Impact on using cryopreservation of testicular or epididymal sperm upon intracytoplasmic sperm injection outcome in men with obstructive azoospermia: a systematic review and meta-analysis

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Received: 8 July 2020 / Accepted: 7 September 2020 / Published online: 15 September 2020
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Abstract

Purpose To determine whether there was a significant impact on using cryopreservation of testicular or epididymal sperm upon the outcomes of intracytoplasmic sperm injection (ICSI) in patients with obstructive azoospermia (OA).

Method Systematic review and meta-analysis of 20 retrospective studies in databases from January 1, 1995, to June 1, 2020.

Result Twenty articles were included in this study. A total of 3602 (64.1%) of 5616 oocytes injected with fresh epididymal sperm were fertilized, compared with 2366 (61.2%) of 3862 oocytes injected with cryopreserved sperm (relative risk ratio (RR) 0.96, 95% confidence interval (CI) (0.90, 1.02), $P > 0.05$). A total of 303 (44.1%) of 687 ICSI cycles using fresh epididymal sperm resulted in a clinical pregnancy, compared with 150 (36.6%) of 410 ICSI cycles using cryopreserved epididymal sperm (RR 0.84, 95% CI (0.72, 0.97), $P < 0.05$). In the testis, a total of 2147 (68.7%) of 3125 oocytes injected with fresh sperm were fertilized, compared with 1623 (63.5%) of 2557 oocytes injected with cryopreserved sperm (RR 0.97, 95% CI (0.90, 1.06), $P > 0.05$). A total of 151 (47.8%) of 316 ICSI cycles using fresh testicular sperm resulted in a clinical pregnancy, compared with 113 (38.2%) of 296 ICSI cycles using cryopreserved sperm (RR 0.87, 95% CI (0.72, 1.05), $P > 0.05$).

Conclusions In men with OA, there was a statistical lower clinical pregnancy rate (CPR) by using frozen epididymal sperm compared with fresh epididymal sperm, but showing no difference on fertilization rate (FR). Additionally, FR and CPR were not affected by whether the retrieved testicular sperm was frozen or fresh.

Keywords Cryopreservation · Epididymal spermatozoa · Intracytoplasmic sperm injection · Obstructive azoospermia · Testicular spermatozoa

Hanchao Liu, Yun Xie and Linzhi Gao contributed equally to this work.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10815-020-01940-1>) contains supplementary material, which is available to authorized users.

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Introduction

Obstructive azoospermia (OA) is the absence of spermatozoa in the ejaculate due to obstruction of the male reproductive tract and comprises approximately 40% of all azoospermic cases [1]. OA can occur anywhere along the course of the male reproductive tract and is classically characterized by normal spermatogenesis [2]. Microsurgical reconstruction of blocked tract is recommended for many cases of OA by the European Association of Urology (EAU) guidelines on male infertility 2018. But if microsurgical reconstruction is unsuccessful or not suitable, through testicular or epididymal spermatozoa in combination with intracytoplasmic sperm injection (ICSI), OA patients could also achieve biological parenthood [3].

Previous studies have shown that surgically retrieved testicular or epididymal spermatozoa used for ICSI have similar outcomes whether fresh or frozen sperm are used [4–6].

However, other studies concluded that cryopreservation of testicular or epididymal spermatozoa may reduce fertilization rate (FR) or clinical pregnancy rate (CPR) [7, 8]. Whether there is a significant impact on the results of ICSI using fresh or frozen sperm on the site of sperm retrieval in OA patients remains controversial. In order to shed light on this topic, a systematic review and meta-analysis was performed. Unlike previous meta-analyses focused on non-obstructive azoospermia (NOA) or did not distinguish the source of sperm from the testis or epididymis [5, 9, 10], we reviewed the outcomes of ICSI on using cryopreservation of testicular or epididymal sperm in patients with OA in this study. And the outcome parameters were FR and CPR.

Materials and methods

Search strategy

We searched the PubMed, EMBASE, Web of Science, and Cochrane Library database. The MeSH terms applied were listed as follows: (((((obstructive azoospermia) OR azoospermia)) AND (((((cryopreservation) OR frozen) OR cryopreserved) OR freeze) OR fresh)) AND (((reproductive techniques, assisted) OR assisted reproductive technology) OR intracytoplasmic sperm injection))) AND (((((outcome) OR fertility)) OR fertilization) OR clinical pregnancy). The aim was to search for all available English-language literatures related to the outcomes of ICSI on using fresh or frozen spermatozoa from the testis or epididymis in patients with OA. Researches were limited to retrospective cohort studies or case-control studies. Since ICSI was started to be used in 1994 [11], the start and end dates of this search are from January 1, 1995, to June 1, 2020.

Study selection: inclusion and exclusion criteria

We strictly followed the participants-interventions-comparisons-outcomes-study design (PICOS) principle, and all retrospective cohort researches or case-control researches were eventually included in this study. Inclusion criteria were as follows: (1) Patients were diagnosed with OA. (2) Sperm retrieval techniques were limited to microsurgical epididymal sperm aspiration (MESA), percutaneous epididymal sperm aspiration (PESA), testicular sperm extraction (TESE), or testicular sperm aspiration (TESA). (3) Fresh and frozen spermatozoa were extracted from the epididymis or testis in OA patients without female infertility factor. (4) The index of ICSI outcomes at least included FR or CPR. (5) Available data could be extracted in the literatures. Exclusion criteria were as follows: (1) Patients with NOA. (2) Not the surgical methods mentioned in the

inclusion criteria. (3) The source of frozen or fresh sperm is not explicitly stated to be derived from the epididymis and testis, respectively. (4) The follow-up procedure is not ICSI, and there are no reports of FRs and CPR. (5) We could not extract valid data, non-English-language literature, repeated published research, meta-analysis, and review articles. The inclusion and exclusion criteria of these studies were independently performed by two authors (Hanchao Liu and Linzhi Gao). When disagreements occurred, an agreement was reached through discussion and consultation with another author (Yun Xie).

Quality assessment of the data

Two researchers (Hanchao Liu and Linzhi Gao) independently conducted literature retrieval and extracted data. Newcastle-Ottawa scale (NOS) scale was used to evaluate the methodological quality of selected experimental studies [12]. Specifically, the patient selection, comparability of the study group, and assessment of outcome were made up of 8 items. Using the semi-quantitative principle of the star system, a perfect score of 9 stars (9 points) and a score of ≥ 6 as the high-quality study were included. When there is a disagreement, reach an agreement by discussing it with another author (Yun Xie).

Statistical analyses

This meta-analysis was based on the Preferred Reporting Items of the System Review and Meta-Analysis (PRISMA) guidelines [13]. The RevMan software (version 5.3) provided by Cochrane collaboration network is used for the analysis (Cochrane Collaboration, Oxford, UK). The counting data were expressed by the relative risk ratio (RR) value and its 95% confidence interval (CI), and the measurement data were expressed by the weighted mean difference (WMD) value and its 95% CI value. $P < 0.05$ means that the difference was statistically significant. The heterogeneity of the effect was tested by forest map [14]. Chi-square test was used to test the heterogeneity of the included study. If heterogeneity was not statistically significant ($P > 0.05$, $I^2 < 50\%$), the fixed effect model was used for meta-analysis. If heterogeneity was statistically significant ($P < 0.05$, $I^2 > 50\%$), sensitivity analysis was carried out to find the causes of heterogeneity. If the cause of heterogeneity could not be explained by any factors, the random effect model was used for meta-analysis, and the results were interpreted carefully. The funnel plot was drawn by Stata 14.0. We used Egger's test to evaluate the publication bias. And the publication bias significance level was set $P < 0.05$.

Results

Study selection process and characteristics of the included studies

We collected 1392 articles from PubMed, EMBASE, Web of Science, Cochrane Library database initially. After the screening of inclusion and exclusion criteria, 20 articles were obtained to meet the requirements (Fig. 1) [7, 15–33]. The main characteristics and NOS scores of each article included in this study were shown (Table 1 and Supplementary Table S1).

The FR and CPR on using epididymal sperm in ICSI

There were 13 related studies of FR on using epididymal sperm in ICSI. The FR of OA patients on using frozen or fresh sperm from epididymis was compared. The results of each experiment were statistically heterogeneous ($I^2 > 50\%$). Therefore, random effect model was used for meta-analysis. The results showed that there was no significant difference in FR between the epididymal frozen sperm group and epididymal fresh sperm group (RR = 0.96, 95% CI (0.90, 1.02), $P = 0.16$). The details are listed in Fig. 2a.

There were 12 related studies of CPR on using epididymal sperm in ICSI. The CPR of OA patients on using frozen or fresh sperm from epididymis was compared. The statistical heterogeneity of each experimental result was less than 50% ($I^2 < 50\%$), and the fixed effect model was used for meta-analysis. The results showed that there was a significant difference in CPR between the epididymal frozen sperm group and epididymal fresh sperm group (RR = 0.84, 95% CI (0.72, 0.97), $P = 0.02$). The results suggested that the CPR on using epididymal fresh sperm was higher than that of epididymal frozen sperm (Fig. 2b).

The FR and CPR on using testicular sperm in ICSI

There were 4 related studies of FR on using testicular sperm in ICSI. The FR of OA patients on using frozen or fresh sperm in the testis was compared. There was statistical heterogeneity ($I^2 > 50\%$) in each experiment, and random effect model was used for meta-analysis. The results demonstrated that there was no significant difference in FR between the testicular frozen sperm group and testicular fresh sperm group (RR = 0.97, 95% CI (0.90, 1.06), $P = 0.52$). The details are listed in Fig. 3a.

There were 7 related studies of CPR on using testicular sperm in ICSI. The CPR of OA patients on using frozen or fresh sperm in testis was compared. The statistical heterogeneity of each experimental result was less than 50% ($I^2 < 50\%$), and the fixed effect model was used for meta-analysis. The results demonstrated that there was no significant difference in CPR between the testicular frozen sperm group and

testicular group (RR = 0.87, 95% CI (0.72, 1.05), $P = 0.15$). The details are listed in Fig. 3b.

Publication bias

The funnel plots were presented in Supplementary Fig. 1. There was no significant publication bias of studies included in our study. In FR and CPR of the epididymis, $P = 0.518$ and 0.098. And in FR and CPR in the testis, $P = 0.946$ and 0.729.

Discussion

Sperm cryopreservation is an effective method to preserve male fertility [34]. Having frozen sperm allows more flexibility before undergoing ICSI, which means that there is no need to extract sperm and oocyte at the same time [23]. In addition, sperm cryopreservation could avoid the repeated sperm retrieval technique for each ICSI cycle, thereby minimizing damage to testicular function [35]. However, whether the use of frozen sperm would affect the ICSI outcomes is still controversial. In this study, we found that in men with OA, using cryopreservation of epididymal sperm reduced the CPR in couples undergoing ICSI, but showing no difference between the use of fresh and frozen epididymal sperm when assessing FR. It also showed that the ICSI outcomes, including FR and CPR, were not affected by whether the retrieved testicular sperm was fresh or frozen.

In females, previous studies have reported clearly that the ICSI outcomes in cryopreservation of embryos were better than those of fresh embryos [36, 37]. However, in males, there is a vigorous debate about the impact of using fresh or frozen sperm with regard to ICSI outcomes. By consulting the related literatures published from 1995 to 2020, we found that this situation could be attributed to the following aspects. Firstly, numerous studies confusedly analyze OA and NOA at the same group. Actually, the OA patients, who generally exhibit normal spermatogenesis, are the more ideal objects on studying the impact of sperm cryopreservation concerning ICSI outcomes. Secondly, few studies strictly distinguish the source of sperm, including testicular sperm and epididymal sperm. Finally, insufficient sample size and large differences in female factors would affect the results. Consequently, we hold that to determine whether there is a significant impact on using cryopreservation of sperm upon the outcomes of ICSI, performing a systematic review and meta-analysis between the ICSI outcomes of fresh versus frozen sperm from the different site of retrieval in OA patients is one of the best ways.

The results of this study demonstrated that the CPR was reduced by using the frozen epididymal sperm when compared with fresh epididymal sperm, but there was no statistical

Table 1 Baseline characteristics of included studies

References	Year	Source	Surgery	Intervention	Female age	Male age	No. of cycles	FR (%)	CPR (%)
Devroey P et al.	[27]	1995	Epididymis	PESA	31.2				
				Fresh			7	39/77	
				Frozen			7	28/68	
Silber SJ et al.	[29]	1995	Epididymis	MESA					
				Fresh			33		20/31
				Frozen			7		4/7
Nagy Z et al.	[28]	1995	Epididymis	MESA					
				Fresh	31.7	38.7	43	306/547	12/40
				Frozen	30.6	33.2	9	59/106	3/9
Oates RD et al.	[20]	1996	Epididymis	MESA					
				Fresh	32.0		18	41/145	
				Frozen	32.0		11	28/103	
Sharma RK et al.	[21]	1997	Epididymis	MESA					
				Fresh				52/101	
				Frozen				20/30	
Friedler S et al.	[30]	1998	Epididymis						
			MESA	Fresh			7	33/60	2/6
				Frozen			11	62/114	5/11
			PESA	Fresh			17	75/134	5/16
				Frozen			10	51/98	2/8
Shibahara H et al.	[18]	1999	Epididymis	MESA					
				Fresh			5	35/51	3/5
				Frozen			13	34/75	3/13
Tournaye H et al.	[32]	1999	Epididymis	MESA					
				Fresh			157	1125/1821	46/145
				Frozen			118	684/1255	28/100
Palermo GD et al.	[31]	1999	Epididymis	MESA					
				Fresh	33.7 ± 5		129	1027/1429	83/129
				Frozen	35.4 ± 5		112	747/1006	52/112
Habermann H et al.	[33]	2000	Testis	TESE					
				Fresh			9	50/86	3/9
				Frozen			25	152/238	8/25
Cayan S et al.	[15]	2001	Epididymis	MESA					
				Fresh	34.3 ± 5.6		19	139/238	6/19
				Frozen	35.5 ± 5.4		19	194/313	7/19
Wood S et al.	[7]	2002	Epididymis	PESA	31.9	41.5			
				Fresh			13	58/90	2/13
				Frozen			13	57/96	3/13
Park YS et al.	[25]	2003	Testis	TESE	37.5 ± 5.7	34.0 ± 5.5			
				Fresh			73	610/958	31/70
				Frozen			114	789/1370	37/109
Wu B <i>et al.</i>	[26]	2005	Testis	TESE		29–56			
				Fresh			16	174/243	11/16
				Frozen			12	100/147	5/12
Kalsi J et al.	[16]	2010	Epididymis	MESA, PESA					
				Fresh	34.03 ± 4.8		173		80/173
				Frozen	33.77 ± 5.2		42		15/42
			Testis	TESE					
				Fresh	36.48 ± 5.7		28		9/28

Table 1 (continued)

References	Year	Source	Surgery	Intervention	Female age	Male age	No. of cycles	FR (%)	CPR (%)
Abdel Raheem A et al. [22]	2013	Testis	TESE	Frozen	35.57 ± 3.1		15		9/15
				Fresh			19		8/19
				Frozen			41		14/41
Karacan M et al. [23]	2013	Testis	TESE	Fresh	31.7 ± 4.8	37.2 ± 8.3	67		23/67
				Frozen	37.9 ± 4.4	30.9 ± 4.4	45		13/45
Kovac JR et al. [17]	2014	Epididymis	PESA	Fresh	40 ± 1	34 ± 1	40	241/310	17/35
				Frozen	37 ± 1	34 ± 1	11	91/124	6/11
Yu G et al. [19]	2018	Testis	TESA	Fresh	30.45 ± 5.03	31.85 ± 7.08	194	1313/1838	66/107
				Frozen	29.43 ± 5.40	30.30 ± 5.92	79	589/802	27/49
Javed A et al. [24]	2019	Epididymis	PESA	Fresh	31.5 ± 4.0	35.1 ± 2.0		431/613	27/73
				Frozen				311/474	22/65

CPR, clinical pregnancy rate; FR, fertilization rate; MESA, microsurgical epididymal sperm aspiration; PESA, percutaneous epididymal sperm aspiration; TESA, testicular sperm aspiration; TESE, testicular sperm extraction

difference when assessing FR in OA patients. Previous studies also hold that the CPR of using frozen epididymal sperm in ICSI was actually weaker than that of fresh epididymal sperm

[16, 18]. Meanwhile, a considerable number of clinical and basic research have found that cryopreservation does damage sperm, in which the mechanism may be associated with the

Fig. 1 Flow diagram of the studies identified, included, and excluded

Fig. 2 Forest plot and meta-analysis of fertilization rate and clinical pregnancy rate on using epididymal sperm in ICSI. **a** Forest plot for fertilization rate. **b** Forest plot for clinical pregnancy rate. Experimental means

frozen group. Control means fresh group. CI, confidence interval; M-H, Mantel-Haenszel method

Fig. 3 Forest plot and meta-analysis of fertilization rate and clinical pregnancy rate on using testicular sperm in ICSI. **a** Forest plot for fertilization rate. **b** Forest plot for clinical pregnancy rate. Experimental means frozen

group. Control means fresh group. CI, confidence interval; M-H, Mantel-Haenszel method

formation of intracellular ice crystals and accumulation of reactive oxygen species (ROS) [18, 31, 38–42]. A study in deer epididymal sperm showed that the addition of enzyme antioxidants to the cryopreservation expander could increase the survival rate of frozen sperm and improve the motility, acrosome integrity and mitochondrial status of thawed sperm [40]. In the same year, the other study also presented that the use of cryoprotectants could improve sperm motility, plasma membrane integrity, DNA integrity, FR, and quality of embryo [43]. Rezaei N et al. demonstrated that the addition of cryoprotectants could improve the quality of frozen-thawed epididymal sperm in mouse, which was characterized by decreasing the level of ROS and expression of Bax, and increasing the mitochondrial activity and expression of Bcl-2 [44]. Therefore, it is reasonable to consider that epididymal sperm may be greatly affected by cryopreservation in men with OA.

The results of this study also showed that there was no significant difference in FR and CPR between frozen sperm versus fresh sperm from testis in OA patients, which was consistent with the previous studies. When the motility of sperm can be identified, the identified fresh sperm obtained by TESE has better fertilization rate and pregnancy rate than frozen sperm [25]. In other words, the sperm selected during ICSI with good motility contributed to good results in fertilization and pregnancy [45]. For example, hypo-osmotic swelling test (HOST) is related to the *in vitro* fertilization ability of male spermatozoa, and there is a strong correlation between HOST and *in vitro* fertilization [46]. Researchers believed the application of HOST may be an important tool for routine identification and selection of active and DNA intact individual spermatozoa to a better ICSI outcome [47].

Besides, it is very important to be able to distinguish the effects of sperm sources on sperm quality. It is well known that the beginning of the epididymis plays an important role in sperm maturation. This is one of the reasons why we divided the testis and epididymis into two groups and compared fresh and frozen sperm in each group. We screened frozen semen and fresh sperm for obstructive azoospermia in epididymis or testis through strict inclusion and exclusion criteria. According to Table 1, we can learn that there is only one study in the literatures we have included that can be used by us to compare epididymis and testis sperm [16]. When researchers defaulted that frozen sperm and fresh sperm are the same sperm, Madelon van Wely et al. proved that the ICSI outcome of epididymal sperm is better than that of testicular sperm in 2015 [48]. Therefore, more studies should be launched to prove that epididymal sperm is better than testicular sperm in OA patients on the basis of strict inclusion and exclusion standard.

According to EAU guidelines on male infertility (2018), when the etiological treatment of OA is ineffective or not

suitable, sperm retrieval techniques, such as MESA, PESA, TESE, and TESA, could be taken for the purpose of assisting reproduction. In fact, various sperm retrieval techniques are re-shaping the treatment of OA. Based on this, the conclusions of our study would contribute to guiding the clinical practice in couples undergoing ICSI. For OA patients receiving MESA or PESA, using the fresh epididymal sperm is recommended to minimize the damage to sperm and improve the outcomes of ICSI. While for OA patients receiving TESE or TESA, there is no difference between the use of fresh and frozen testicular sperm. In order to avoid the potential repeated sperm retrieval technique for each ICSI cycle, and keep away from extracting sperm and oocyte at the same time, to our minds, the use of frozen testicular sperm is still recommended in ICSI. Overall, no matter what sperm retrieval technique the OA patients received, the continuous improvement of sperm freezing methods is needed. Additionally, the mechanism of using cryopreservation of epididymal sperm affecting the outcomes of ICSI also needs further explorations in the future.

Although this study makes significant contributions to understanding the impact on using cryopreservation of testicular or epididymal sperm upon the outcomes of ICSI in patients with OA, it still has some limitations. In our meta-analysis, the data presented are based on the previous publications, which all the data and details cannot be extracted completely. There were 12 and 14 studies included in epididymis and only 4 and 7 studies included in testis. We had the result of $P < 0.05$ in epididymis CPR group. However, it is important to note that some other factors from successful fertilization to clinical pregnancy, such as maternal factors, and embryo quality, may affect the process of implantation and embryo development in each included study. These factors may interpret that some studies have similar fertilization rate but vary widely in clinical pregnancy rate. It is also the limitation of published clinical studies and our meta-analysis. Then, our meta-analysis results in testes of patients with obstructive azoospermia were $P > 0.05$ in FR and CPR groups. However, this does not mean that freezing does not damage testicular sperm, which may be due to the limitation of study sizes on testicular sperm in patients with OA, the number of cases or some other reasons. And we have mentioned that selecting good-quality sperm is beneficial to the results of ICSI. Therefore, more clinical and experimental studies with strict inclusion and exclusion criteria are needed to further prove this. All the studies included are retrospective studies, and the deviations of individual studies are not controlled by us, such as female factors, detailed clinical characteristics of OA patients. Also, the absence of age, history of recurrent ICSI failure, and other clinical factors of the studies which might adversely affect the outcome. Furthermore, the sample size of clinical data is still limited.

Conclusions

In men with OA, using cryopreservation of epididymal sperm reduced the CPR in couples undergoing ICSI, but showing no impact when assessing FR. Furthermore, the FR and CPR were not affected by whether the retrieved testicular sperm is frozen or fresh.

Authors' contributions L.H.C., X.Y., and G.L.Z. made the literature search and data analysis. S.X.Z., L.X.Y., and D.C.H. made study design. L.H.C., X.Y., and G.Y. drew the figures and tables. L.G.H. and G.Y. made the revisions. All authors approved the final version of the paper.

Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

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