groups [7]. Comparison between cycles with fresh and frozen-thawed spermatozoa demonstrated higher FR and CPR with fresh

Fig. 2 Egger's adjusted rank correlation test

Fig. 3 Forest plot of summary risk estimates for fertilization rate (FR). RR = relative risk; CI = confidence interval

spermatozoa injection [8]. Wu *et al.* reported that the IR differed significantly between fresh and frozen-thawed cycles [16].

The pathological basis of microTESE was focal spermatogenesis in the testicles. The number of sperms retrieved in few patients could be extremely scarce. To some extent, the loss of few sperms during frozen-thawed processes showed tremendous influence. It should be noted that freezing testicular tissue may result in some degree of damage to spermatozoa [16]. Sperm cryoinjury during cooling, thawing, and cryoprotectant exposure resulted in dramatic functional and structural changes [17, 18]. Sperm freezing can cause swelling and rupture of inner and outer acrosomal and plasma membranes [19]. Moreover, the production of oxygen free radicals was increased during both cooling and thawing processes, leading to free radical injury secondary to plasma membrane lipid peroxidation [20]. The rare cryopreserved spermatozoa did not maintain all the properties after thawing [8]. However, Friedler *et al.* [15] suggested that cryopreservation ought to be considered in every surgical sperm retrieval case, making it feasible even in patients with few testicular sperm retrieved.

According to the literature, the ICSI outcomes were improved when the cryopreserved testicular spermatozoa exhibited any kind of motility [21], although other studies suggested no major difference between the use of motile or immotile testicular spermatozoa [22, 23]. Sperm motility may be lost during the cryopreservation process. Embryos cannot be implanted when the immotile spermatozoa were microinjected [21]. Nijs *et al.* [24] demonstrated that 65% of oocytes were fertilized when totally immotile testicular spermatozoa were injected, but still the overall embryo quality was low. Fundamentally, the significant reduction of FR achieved by embryos resulting from the

microinjection of immotile spermatozoa may be due to higher incidence of chromosomal abnormalities [25].

The injection of immotile spermatozoa results in an inferior ICSI outcome emphasizing the importance of viability of spermatozoa. To improve the outcome of ICSI carried out with immotile spermatozoa, addition of agents that induce motility like relaxin [26], deoxyadenosine [27], caffeine [28], pentoxifyllin [29] and theophylline [30] were introduced. Majority of such agents belong to methylxanthine group, triggering the movement in immotile but alive spermatozoa for short period of time, making the process of identification and selection far easier [31]. Kovacic *et al.* [32] have concluded that pentoxifyllin introduction into the thawed testicular spermatozoa could induce motility, consequently resulting in higher FR. However, smaller studies where cycles did not use activating substances achieved a slight but not significantly higher FR, CPR and LBR when compared with the cycles which used pentoxifylline [32].

The latest World Health Organization (2010) manual for the examination and processing of human semen recommended hypo-osmotic swelling (HOS) test as an alternative to eosin test for the diagnostic evaluation of vitality. The HOS test can give information on viability and functionality of spermatozoa through exposing to hypo-osmotic conditions along with functional membranes [33, 34]. The application of HOS test leads to higher developmental potential and IR as well as CPR of the

Fig. 4 Meta-analysis of ICSI outcome using fresh or frozen-thawed testicular sperm in men with NOA. **a** Cleavage rate (CR), **b** good embryo rate, and **c** implantation rate (IR). RR = relative risk; CI = confidence interval

(A)

(B)

(C)

Fig. 5 Meta-analysis of ICSI outcome using fresh or frozen-thawed testicular sperm in men with NOA. **a** Clinical pregnancies rate (CPR) and **b** live birth rate (LBR) per ICSI cycle. RR = relative risk; CI = confidence interval

embryos [35]. FR was increased from 30.3 to 44.0% in the fresh and from 25.7 to 42.7% in the frozen TESE group by HOS test selection [36]. However, Check *et al.* argued that even spermatozoa with low HOS test grade has the similar fertility potential [37], and poor HOS test results from cryopreserved spermatozoa despite preservation demonstrated sperm motility [38].

Based on the flexible tail of immotile spermatozoa, the sperm tail flexibility (STF) test was performed to choose viable spermatozoa for injection. More specifically, the sperm tail which is able to move without the movement of the head with an ICSI needle is considered to be viable [39]. With the use of STF test, both fresh and frozen cycles have been found to

result in same FR whether motile or immotile spermatozoa were injected [40].

Laser-assisted immotile spermatozoa selection (LAISS) is a novel technique used for identifying the viability of immotile spermatozoa. In vital spermatozoa, the tail starts to curl and coil almost immediately after a single laser shot. The use of LAISS on ejaculated asthenozoospermic and immotile spermatozoa is equally effective compared to HOS test [41]. In addition, Chen et al. [42] has reported a successful pregnancy using completely immotile but viable frozen-thawed spermatozoa selected by laser.

Only a few series presented detailed ICSI outcomes according to pathological type. It was not possible to perform a meta-analysis on this issue due to lack of adequate data. Although the CPR was lower (20% in SCOS, 35% in MA, 32.1% in HS) in the reported 87 ICSI cycles, the FR, CR and high embryo quality were relatively higher in subgroups of NOA (63.5, 85, 88.2% respectively in SCOS, $n = 12$; 66.3, 84.1, 86.2% respectively in MA, $n = 22$, and 74.8, 95.5, 85.4% respectively in HS, $n = 55$) [43]. Other studies have demonstrated lower FR and CPR in spermatozoa obtained from patients with MA versus HS, as well as other differences using spermatozoa from patients with MA versus SCO [44]. However, Raheem et al. [7] have reported no significant differences in the laboratory results in successfully obtaining the spermatozoa. Although testicular histopathology may be considered as the strongest predictor of positive sperm retrieval in patients with NOA, different histopathological subsets of NOA did not affect the ICSI outcomes [32].

Regarding the cryopreservation object being used in our included studies, the two choices accepted for sperm preservation were sperm-containing suspensions and testicular tissue sample. The cryoprotectants application, cryopreservation protocol varied by laboratory and individual, but the outcome appears to be satisfactory. However, in clinical practice, surgical retrieve of rare or very low concentrations of sperm could happen. Because conventional cryopreservation presents a technical limitation to those patients with rare quantities of sperm, newer technologies have been developed to address this clinical scenario. Over the years, a number of devices have been trialed to cryopreserve scarce spermatozoa, included Cryolock [45], Cell Sleepers [46], and other biological or nonbiological devices. None of these have yet achieved widespread application, perhaps due to technical requirement and cost concerns.

Impaired spermatogenesis is negatively related to fertilization and embryo development potential. NOA showed lower diploid fertilization, blastocyst rates and longer timing of development to the blastocyst stage than normozoospermia [47–49]. It has been found that men with NOA often have aneuploidy, mosaicism and Y microdeletions and DNA damage that contribute to decreased FR [49, 50]. However, once a blastocyst is obtained, the male factor does not affect the aneuploidy rate,

eventually the CR, good embryo rate, IR and possibly the post-implantation development of euploid embryos [47, 48]. As for pregnancy results, majority of studies in literature confirmed that the CPR and LBR were significantly reduced in NOA compared with obstructive azoospermia [47–49].

Limitations of this study include small sample sizes in some included studies that were used for fertility outcome analysis. Moreover, the analyzed studies were retrospective in nature and limited in number. Therefore, we could not control the biases of individual studies. Undoubtedly, there was also publication bias in reporting the splendid outcome, despite the bias being obviously noticed in our statistical analysis. Besides, ICSI outcomes may be affected by many confounding factors. However, almost all the included studies ignored the influence of female factor. Six studies in this meta-analysis included age, but showed no significant differences between fresh or frozen-thawed groups. Information about other baseline characteristics or confounding factors were scarcely provided in the included studies. Furthermore, the impact of lifestyle behaviors, such as regular smoking, alcohol and drug consumption for basic disease like diabetes, hypertension, hyperlipidemia on ICSI outcome remains further research. Therefore, we recommend that future studies include data on male factor that affect the sperm quality, the characteristics of the female population in detail to evaluate a potential positive or negative impact on clinical outcome as well as the blastocyst euploid rate. These aspects could be another important new element to better understand the real effect of sperm cryopreservation.

In conclusion, this systematic review and meta-analysis of ICSI outcome in patients with NOA showed no discernible differences in FR, CR, good embryo rate, IR, CPR associated with LBR when compared to the use of fresh versus frozen-thawed testicular spermatozoa. In view of these findings, it is important to develop consensus regarding the cryopreservation of spermatozoa. Effective doctor-patient communication is suggested to avoid any communication pitfalls. Sperm cryopreservation ought to be considered in every surgical sperm retrieval case due to its advantages.

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Authors' roles ZY made substantial contributions to define the research question, acquisition and analyses of data as well as manuscript preparation. ZY and ZWW designed the strategy for literature search, acquisition and interpretation of data. JY and TW assessed eligibility of studies for inclusion to the systematic review. Statistical analyses were performed by HYJ, HL and ZT. SGW and JHL contributed to revision of the manuscript and final approval. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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