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ORIGINAL ARTICLE

Male Infertility

Reproductive outcomes of intracytoplasmic sperm injection using testicular sperm and ejaculated sperm in patients with AZFc microdeletions: a systematic review and meta-analysis

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Studies have explored the assisted reproductive technology (ART) outcomes of Y-chromosome azoospermia factor c (AZFc) microdeletions, but the effect of sperm source on intracytoplasmic sperm injection (ICSI) remains unknown. To determine the ART results of ICSI using testicular sperm and ejaculated sperm from males with AZFc microdeletions, we searched Embase, Web of Science, and PubMed to conduct a systematic review and meta-analysis. The first meta-analysis results for 106 cycles in five studies showed no significant differences in the live birth rate between the testicular sperm group and the ejaculated sperm group (risk ratio: 0.97, 95% confidence interval [CI]: 0.73–1.28, *P* = 0.82). The second meta-analysis of 106 cycles in five studies showed no difference in the abortion rate between the testicular sperm group and ejaculated sperm group (risk ratio: 1.06, 95% CI: 0.54–2.06, *P* = 0.87). The third meta-analysis of 386 cycles in seven studies showed no significant difference in clinical pregnancy rates between the testicular sperm group and the ejaculated sperm group (risk ratio: 1.24, 95% CI: 0.66–2.34, *P* = 0.50). Inevitable heterogeneity weakened our results. However, our results indicated that testicular sperm and ejaculated sperm yield similar ART outcomes, representing a meaningful result for clinical treatment. More properly designed studies are needed to further confirm our conclusions.

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INTRODUCTION

Infertility is defined as the inability to conceive or deliver descendants within 1 year of regular (scheduled) unprotected sexual intercourse.[1] Approximately 15% of couples suffer from infertility,[2] and male infertility accounts for approximately 50% of these cases. In total, 15%–30% of male infertility is attributed to genetic factors.[3] Genetic causes, including Y chromosome microdeletions and chromosomal aberrations, represent the primary reasons for male infertility due to azoospermia and severe oligozoospermia.[4]

The locus defined as azoospermia factor (AZF) in Yq11 contains the genes necessary for normal spermatogenesis, and deletions in this locus have been correlated with male infertility. This locus is divided into three major regions named AZFa, AZFb, and AZFc.[5] The AZFa region was associated with Sertoli-cell-only syndrome (SCOS),[5,6] the AZFb region was associated with meiotic arrest (MA),[5,7,8] and the AZFc region was associated with a variable phenotype, ranging from severe oligozoospermia (SOZ) to secretory azoospermia (SAZ).[5,9] Within the AZFc region, it is located in the deleted azoospermia (*DAZ*) gene family, with the identification of four loci, *DAZ1–4*.[10] Deletions of the *DAZ1*/*DAZ2* gene doublet were found to be responsible for severe oligozoospermia, with possible evolution to secretory azoospermia with different testicular phenotypes,[11] but later demonstrated that only *DAZ1* was responsible for this phenotype.[12] The impact on infertility treatments using testicular and ejaculated sperm in patients with AZF microdeletions was recently reviewed.[13,14]

Sperm retrieval is unlikely in AZFa or AZFb microdeletion patients, while sperm is relatively likely to be retrieved by operation in men with AZFc microdeletions (up to 70%).[15] Some studies have explored the results of intracytoplasmic sperm injection (ICSI) in males suffering from Y chromosome microdeletions. A previous study showed that in males with high DNA fragmentation, both clinical pregnancy rate and live birth rate were significantly increased in the testicular sperm group compared with those in the ejaculated sperm group.[16]

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AZFc microdeletions may cause azoospermia or severe oligozoospermia. In men with azoospermia or oligozoospermia caused by AZFc microdeletions, the choice of testicular sperm or ejaculated sperm to perform ICSI is debated. Sabbaghian *et al*.[17] found that the pregnancy rate with ICSI for the ejaculated sperm group was increased compared with that of the testicular sperm group. The relative success rates of testicular sperm and ejaculated sperm from AZFc microdeletion patients need to be clarified. Considering the risks of clinical complications of testicular puncture, studies of assisted reproductive outcomes for testicular sperm and ejaculated sperm from men with severe oligozoospermia are important.

In this study, we compared the assisted reproductive technology (ART) outcomes for testicular sperm and ejaculated sperm for ICSI in AZFc microdeletion patients. The ART outcomes compared include live birth rates, clinical pregnancy rates, and abortion rate.

MATERIALS AND METHODS

Meta-analyses were performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) and the Meta-analysis of Observational Studies in Epidemiology (MOOSE) recommendations for study reporting.[18–20]

Data sources and search strategy

Electronic searches were conducted in PubMed, Web of Science, and Embase databases without restriction of region, publication type, or language. The following MeSH terms and their combinations were searched in (title/abstract): (AZFc) AND (((((((Oligozoospermia) OR Low Sperm Count) OR Sperm Count, Low) OR Oligoasthenoteratozoospermia) OR Hypospermatogenesis)) OR azoospermia). Related articles were searched to broaden our study. Full texts of non-English studies were searched in the library of Jinan University (Guangzhou, China). The most recent article was considered when multiple articles shared the same population.

Study selection: inclusion and exclusion criteria

This analysis included prospective or retrospective comparative cohort studies that compared clinical pregnancy, pregnancy loss, or live birth rates. Only studies that controlled for baseline factors (such as age, follicle-stimulating hormone [FSH], luteinizing hormone [LH], or testosterone) were included. In all included cohort studies, infertile couples received ART treatment in the form of ICSI.

Regarding exclusion criteria, editorials, case reports, reviews, animal studies, and letters were excluded. Second, studies for which the full text could not be identified were excluded. Third, studies for which the data could not be extracted were excluded. The studies were assessed for the inclusion criteria and exclusion criteria separately by two authors (YZ and HL). All differences were settled by senior authors through discussion and arbitration (JHD and CCD).

Data quality assessment

The methodological quality of studies was assessed using a modified Newcastle–Ottawa scale (NOS). This scale contains three factors: patient selection, comparability of research groups, and outcome evaluation.[21] NOS scores were adjusted for the weight of representative values, subjects, and confounding factors associated with the subjects. Studies received high NOS scores if bias factors were well controlled. Evidence quality assessments were performed separately by two reviewers (WJL and HBZ), and differences were settled through discussion with senior authors (XZZ and JHD).

Retrospective cohort studies receiving NOS scores of 6 or greater were regarded as high-quality studies.

Data extraction and concerned outcome

Data from included studies were extracted by two primary authors (YZ and YGT). Differences were settled by discussion with senior authors (CCD and XZZ). Data from studies in which the outcome of ICSI was compared between testicular sperm and ejaculated sperm were extracted. The extracted data were recorded in 2×2 tables.

The live birth rate was the outcome of greatest interest. Live birth refers to achieving at least one live birth in one ICSI cycle. The secondary outcome investigated was abortion rate. A clinical pregnancy that is aborted before 12 weeks of gestation is defined as an abortion. The tertiary outcome investigated was the clinical pregnancy rate.

Statistical analyses

Our meta-analyses were based on the PRISMA guidelines.[18] Meta-analysis was performed using Review Manager 5.3 (Cochrane Collaboration, Oxford, UK). Relative risk was used to compare the live birth, clinical pregnancy, and abortion rates in the ejaculated sperm group and the testicular sperm group in individual studies. Meta-analysis results were described using 95% confidence intervals (CIs). $P < 0.05$ indicated a statistically significant result.

Forest plots were used to evaluate the heterogeneity of ICSI outcome.[22] The statistical heterogeneity of the included studies was evaluated at a significance level of $P < 0.10$ through I^2 statistics and Chi-square tests. A random-effects model was adopted when heterogeneity was noted; otherwise, the fixed-effects model was adopted.[23] Sensitivity analyses were performed using Review Manager 5.3. To evaluate potential publication bias, StataSE 12.0 (StataCorp, College Station, TX, USA) was used to produce funnel plots.[24]

RESULTS

Collection and inclusion of studies for meta-analyses

In our meta-analyses, 859 studies were identified from electronic databases. First, 177 duplicate studies were deleted. Second, 457 unrelated studies, two studies using animal models, and 65 noncomparative studies were excluded. Third, 32 reviews or guidelines, 101 meeting abstracts, and two letters or comments were excluded. Fourth, 14 studies with unextractable data were excluded. Finally, seven studies meeting inclusion criteria were included in the meta-analyses (**Figure 1**).

Characteristics of the included studies

To detect Y chromosome microdeletions, polymerase chain reaction (PCR) was performed in all included studies. The ages of patients with AZFc deletions in the testicular sperm group and the ejaculated group in included studies are similar. Patients of all included studies received no hormonal treatment before sperm collection or retrieval. No severe female factors that would influence the assisted reproductive outcome were mentioned in any of the included studies.

The major characteristics of the seven included studies and NOS scores are presented in **Table 1**. Details of the modified NOS assessment are shown in **Supplementary Table 1**. All included studies were retrospective cohort studies. The patients included in the study came from different countries around the world. All seven studies were written in English. The size of the effective cycles in included studies varied from 8 effective cycles[25] to 195 effective cycles.[13] The meta-analysis of live birth rate included five studies containing 106 effective cycles.[13,17,26–28] The meta-analysis of abortion rate included five studies containing 106 effective cycles.[13,17,26–28] The meta-analysis of clinical pregnancy rate included seven studies with 386 effective cycles.[13,17,25–29]

NOS of included studies

We considered studies with scores greater than or equal to 6 as high-quality studies. The studies achieved high-modified NOS scores: two studies scored 9, four studies scored 8, and one study scored 7. No studies received scores lower than 6.

Major result of meta-analysis: live birth rate

Five studies with 106 cycles were included in the meta-analysis of live birth rate. The live birth rate of the testicular sperm group was not different from that of the ejaculated sperm group (risk ratio: 0.97, 95% CI: 0.73–1.28, $P = 0.82$; **Figure 2**).

Secondary outcome of meta-analysis: abortion rate

Pooling the data from five studies with 106 cycles reporting abortion rate, our meta-analysis showed that the abortion rate was not different between the testis sperm group and the ejaculated sperm group (risk ratio: 1.06, 95% CI: 0.54–2.06, $P = 0.87$; **Figure 3**).

Tertiary meta-analysis result: clinical pregnancy rate

Seven studies with 386 cycles were included in the meta-analysis of clinical pregnancy rate. The clinical pregnancy rate exhibited no significant difference between the testis sperm group and the ejaculated sperm group (risk ratio: 1.24, 95% CI: 0.66–2.34, $P = 0.50$; **Figure 4**).

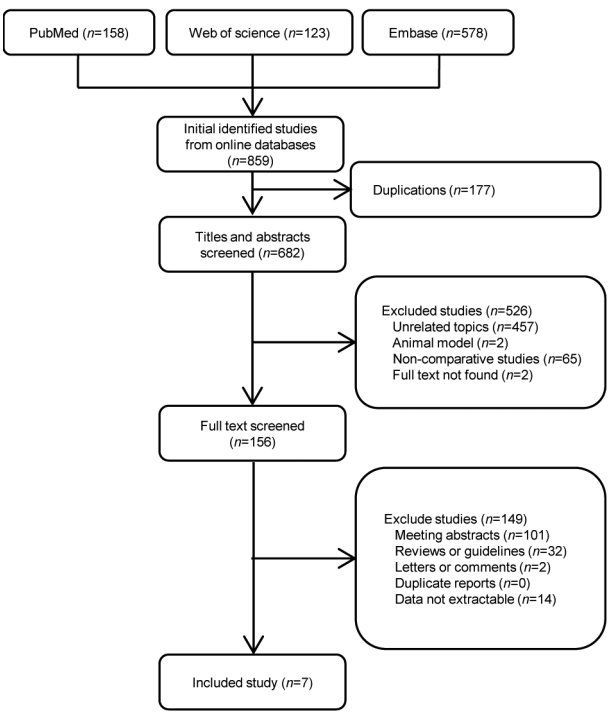

Figure 1: Flow diagram of the studies identified, included, and excluded.

Sensitivity analysis and publication bias

Seven cohort studies with high NOS scores were included in the sensitivity analysis. There was no change in the significance of live birth rate, abortion rate, or clinical pregnancy rate.

Funnel plots of studies included in meta-analyses of live birth rate showed no significant publication bias ($P = 0.37$; **Figure 5**). There was no significant publication bias in studies on abortion rate ($P = 0.796$) or clinical pregnancy rate ($P = 0.59$).

DISCUSSION

Herein, we performed the first systematic review and meta-analysis to compare ART outcomes between testicular sperm and ejaculated sperm of infertile patients with AZFc region microdeletions. Our meta-analyses showed that live birth rate, clinical pregnancy rate, and abortion rate were not different between testicular sperm and ejaculated sperm groups among men with AZFc microdeletions.

AZFc microdeletions play an important role in spermatogenic failure, resulting in oligozoospermia or nonobstructive azoospermia. However, patients with AZFc microdeletions are able to reproduce by ICSI. In previous studies, the influences of spermatozoa source on ART outcome remain unclear. Esteves *et al.*[16] revealed that testis sperm tends to achieve better clinical outcomes than ejaculated sperm among patients with high DNA fragmentation. However, Tsai *et al.*[30] showed that the clinical outcome was not different between testicular sperm or ejaculated sperm for ICSI in extreme severe oligoasthenoteratozoospermia patients. Kihaile *et al.*[25] reported that as long as sperm could be obtained for ICSI cycles in males with AZFc microdeletions, the embryo quality was the same as that traditionally obtained with *in vitro* fertilization (IVF). Gonçalves *et al.*[13] indicated that there were no significant differences in embryological and clinical parameters between testicular sperm extraction (TESE) and ejaculated sperm (EJAC) cycles.[13]

The effect of sperm source on embryo development and ART outcome is worthy of attention. Some previous studies[31,32] generally did not focus on the sperm source, so azoospermia patients with testicular sperm and oligozoospermia patients with ejaculated sperm were included in one group. It is not clear whether sperm source influences embryo development and clinical outcome in patients with AZFc microdeletions. Therefore, in our meta-analysis, to evaluate the effect of sperm source on ART outcome in males with AZFc microdeletions, we divided patients with AZFc microdeletions into a testicular sperm group and an ejaculated sperm group. Our meta-analyses showed that clinical pregnancy rate, abortion rate, and live birth rate were not significantly different between the testicular sperm group and the ejaculated sperm group. According to our meta-analysis data, the sperm

Table 1: The main characteristics and the Newcastle–Ottawa scale scores of included studies

Article	*Year*	*Area*	*Study*	*Participant (n)*	*Matching*[a]	*Treatment*	*Outcome measurement*	*Modified quality scores*
Oates *et al.*[27]	2002	USA	Retrospective cohort study	44	1, 2, 3, 4	ICSI	CP, PL, LB	9
Choi *et al.*[26]	2004	USA	Retrospective cohort study	22	1, 2	ICSI	CP, PL, LB	9
Kihaile *et al.*[25]	2004	Japan	Retrospective cohort study	8	1	ICSI	CP	7
Stouffs *et al.*[28]	2005	Belgium	Retrospective cohort study	40	1	ICSI	CP, PL, LB	8
Patrat *et al.*[29]	2010	France	Retrospective cohort study	39	1	ICSI	CP, PL, LB	8
Sabbaghian *et al.*[17]	2018	Iran	Retrospective cohort study	38	1	ICSI	CP, PL, LB	8
Gonçalves *et al.*[13]	2017	Portugal	Retrospective cohort study	195	1	ICSI	CP, PL, LB	8

[a]1: age (year); 2: FSH (mIU ml^{-1}); 3: LH (mIU ml^{-1}); 4: testosterone (ng dl^{-1}). CP: clinical pregnancy; PL: pregnancy loss; LB: live birth; ICSI: intracytoplasmic sperm injection; FSH: follicle-stimulating hormone; LH: luteinizing hormone

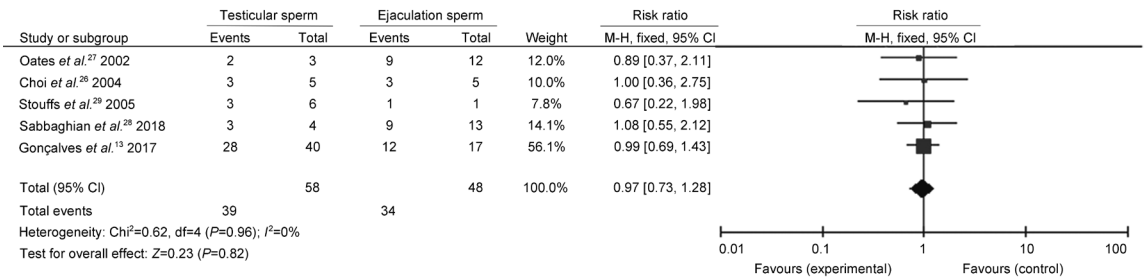

Figure 2: Forest plot and meta-analysis of the live birth rate. CI: confidence interval; M-H: Mantel-Haenszel method; df: degree of freedom.

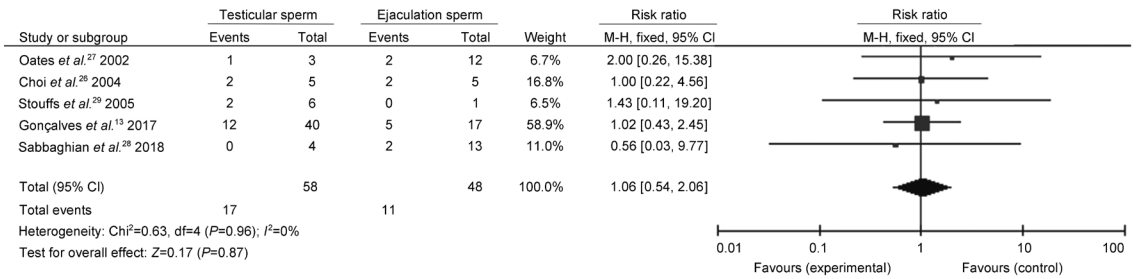

Figure 3: Forest plot and meta-analysis of the abortion rate. CI: confidence interval; M-H: Mantel-Haenszel method; df: degree of freedom.

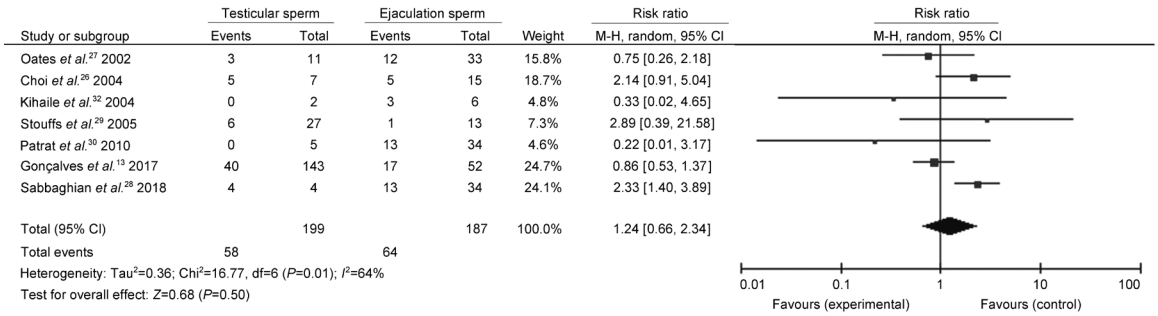

Figure 4: Forest plot and meta-analysis of the clinical pregnancy rate. CI: confidence interval; M-H: Mantel-Haenszel method; df: degree of freedom.

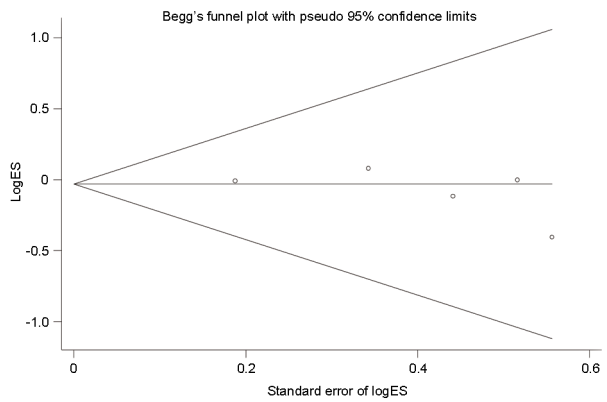

Figure 5: Funnel plots illustrating the meta-analysis of live birth rate. ES: effect size.

source may not affect the clinical outcome. Consistent with our meta-analysis, Zhu *et al*.[33] reported that their testicular sperm group exhibits comparable clinical outcomes to their ejaculated sperm group in AZF microdeletion patients. The fertilization rate and cleavage rate in the testicular sperm group were the same as those noted in the ejaculated sperm group, and the abortion rate, clinical pregnancy rate, and live birth rate were also similar.[33]

AZFc microdeletions were the major causes of spermatogenesis failure. Four effective protein-coding genes correspond to the AZFc interval: *DAZ*, chromodomin Y-linked 1 gene (*CDY1*), protein tyrosine phosphatase-non-receptor type 13 like on the Y chromosome 2 gene (*PRY2*) and basic protein on Y chromosome 2 gene (*BPY2*).[34] Studies show that AZFc-related gene families encode proteins with definite spermatogenesis functions, such as (1) protein ubiquitination;[35] (2) germ cell apoptosis;[36] (3) transcriptional regulation and chromatin remodeling;[37] and (4) transport, storage, and translation activation of developmental regulatory transcripts.[38] Although the loss of AZFc genes may influence spermatogenesis, mature sperm can be generated. AZFc microdeletions contribute to 5%–10% of azoospermia and 2%–5% of severe oligozoospermia cases. Men with AZFc microdeletions can achieve pregnancy by ICSI

with fresh ejaculated sperm at rates similar to those of men without AZFc microdeletions.[31]

Testicular-retrieved sperm are widely used in cases of obstructive azoospermia and nonobstructive azoospermia. SAZ was suggested to be a treatable situation after it was shown that sperm could be retrieved from the testis in cases of MA and hypospermatogenesis (HP).[39] This was then extended to cases with SCOS and ICSI.[40–42] Studies also provided probabilities of successful TESE and ICSI in SCOS, MA, and HP.[43–47] For patients suffering from poor sperm parameters or having experienced previous failed ART cycles, the use of testicular spermatozoa for ICSI might result in better pregnancy rates than ejaculated sperm.[48,49] In contrast to these studies,[48,49] our meta-analysis showed that ART outcomes were not different between testicular sperm and ejaculated sperm groups. AZFc might not influence sperm DNA fragmentation; thus, the ART results of testicular sperm from males with AZFc microdeletions were not better than those of ejaculated sperm. This finding is important for clinical treatment. Testicular sperm retrieval is an invasive surgical procedure for male patients and is associated with complications including bleeding, infection, and irreversible testicular tissue damage. Given that the ART outcomes were not different between testicular sperm and ejaculated sperm in patients with AZFc microdeletions, ejaculated sperm should be prioritized over testicular puncture for ICSI.

The advantages of our meta-analysis were as follows. First, our meta-analysis was the first to compare ART outcomes of testis sperm and ejaculated sperm in AZFc microdeletion patients. Second, our meta-analysis innovatively compared ART outcomes, including abortion, clinical pregnancy, and live birth rate. The clinical pregnancy rate, abortion rate, and live birth rate are crucial ART metrics that represent various stages of the ART treatment. A combination of the ART outcomes provides more credible predictions of ART outcomes. Third, publication bias was assessed by StataSE 12.0 with exact *P* values and a funnel plot. The limitations of this meta-analysis should be noted. First, the major limitation was the fact that all of the six included studies were retrospective studies. Second, the heterogeneity of included studies, which is inevitable due to the inclusion of distinct participants, might increase experimental bias. Although heterogeneity exists, confounding factors were controlled to some extent, and ART progression and outcome evaluation standards were adopted in all included studies.

CONCLUSIONS

This systematic review and meta-analysis concluded that clinical pregnancy rate, abortion rate, and live birth rate in ICSI cycles did not differ between testicular sperm and ejaculated sperm groups among males exhibiting AZFc microdeletions. Given that the source of sperm did not influence ART outcome, ejaculated sperm should be given priority over testicular puncture for ICSI, and this finding is important for the clinical treatment of severe oligozoospermia patients with AZFc microdeletions. The inevitable heterogeneity of the included studies prevented us from drawing precise conclusions. Properly designed and rigorously controlled studies, especially randomized controlled trials, should be performed to further confirm our meta-analysis results.

AUTHOR CONTRIBUTIONS

JHD took responsibility for data integrity and the accuracy of this meta-analysis. CCD and JHD designed the study. YZ and HL carried out the execution of the inclusion criteria and exclusion criteria and collected data from included studies. WJL and HBZ carried out the evidence quality assessments. YZ and YGT performed the data analysis and explanation. YZ and CCD wrote the manuscript and performed the statistical analysis. YZ, CCD, and WJL revised the manuscript. XZZ, JHD, YZ, and CCD provided the administrative and technical support. CCD, XZZ, and JHD are the supervisor of this study. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 1: Quality assessment of the data according to the modified Newcastle–Ottawa scale

Article	*Selection*				*Comparability*	*Outcome*			*Modified quality scores*
	Representativeness of the exposed cohort	*Selection of the nonexposed cohort*	*Ascertainment of exposure*	*Demonstration that outcome of interest was not present at start of study*	*Comparability of cohorts on the basis of the design or analysis*	*Assessment of outcome*	*Was follow-up long enough for outcomes to occur*	*Adequacy of follow-up of cohorts*	
Oates *et al*. 2002[27]	a	a	a	a	ab	a	a	a	9
Choi *et al*. 2004[26]	a	a	a	a	ab	a	a	a	9
Kihaile *et al*. 2004[25]	a	a	a	a	a	a	a	b	7
Stouffs *et al*. 2005[28]	a	a	a	a	a	a	a	a	8
Patrat *et al*. 2010[29]	a	a	a	a	a	a	a	b	8
Sabbaghian *et al*. 2018[17]	a	a	a	a	a	a	a	a	8
Gonçalves *et al*. 2017[13]	a	a	a	a	a	a	a	a	8