Asian Journal of Andrology (2022) 24, 422–429
www.asiaandro.com; www.ajandrology.com

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ORIGINAL ARTICLE

Male Infertility

The association between mutations in ubiquitin-specific protease 26 (*USP26*) and male infertility: a systematic review and meta-analysis

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During recent decades, the association between mutations in ubiquitin-specific protease 26 (*USP26*) and male infertility remains doubtful. We conducted this meta-analysis to evaluate the association between mutations in *USP26* and male infertility according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. It was registered in the International Prospective Register of Systematic Reviews (PROSPERO; CRD42021225251). PubMed, Web of Science, and Scopus were systematically searched for comparative clinical studies, which were written in English and provided eligible data. Studies were included when they compared *USP26* mutations in azoospermic, oligozoospermic, and asthenozoospermic patients with controls with normal sperm parameter values or whose partners had experienced spontaneous pregnancy. Pooled odds ratio (OR) with 95% confidence interval (CI) was calculated with random effect models. Overall, twelve studies with 3927 infertility patients and 4648 healthy controls were included. The association between overall *USP26* mutations and infertility was not significant (OR = 1.60, 95% CI: 0.51–5.01). For specific mutations, the pooled ORs were 1.65 (95% CI: 1.02–2.69) for cluster mutation (including 370–371insACA, 494T>C, and 1423C>T), 1.80 (95% CI: 0.35–9.15) for c.576G>A, 1.43 (95% CI: 0.79–2.56) for c.1090C>T, and 3.59 (95% CI: 2.30–5.59) for c.1737G>A. Our results suggest that several mutations (cluster mutation, c.1737G>A) may play roles in male infertility, while others (c.576G>A and c.1090C>T) do not show notable associations with male infertility. More high-quality clinical researches are needed for validation.

Asian Journal of Andrology (2022) **24**, 422–429; doi: 10.4103/aja2021109; published online: 21 January 2022

Keywords: azoospermia; haplotype; male infertility; mutation; ubiquitin-specific protease 26

INTRODUCTION

Infertility is characterized as the failure to obtain a pregnancy after undergoing regular and unprotected sexual behavior for 1 year. About 8%–12% of reproductive-aged couples are affected all over the world.[1] Furthermore, the existing body of researches suggests that 20%–30% of infertility cases result solely from males, and 50% of infertility cases are associated with male infertility.[2] In the last few years, a high prevalence of genetic causes for spermatogenic impairment has been reported.[3] Therefore, there has been increasing attention to the genetic landscape of the patients. This landscape consists of a small number of altered genes (gene mutations) in a high percentage of cases and a much larger number of genes which are altered infrequently.[4] Owing to the complexity of spermatogenesis, semen composition, and testicular histological diversity, the gene landscape of male infertility is very complicated. Actually, more than 2000 genes are involved in spermatogenesis.[5] Genetic abnormalities are responsible for about 25% of azoospermic cases, and the number of genetic abnormalities that are identified in the semen composition and etiological categories is steadily growing.[5] Genetic screening is associated with diagnosis, genetic counseling, and clinical decision-making.[5] However, even with a huge development of detecting techniques, the etiology of infertility in numerous patients remains unknown. The identification of novel genetic factors in idiopathic infertility, which refers to infertility without a clear-cut cause identified,[6] is crucial for the treatment of these patients.[5]

Male infertility mainly results from spermatogenic defects, to which X chromosomal dosage is closely connected.[7] Since only one X chromosome exists in males, there is no normal allele that can compensate for loss-of-function mutations in single-copy X chromosomal genes. Therefore, X-linked genes are thought to play vital roles in male spermatogenic failure. Furthermore, with the development of next-generation sequencing (NGS), increasingly, novel genes have been discovered to be associated with male infertility, such as bromodomain and WD repeat domain containing 1 (*BRWD1*), DNA methyltransferase 1 (*DNMT1*), DNA methyltransferase 3 beta (*DNMT3B*), ring finger protein 17 (*RNF17*), ubiquitin protein ligase E3 component n-recognin 2 (*UBR2*), ubiquitin-specific peptidase 1 (*USP1*), and ubiquitin-specific peptidase 26 (*USP26*).[8] However,

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Received: 10 June 2021; Accepted: 22 November 2021

definite evidence is still lacking for a gene–disease relationship of these genes.[9] *USP26* is one of the genes, which is assumed to be relevant to male infertility. The *USP26* gene, located at Xq26.2, presents as a single exon on the X chromosome and encodes a protein containing 913 amino acids. It is a member of a large family of de-ubiquitinating enzymes and is exclusively expressed in the testis.[10] Ubiquitination is an important biological process that is involved in the stability and degradation of cellular proteins.[11] The addition of ubiquitin to substrate proteins can enhance the degradation of target proteins.[12] Conversely, the removal of ubiquitin from substrate proteins (also called de-ubiquitination) can prevent these proteins from being degraded.[12] The balance between ubiquitination and de-ubiquitination is crucial for biological activity, including the completion of spermatogenesis.[13,14] The *USP26* gene was first reported in 2001.[15] Owing to the importance of the de-ubiquitinating enzymes in male infertility, more and more attention has been paid to the association between *USP26* and male infertility.

In recent years, over 20 mutations in the *USP26* have been reported to be associated with male infertility.[16] Several mutations are mentioned frequently, such as the cluster mutation (370–371insACA, 494T>C, and 1423C>T in the same allele, causing the amino acid changes including T123–124ins, L165S, and H475Y), c.576G>A,[17–21] c.1090C>T,[22] and c.1737G>A.[22] The cluster mutation is presumed to be concerned with Sertoli cell-only syndrome.[17] Several research papers describe a remarkable relationship between the *USP26* mutations and male infertility,[17,19,22,23] while others do not identify a significant association between them.[20,24–26] Whether specific single-nucleotide polymorphisms, such as c.576G>A, play a crucial role in male infertility remains unknown.

Although two meta-analytic studies on this issue have been performed, the conclusions from them are different. Xia *et al*.[27] reported an association of *USP26* mutations with male infertility, while the result reported by Zhang *et al*.[28] did not support a significant association between *USP26* mutations and male infertility. Moreover, an increasing number of single-nucleotide polymorphisms of *USP26* have recently been found. Therefore, our study included more records and investigated some of the new single-nucleotide polymorphisms (SNPs) of *USP26* to clarify the association between *USP26* mutations and male infertility.

MATERIALS AND METHODS

This work was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. According to PRISMA 2020 guidelines, as shown in **Supplementary Table 1**, the populations were adult males, the exposures were patients with azoospermia, oligozoospermia, or asthenozoospermia, the comparators were individuals with normal fertility or normal spermatogenic function, and the outcomes were the results of the assessment of mutations of *USP26*. It was also registered in the International Prospective Register of Systematic Reviews (PROSPERO; CRD42021225251).

Search strategy

Studies published before March 2021 were identified from PubMed, Scopus, and Web of Science. No retrieval limitation was applied. The searching strategy was ("infertility" OR "azoospermia" OR "oligozoospermia" OR "oligoasthenozoospermia" OR "asthenozoospermia") AND ("USP26" OR "ubiquitin-specific protease 26"). Furthermore, the reference lists of the included studies and related reviews and reports were also screened.

Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) clinical studies reported the association between *USP26* mutations and male infertility; (2) both the number of patients and controls, and the number of patients and controls with *USP26* polymorphisms can be extracted according to reported data; (3) the studies were published in English; (4) the studies included patients which were azoospermia, oligozoospermia, and/or asthenozoospermia; and (5) the studies included controls with normal sperm parameter values or had experienced a partner's spontaneous pregnancy.

The exclusion criteria were as follows: (1) the article was a review, comment or abstract; or (2) when there were multiple publications from the same study group, the study that reported most complete and recent results was included.

Data extraction and quality assessment

Two authors (QYL and YCZ) extracted the following information from each study independently: name of first author, publication year, country, age, description of cases (infertility and subgroups), description of control, the sample size, the number of patients and controls, the number of patients and controls with *USP26* polymorphisms, genotyping method, and the result of genotyping method (the mutations of *USP26*). In the end, the accuracy of the data extraction was checked again.

Quality assessment was independently conducted by two investigators (XML and LCW) according to the National Institutes of Health (NIH) Quality Assessment tool for case-control studies (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools). The NIH's scales consist of twelve parameters of quality (**Supplementary Table 2**). We scored these items and classified the included studies as "good", "no", or "others (CD: cannot determine, NA: not applicable, and NR: not reported)" according to the scales.

Statistical analyses

For each *USP26* mutation reported in the included study, we estimated the odds ratio (OR) and the 95% confidence interval (CI), by retrieving the number of cases and controls. To obtain conservative results, the random-effects model was employed for pooled analysis. The Chi-square test and inconsistency index (I^2) were applied to evaluate the heterogeneity. Subgroup analyses were performed according to different case types (azoospermia, oligozoospermia, and unselected cases). Unselected cases are those in which the detailed numbers of patients with azoospermia or oligozoospermia were not reported in the included article, while the study reported the total number of patients with azoospermia or oligozoospermia instead. All statistical analyses were executed by using Review Manager 5.3 (Cochrane Collaboration, Oxford, UK), and $P < 0.05$ was considered to be statistically significant. Funnel plots were applied for the assessment of publication bias. In addition, a sensitivity analysis was performed by excluding the lowest-weighted or lowest-scored studies.

RESULTS

Characteristics of the included studies

The search and screening process is shown in **Figure 1**. Studies included in our analysis were published between March 2005 and December 2016. A total of 12 studies[8,17–26,29] with 3927 infertility patients and 4648 healthy controls were included in this meta-

analysis. The characteristics of the included studies are summarized in **Table 1**. Patients were from six countries including the USA, Belgium, France, China, Israel, and Iran, which mainly contained Caucasian and Asian. According to the NIH's scales, all included studies were of high quality (**Table 2**).

The association between overall mutations of USP26 and male infertility

Six studies[8,20–23,29] containing 2943 patients and 2478 controls were involved in the analysis of the association between the overall mutations of *USP26* and male infertility. The pooled OR was 1.60 (95% CI: 0.51–5.01, $P = 0.42$; **Figure 2**). However, heterogeneity among these studies was significant ($P < 0.01$; **Supplementary Figure 1**).

The associations between cluster mutation of USP26 and male infertility

Ten studies[17–26] containing 1637 patients and 4052 controls were involved in the analysis of the cluster mutation of *USP26*. The cluster mutation of *USP26* included the mutations of 370–371insACA, 494T>C, and 1423C>T. The pooled OR for overall results was 1.65 (95% CI: 1.02–2.69, $P = 0.04$), which indicated that the cluster mutation of *USP26* was associated with overall male infertility (**Figure 3a**). In subgroup analysis, the pooled ORs were 1.97 (95% CI: 0.96–4.04, $P = 0.06$) for azoospermia, 0.88 (95% CI: 0.24–3.25, $P = 0.85$) for oligozoospermia, and 3.00 (95% CI: 0.35–25.61, $P = 0.32$) for unselected cases (**Figure 3a**). Although the results of the subgroup analysis did not show a significant association between cluster mutation of *USP26* and male infertility, the overall results showed a statistical significance which revealed a potential association between them (**Figure 3a**). Moreover, the funnel plot suggested that the publication bias was limited ($P = 0.45$; **Supplementary Figure 2**).

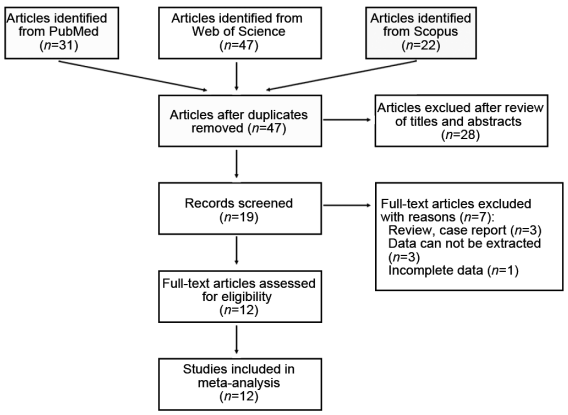


Figure 1: Flow diagram of the included study in this meta-analysis.

The associations between c.576G>A and male infertility

Five studies[17–21] containing 781 patients and 772 controls were involved in the analysis of the c.576G>A mutation of *USP26*. The pooled OR was 1.01 (95% CI: 0.45–2.24, $P = 0.98$; **Figure 3b**). For the subgroup, the pooled ORs were 1.21 (95% CI: 0.40–3.63, $P = 0.73$) for azoospermia, 0.40 (95% CI: 0.11–1.46, $P = 0.17$) for oligozoospermia, 1.80 (95% CI: 0.35–9.15, $P = 0.48$) for unselected cases (**Figure 3b**). Neither overall nor subgroup analysis showed an obvious association between c.576G>A and male infertility. The funnel plot suggested that the publication bias may exist ($P < 0.01$; **Supplementary Figure 3**).

The association between c.1090C>T and male infertility

Five studies[18,21–23,26] containing 828 patients and 532 controls were involved in the analysis of the c.1090C>T mutation of *USP26*. The pooled OR was 1.43 (95% CI: 0.79–2.56, $P = 0.24$; **Figure 4a**). For the subgroup analysis, the pooled ORs were 2.61 (95% CI: 0.73–9.36, $P = 0.14$) for azoospermia, and 1.21 (95% CI: 0.63–2.34, $P = 0.57$) for unselected cases (**Figure 4a**). The results indicated that the c.1090C>T was not significantly associated with male infertility. The funnel plot suggested that the publication bias was limited ($P = 0.81$; **Supplementary Figure 4**).

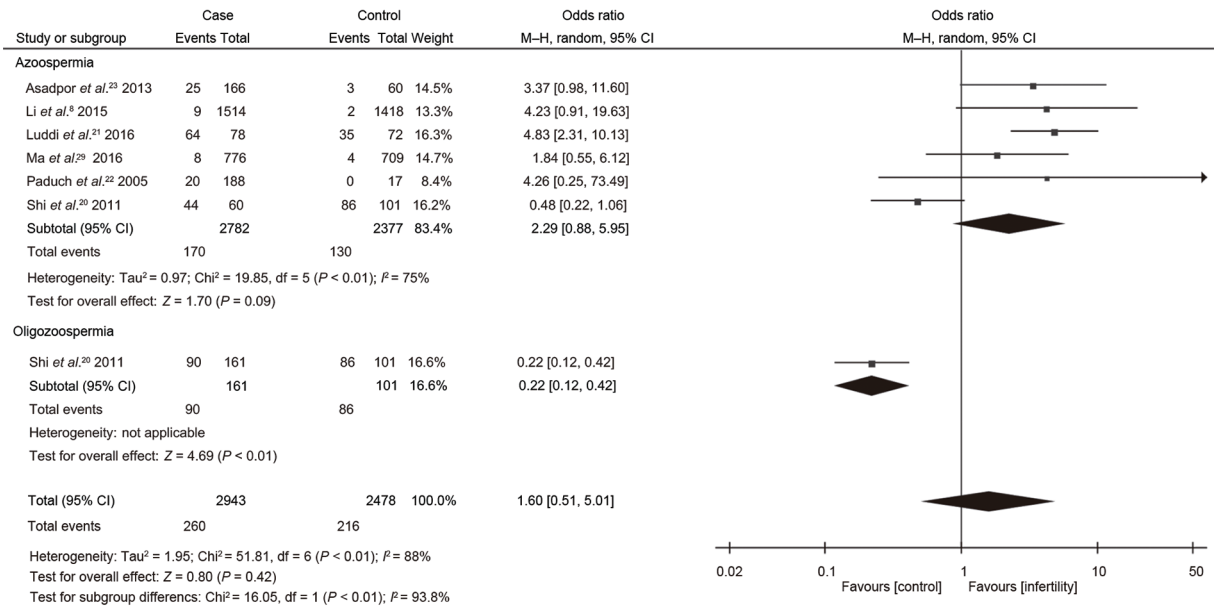


Figure 2: Forest plot of the association between overall mutations of ubiquitin-specific protease 26 (*USP26*) and male infertility. CI: confidence interval; df: degrees of freedom; M–H: Mantel–Haenszel.

Table 1: Main characteristics of studies in meta-analysis

Reference	*Country*	*Ethnic group*	*Number of cases (age [year], range)*	*Number of controls (age [year], range)*	*Genotyping method*	*Diagnostic examination*	*Patient description*	*Control description*
Paduch *et al.*[22] 2005	USA	Caucasian	188 (NM)	17 (NM)	PCR, HPLC	Semen analysis, serum hormones, karyotype and physical examinations	Patients with azoospermia or severe oligozoospermia	Fertile men
Stouffs *et al.*[17] 2005	Belgium	Caucasian	143 (NM)	152 (NM)	PCR, TaqMan	Histological examination of testicular tissues	Diagnosed with Sertoli cell-only syndrome	Normal sperm parameters
Ravel *et al.*[24] 2006	France	Mixed	99 (NM)	1334 (NM)	PCR, TaqMan	Semen analysis	Azoospermia, extreme oligozoospermia ($<1\times10^6$ sperm per ml), severe oligozoospermia (1×10^6–5×10^6 sperm per ml), moderate oligozoospermia (5×10^6–10×10^6 sperm per ml), mild oligozoospermia (10×10^6–20×10^6 sperm per ml)	Fertility status or sperm count of the individual was known
Stouffs *et al.*[25] 2006	Belgium	Caucasian	146 (NM)	202 (NM)	PCR, TaqMan	Semen analysis, karyotype examinations, testicular biopsy	Cryptozoospermia (sperm concentration $<0.1\times10^6$ ml^{-1}), severe oligozoospermia (sperm concentration of 0.1×10^6–5×10^6 ml^{-1})	Normal spermatogenesis on testicular biopsy, normal sperm parameters or proven fertility
Lee *et al.*[19] 2008	China	Asian	200 (NM)	200 (NM)	PCR, TaqMan	Detailed history, physical examination, semen analyses, hormone assays, karyotyping test, molecular test, testicular biopsies	Severe oligozoospermia (spermatozoa count $<5\times10^6$ ml^{-1}) or nonobstructive azoospermia	Proven fertility
Christensen *et al.*[18] 2008	USA	Caucasian	96 (NM)	96 (NM)	PCR	Semen analysis	Severely oligozoospermic (<5 million ml^{-1}) or azoospermic patients	Known paternity
Ribarski *et al.*[26] 2009	Israel	Caucasian	300 (NM)	287 (NM)	PCR	Semen analysis, karyotype analysis	Azoospermic, oligozoospermic, infertile with unknown semen parameters	Sperm bank donors, known fertile men with at least 2 children
Shi *et al.*[20] 2011	China	Asian	221 (24–42)	101 (25–40)	PCR	Detailed history, physical examination, semen analyses, hormone assays, karyotyping test, semen plasma biochemical analysis	Azoospermia, oligoasthenozoospermia, asthenozoospermia, oligozoospermia	Normozoospermia
Asadpor *et al.*[23] 2013	Iran	Asian	166 (25–46)	60 (24–46)	PCR-SSCP, TaqMan	Detailed history, physical examination, semen analyses, hormone assays, karyotyping test, molecular test, testicular biopsies	Nonobstructive azoospermia	Normal semen analysis, at least one child within 3 years without assisted reproductive technologies and no history of miscarriages
Li *et al.*[8] 2015	NM	NM	1514 (NM)	1418 (NM)	PCR, Custom-designed capture array	Semen analyses, karyotyping test	Nonobstructive azoospermia	Proven fertile
Luddi *et al.*[21] 2016	NM	Caucasian	78 (25–44)	72 (28–42)	HRM analysis, direct sequencing	Semen analysis	Azoospermic patients	Normal sperm parameters
Ma *et al.*[29] 2016	China	Asian	776 (24–46)	709 (29–51)	AllPrep DNA/RNA Mini Kit, Illumina HiSeq 2000 platform	Semen analyses, karyotyping test	Nonobstructive azoospermia	Had fathered at least 1 child without assisted reproductive techniques

NM: not mentioned; PCR: polymerase chain reaction; HPLC: high-performance liquid chromatography; SSCP: single-strand conformation polymorphism; HRM analysis: high-resolution melting analysis

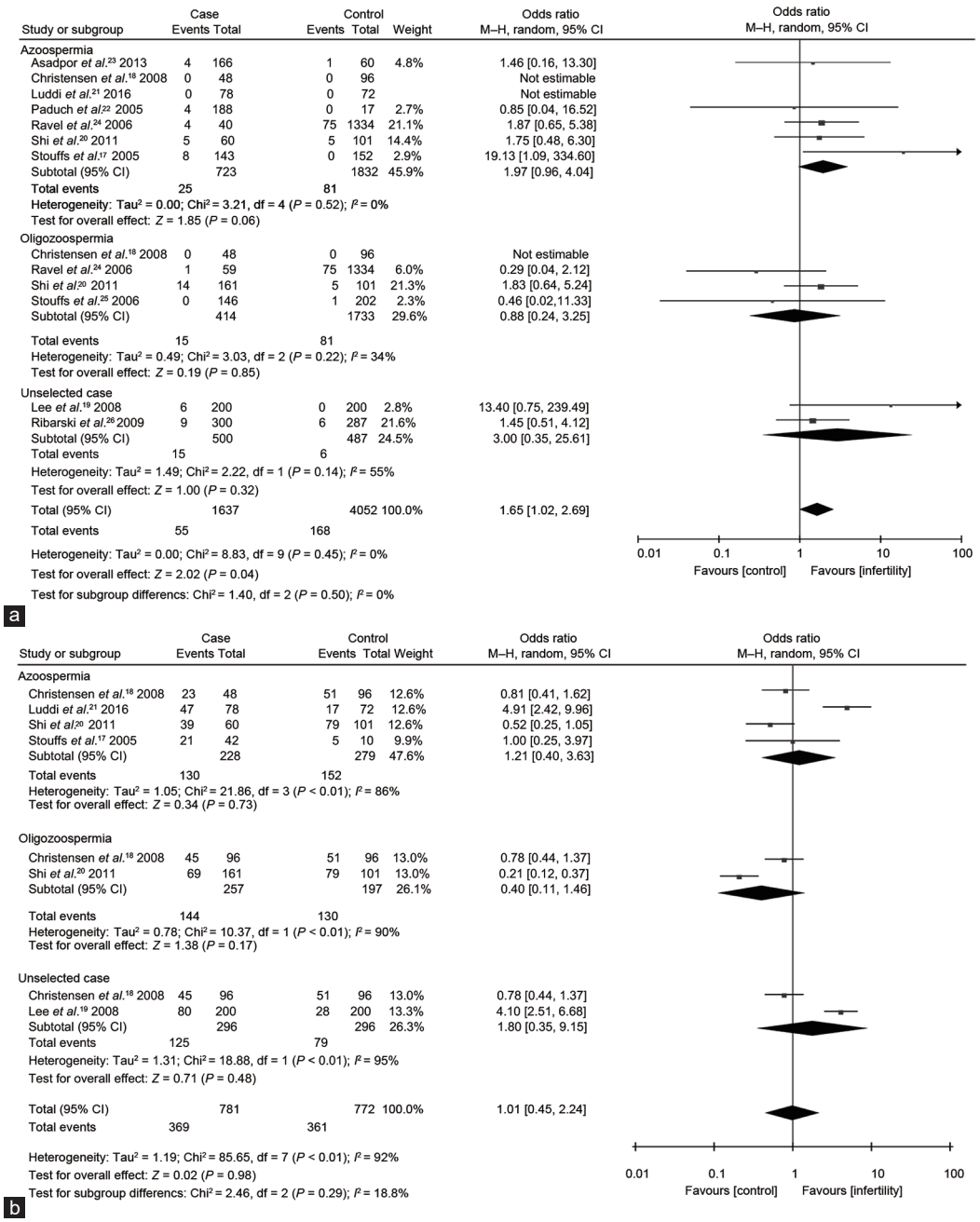


Figure 3: Forest plots of the association between variants of ubiquitin-specific protease 26 (*USP26*) and (**a**) male infertility and (**b**) cluster mutation of *USP26* (c.576G>A). CI: confidence interval; df: degrees of freedom; M–H: Mantel–Haenszel.

The association between c.1737G>A and male infertility

Five studies[19,21–23,26] containing 932 patients and 636 controls were involved in the analysis of the c.1737G>A mutation of *USP26*. The pooled OR was 3.59 (95% CI: 2.30–5.59, $P < 0.01$; **Figure 4b**). For the subgroup, the pooled ORs were 2.31 (95% CI: 0.64–8.34, $P = 0.20$) for azoospermia and 3.52 (95% CI: 1.71–7.24, $P < 0.01$) for unselected cases (**Figure 4b**). Although the results of azoospermia did not indicate the association between c.1737G>A and male infertility, the unselected case and overall cases showed a statistical significance, which revealed a potential relationship between them. The funnel plot suggested that the publication bias was limited ($P = 0.56$; **Supplementary Figure 5**).

Publication bias and sensitivity analysis

Publication bias was assessed by the funnel plot. As shown in **Supplementary Figure 2–5**, the publication bias was limited in our study. In sensitivity analyses, all the outcomes remained consistent with the previous outcomes, suggesting that no individual study significantly affected the pooled OR of the association between mutations in *USP26* and male infertility (**Supplementary Figure 6** and **7**).

DISCUSSION

In recent years, the outcomes of the analysis of the relationship between *USP26* variants and male infertility have been controversial.[27,28] Therefore, a meta-analysis including larger samples and updated

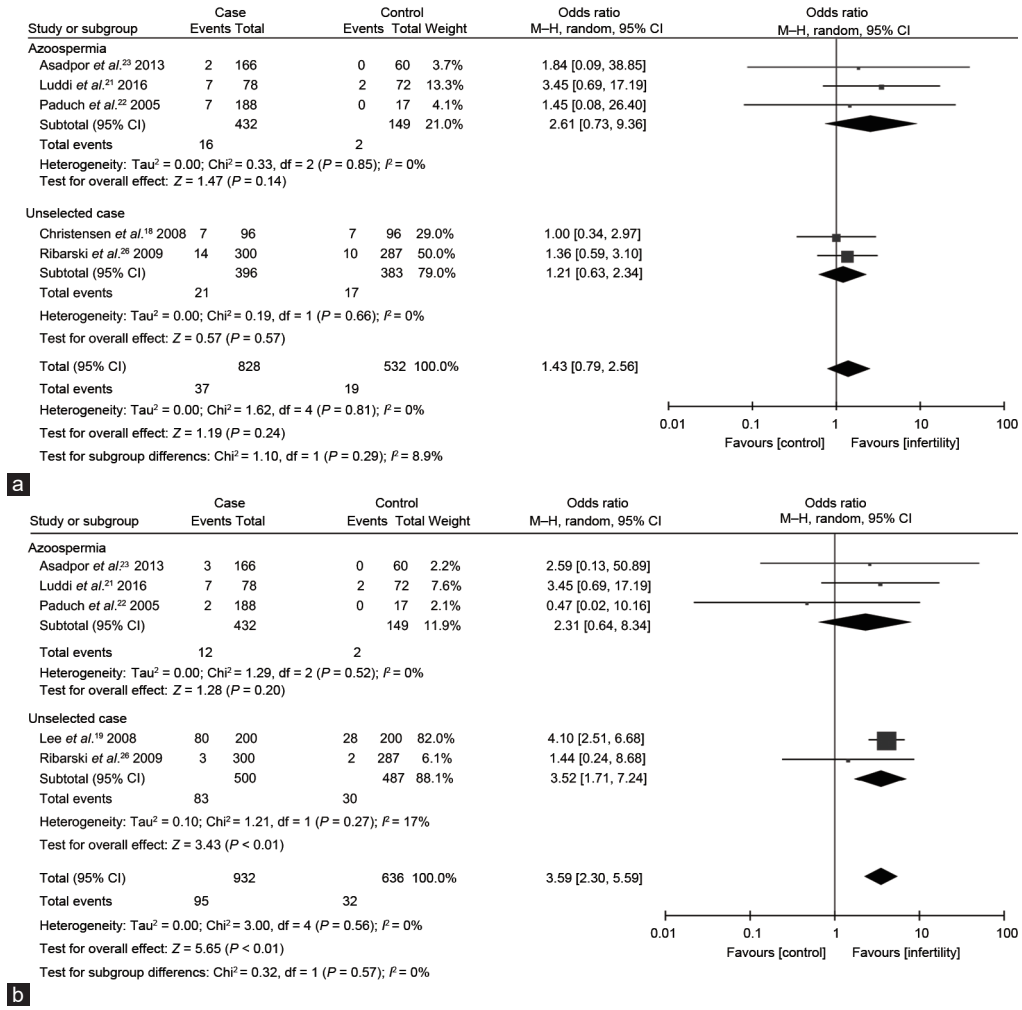


Figure 4: Forest plots of the association between variants of ubiquitin-specific protease 26 (*USP26*) and male infertility: (**a**) c.1090C>T, (**b**) c.1737G>A. CI: confidence interval; df: degrees of freedom; M–H: Mantel–Haenszel.

results is needed. Our analysis involved 3927 patients and 4648 controls from 12 studies. From the result of our analysis, the mutations of *USP26* did not lead to the occurrence of male infertility inevitably. Only some specific mutations play important roles in the male infertility, such as cluster mutation and c.1737G>A. In fact, in the previous study, only one out of nineteen mutations results in the disappearance of the enzyme activity.[30] As for the c.576G>A, it might play a crucial role in male infertility, including asthenozoospermia and oligoasthenozoospermia,[20] though this polymorphism is a synonymous mutation which does not change the amino acid sequence.[21] However, SNPs can influence the gene function through modifying the stability of mRNA.[31] Nevertheless, our results did not show a significant association between mutation of c.576G>A and male infertility, which may be due to we did not conduct the analysis on cases of asthenozoospermia or oligoasthenozoospermia. For the c.1090C>T, our results did not support the association between male infertility and this mutation. This is also consistent with the previous meta-analysis by Zhang *et al*.[28]

Spermatogenesis is an extremely complicated process. Several cell types, hormones, paracrine factors, genes, and epigenetic regulators are involved in the differentiation of spermatogonia into spermatozoa.[32] Such an intricate process requires the precise expression of functional enzymes. Therefore, the balance of ubiquitination and de-ubiquitination plays a critical role in spermatogonia.[33] De-ubiquitinating enzymes are responsible for regulating ubiquitin-dependent processes, which is important for protein stability and activity.[12] USP26 belongs to a de-ubiquitinating enzyme family. In a previous study, mutations of *USP26* were found to affect spermatogenesis and hormone secretion and cause male subfertility.[33] As for the mechanism, androgen receptor (AR) pathway is considered to be involved in the association between *USP26* and male infertility. Androgens such as testosterone are steroid hormones that are essential for normal male reproductive development and function. The AR signaling in the testis is essential for spermatogenesis.[34] Substrates of USP26 include AR, MDM2 proto-oncogene (MDM2), SMAD family member 7 (SMAD7), and protein regulator of cytokinesis 1 (PRC1).[35] Because USP26 can prevent the degradation of AR, the downregulation of USP26 may affect spermatogenesis.[35]

However, the role of *USP26* in male infertility remains doubtful because *USP26*-knockout mice are reported to be fertile.[36] Nevertheless, the function of *USP26* might be different between humans and mice. A frameshift mutation in the *USP26* gene in a patient is reported to result in severe oligozoospermia,[37] which indicates a crucial role of *USP26* in humans.

Table 2: The quality assessment of studies by using the National Institutes of Health's scales

Reference	*Criteria 1*	*Criteria 2*	*Criteria 3*	*Criteria 4*	*Criteria 5*	*Criteria 6*	*Criteria 7*	*Criteria 8*	*Criteria 9*	*Criteria 10*	*Criteria 11*	*Criteria 12*
Paduch *et al*.[22] 2005	Yes	Yes	No	Yes	Yes	Yes	NA	No	Yes	Yes	Yes	Yes
Stouffs *et al*.[17] 2005	Yes	Yes	No	Yes	Yes	Yes	NA	No	Yes	Yes	Yes	Yes
Ravel *et al*.[24] 2006	Yes	Yes	No	Yes	Yes	Yes	NA	No	Yes	Yes	Yes	Yes
Stouffs *et al*.[25] 2006	Yes	Yes	No	Yes	Yes	Yes	NA	No	Yes	Yes	Yes	Yes
Lee *et al*.[19] 2008	Yes	Yes	No	Yes	Yes	Yes	NA	No	Yes	Yes	Yes	Yes
Christensen *et al*.[18] 2008	Yes	Yes	No	Yes	Yes	Yes	NA	No	Yes	Yes	Yes	Yes
Ribarski *et al*.[26] 2009	Yes	Yes	No	Yes	Yes	Yes	NA	No	Yes	Yes	Yes	Yes
Shi *et al*.[20] 2011	Yes	Yes	No	Yes	Yes	Yes	NA	No	Yes	Yes	Yes	Yes
Asadpor *et al*.[23] 2013	Yes	Yes	No	Yes	Yes	Yes	NA	No	Yes	Yes	Yes	Yes
Li *et al*.[8] 2015	Yes	Yes	No	Yes	Yes	Yes	NA	No	Yes	Yes	Yes	Yes
Luddi *et al*.[21] 2016	Yes	Yes	No	Yes	Yes	Yes	NA	No	Yes	Yes	Yes	Yes
Ma *et al*.[29] 2016	Yes	Yes	No	Yes	Yes	Yes	NA	No	Yes	Yes	Yes	Yes

Criteria 1: was the research question or objective in this paper clearly stated and appropriate? Criteria 2: was the study population clearly specified and defined? Criteria 3: did the authors include a sample size justification? Criteria 4: were controls selected or recruited from the same or similar population that gave rise to the cases (including the same timeframe)? Criteria 5: were the definitions, inclusion and exclusion criteria, algorithms, or processes used to identify or select cases and controls valid, reliable, and implemented consistently across all study participants? Criteria 6: were the cases clearly defined and differentiated from controls? Criteria 7: if less than 100% of eligible cases and/or controls were selected for the study, were the cases and/or controls randomly selected from those eligible? Criteria 8: was there use of concurrent controls? Criteria 9: were the investigators able to confirm that the exposure/risk occurred before the development of the condition or event that defined a participant as a case? Criteria 10: were the measures of exposure/risk clearly defined, valid, reliable, and implemented consistently (including the same time period) across all study participants? Criteria 11: were the assessors of exposure/risk blinded to the case or control status of participants? Criteria 12: were key potential confounding variables measured and adjusted statistically in the analyses? If matching was used, did the investigators account for matching during study analysis? NA: not applicable

Interestingly, the defects in fertility caused by *USP26* mutations in an animal study were found to be dependent on the genetic background.[38] A mutation was introduced into the *USP26* gene in mice, and the *USP26*-mutant males backcrossed to a DBA/2 background are found to be sterile with atrophic testes. Xia *et al*.[27] also find that the association between *USP26* mutation and male infertility is significant only in Asian, but not in Euramerican populations. Therefore, further studies should pay more attention to the genetic background of patients and enroll individuals with similar as possible genetic backgrounds. In addition, studies on the relationships between different mutation variants of *USP26* are also needed for a better understanding of male infertility. Although the expression level of *USP26* in different tissues has been explored,[39] comparison of the expression levels of *USP26* in azoospermia patients and healthy individuals is also needed to be further investigated.

The main limitation of our study is that the definitions of case and control were different in the included study. Patients in some studies were azoospermic while others were oligozoospermic, and the definitions of control also varied from different studies. Only one study reported the data on oligoasthenozoospermic and asthenozoospermic patients,[20] so we did not analyze these patients. Furthermore, in some studies such as those reported by Stouffs *et al*.[17] and Shi *et al*.[20] individuals with normal sperm parameter values were defined as controls. However, known fertile men with at least 2 children were enrolled as control group in the study by Ribarski *et al*.[26] As mentioned in the introduction, several studies described a significant association between the mutations and male infertility, while others reported negative outcome. Since researchers are willing to report the positive outcome, more negative outcome may not be reported, suggesting potential publication bias. In addition, the sample sizes of some included studies were limited. We only searched PubMed, Scopus, and Web of Science; some articles in other databases satisfied our criteria may not be included. Although we tried to contact with study authors to identify additional studies, some authors did not reply. In addition, some later studies after March 2021 may not be included. Furthermore, the factors that could influence the male infertility, such as age, were different in cases of each included study. Therefore, more high-quality clinical researches are needed for further validation.

CONCLUSION

Our results suggest that several variants (cluster mutation and c.1737G>A) of *USP26* may play roles in male infertility. More high-quality clinical research is needed for further validation.

AUTHOR CONTRIBUTIONS

QYL and YCZ proposed the conception and design. XML, LCW and JHL provided the administrative support. CW and ZL supplied the study materials. XML and LCW collected and assessed the data. GDS, BLC and ML analyzed the data. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

ACKNOWLEDGMENTS

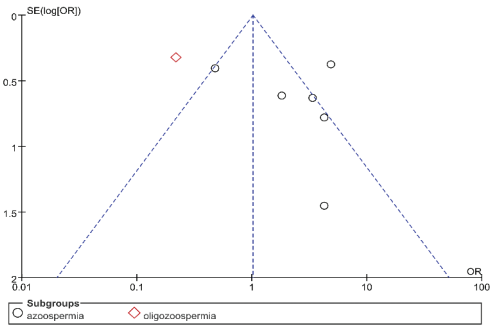
This study was funded by National Natural Science Foundation of China (grant No. 82072838), Tongji Outstanding Young Researcher Funding (grant No. 2020YQ13), and Huazhong University of Science and Technology (grant No. 2019kfyXKJC06).

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

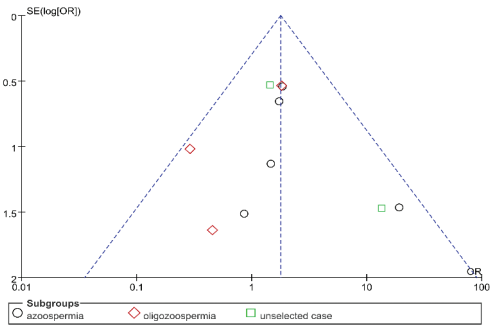
REFERENCES

1 Duca Y, Calogero AE, Cannarella R, Condorelli RA, La Vignera S. Current and emerging medical therapeutic agents for idiopathic male infertility. *Expert Opin Pharmacother* 2019; 20: 55–67.
2 Vander Borght M, Wyns C. Fertility and infertility: definition and epidemiology. *Clin Biochem* 2018; 62: 2–10.
3 Shiraishi K. Genome medicine in male infertility: from karyotyping to single-cell analysis. *J Obstet Gynaecol Res* 2021; 47: 2586–96.
4 Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, *et al.* Cancer genome landscapes. *Science* 2013; 339: 1546–58.
5 Krausz C, Riera-Escamilla A. Genetics of male infertility. *Nat Rev Urol* 2018; 15: 369–84.
6 de Kretser DM. Male infertility. *Lancet* 1997; 349: 787–90.
7 Vockel M, Riera-Escamilla A, Tuttelmann F, Krausz C. The X chromosome and male infertility. *Hum Genet* 2021; 140: 203–15.
8 Li Z, Huang Y, Li H, Hu J, Liu X, *et al*. Excess of rare variants in genes that are key epigenetic regulators of spermatogenesis in the patients with non-obstructive azoospermia. *Sci Rep* 2015; 5: 8785.
9 Oud MS, Volozonoka L, Smits RM, Vissers L, Ramos L, *et al*. A systematic review and standardized clinical validity assessment of male infertility genes. *Hum Reprod*

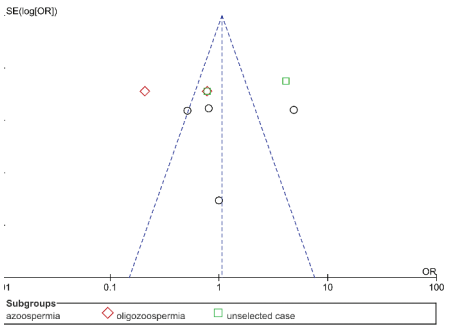
2019; 34: 932–41.
10 Wei L, Shi YC, Cui YX, Huang YF. [Mutation of the *USP26* gene in spermatogenesis dysfunction]. *Zhonghua Nan Ke Xue* 2010; 16: 65–7. [Article in Chinese].
11 Kwon YT, Ciechanover A. The ubiquitin code in the ubiquitin-proteasome system and autophagy. *Trends Biochem Sci* 2017; 42: 873–86.
12 Nijman SM, Luna-Vargas MP, Velds A, Brummelkamp TR, Dirac AM, *et al.* A genomic and functional inventory of deubiquitinating enzymes. *Cell* 2005; 123: 773–86.
13 Richburg JH, Myers JL, Bratton SB. The role of E3 ligases in the ubiquitin-dependent regulation of spermatogenesis. *Semin Cell Dev Biol* 2014; 30: 27–35.
14 He M, Zhou Z, Shah AA, Zou H, Tao J, *et al.* The emerging role of deubiquitinating enzymes in genomic integrity, diseases, and therapeutics. *Cell Biosci* 2016; 6: 62.
15 Wang PJ, McCarrey JR, Yang F, Page DC. An abundance of X-linked genes expressed in spermatogonia. *Nat Genet* 2001; 27: 422–6.
16 Stouffs K, Tournaye H, Liebaers I, Lissens W. Male infertility and the involvement of the X chromosome. *Hum Reprod Update* 2009; 15: 623–37.
17 Stouffs K, Lissens W, Tournaye H, Van Steirteghem A, Liebaers I. Possible role of *USP26* in patients with severely impaired spermatogenesis. *Eur J Hum Genet* 2005; 13: 336–40.
18 Christensen GL, Griffin J, Carrell DT. Sequence analysis of the X-linked *USP26* gene in severe male factor infertility patients and fertile controls. *Fertil Steril* 2008; 90: 851–2.
19 Lee IW, Kuan LC, Lin CH, Pan HA, Hsu CC, *et al.* Association of USP26 haplotypes in men in Taiwan, China with severe spermatogenic defect. *Asian J Androl* 2008; 10: 896–904.
20 Shi YC, Wei L, Cui YX, Shang XJ, Wang HY, *et al.* Association between ubiquitin-specific protease *USP26* polymorphism and male infertility in Chinese men. *Clin Chim Acta* 2011; 412: 545–9.
21 Luddi A, Crifasi L, Quagliarello A, Governini L, De Leo V, *et al.* Single nucleotide polymorphisms of *USP26* in azoospermic men. *Syst Biol Reprod Med* 2016; 62: 372–8.
22 Paduch DA, Mielnik A, Schlegel PN. Novel mutations in testis-specific ubiquitin protease 26 gene may cause male infertility and hypogonadism. *Reprod Biomed Online* 2005; 10: 747–54.
23 Asadpor U, Totonchi M, Sabbaghian M, Hoseinifar H, Akhound MR, *et al.* Ubiquitin-specific protease (*USP26*) gene alterations associated with male infertility and recurrent pregnancy loss (RPL) in Iranian infertile patients. *J Assist Reprod Genet* 2013; 30: 923–31.
24 Ravel C, El Houate B, Chantot S, Lourenco D, Dumaine A, *et al.* Haplotypes, mutations and male fertility: the story of the testis-specific ubiquitin protease *USP26*. *Mol Hum Reprod* 2006; 12: 643–6.
25 Stouffs K, Lissens W, Tournaye H, Van Steirteghem A, Liebaers I. Alterations of the *USP26* gene in Caucasian men. *Int J Androl* 2006; 29: 614–7.
26 Ribarski I, Lehavi O, Yogev L, Hauser R, Bar-Shira Maymon B, *et al. USP26* gene variations in fertile and infertile men. *Hum Reprod* 2009; 24: 477–84.
27 Xia JD, Chen J, Han YF, Chen H, Yu W, *et al.* Association of 370-371insACA, 494T>C, and 1423C>T haplotype in ubiquitin-specific protease 26 gene and male infertility: a meta-analysis. *Asian J Androl* 2014; 16: 720–4.
28 Zhang W, Liu T, Mi YJ, Yue LD, Wang JM, *et al.* Evidence from enzymatic and meta-analyses does not support a direct association between *USP26* gene variants and male infertility. *Andrology* 2015; 3: 271–9.
29 Ma Q, Li Y, Guo H, Li C, Chen J, *et al.* A novel missense mutation in *USP26* gene is associated with nonobstructive azoospermia. *Reprod Sci* 2016; 23: 1434–41.
30 Liu YL, Zheng J, Mi YJ, Zhao J, Tian QB. The impacts of nineteen mutations on the enzymatic activity of *USP26*. *Gene* 2018; 641: 292–6.
31 Capon F, Allen MH, Ameen M, Burden AD, Tillman D, *et al.* A synonymous SNP of the corneodesmosin gene leads to increased mRNA stability and demonstrates association with psoriasis across diverse ethnic groups. *Hum Mol Gene*t 2004; 13: 2361–8.
32 Neto FT, Bach PV, Najari BB, Li PS, Goldstein M. Spermatogenesis in humans and its affecting factors. *Semin Cell Dev Biol* 2016; 59: 10–26.
33 Tian H, Huo Y, Zhang J, Ding S, Wang Z, *et al*. Disruption of ubiquitin specific protease 26 gene causes male subfertility associated with spermatogenesis defects in micedagger. *Biol Reprod* 2019; 100: 1118–28.
34 O'Hara L, Smith LB. Androgen receptor roles in spermatogenesis and infertility. *Best Pract Res Clin Endocrinol Metab* 2015; 29: 595–605.
35 Typas D, Luijsterburg MS, Wiegant WW, Diakatou M, Helfricht A, *et al.* The de-ubiquitylating enzymes USP26 and USP37 regulate homologous recombination by counteracting RAP80. *Nucleic Acids Res* 2015; 43: 6919–33.
36 Felipe-Medina N, Gomez HL, Condezo YB, Sanchez-Martin M, Barbero JL, *et al.* Ubiquitin-specific protease 26 (*USP26*) is not essential for mouse gametogenesis and fertility. *Chromosoma* 2019; 128: 237–47.
37 Li L, Xi Q, Zhang H, Fei J, Jiang Y, *et al*. A novel frameshift mutation in ubiquitin-specific protease 26 gene in a patient with severe oligozoospermia. *Biosci Rep* 2020; 40: BSR20191902.
38 Sakai K, Ito C, Wakabayashi M, Kanzaki S, Ito T, *et al. Usp26* mutation in mice leads to defective spermatogenesis depending on genetic background. *Sci Rep* 2019; 9: 13757.
39 Zhang J, Tian H, Huo YW, Zhou DX, Wang HX, *et al*. The expression of *Usp26* gene in mouse testis and brain. *Asian J Androl* 2009; 11: 478–83.



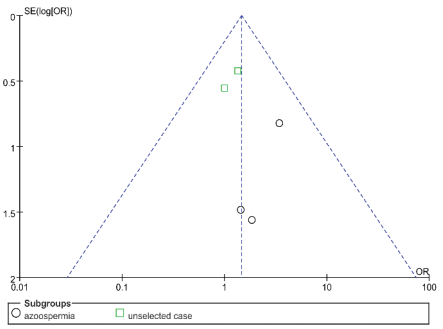
Supplementary Figure 1: Tunnel plot of the overall mutations of USP26. USP26: ubiquitin-specific protease 26.



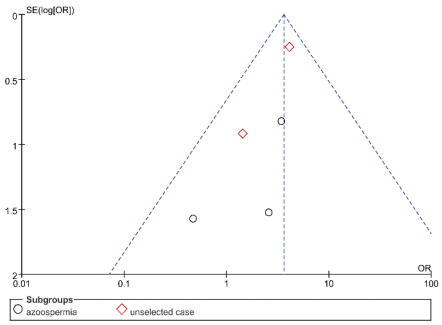
Supplementary Figure 2: Tunnel plot of the cluster mutation of USP26. USP26: ubiquitin-specific protease 26.



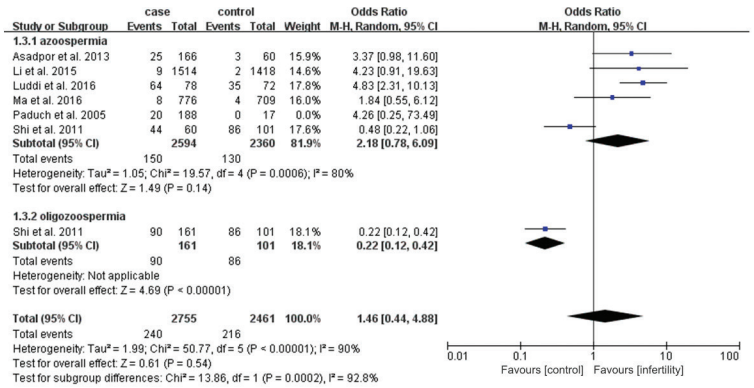
Supplementary Figure 3: Tunnel plot of the c.576G>A.



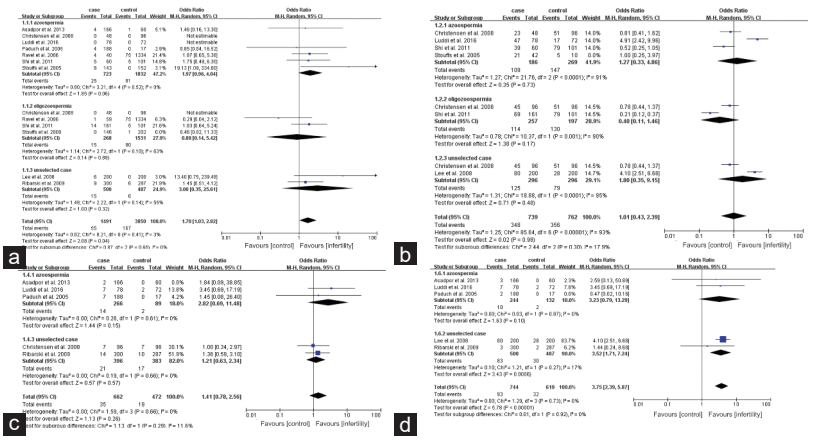
Supplementary Figure 4: Tunnel plot of the c.1090C>T.



Supplementary Figure 5: Tunnel plot of the c.1737G>A.



Supplementary Figure 6: Sensitivity analysis of the association between overall mutations of USP26 and male infertility. USP26: ubiquitin-specific protease 26.



Supplementary Figure 7: Sensitivity analysis of the association between variants of USP26 and male infertility. (**a**) Cluster mutation of USP26, (**b**) c.576G>A, (**c**) c.1090C>T, (**d**) c.1737G>A. USP26: ubiquitin-specific protease 26.

Supplementary Table 1: PECOS table

Participants	*Adult male*
Exposure	Individuals which were azoospermia or oligozoospermia
Comparisons	Individuals which are tested of normal sperm parameters or spontaneous pregnancy occurred
Outcomes	Results of the assessment of mutations of usp26
Study design	Case-control study

PECOS: P: Participants, E: Exposure, C: Comparisons, O: Outcome, S: Study design

Supplementary Table 2: The parameters in National Institutes of Health's scales

(1) Research question
(2) Study population
(3) Sample size justification
(4) Groups recruited from the same population
(5) Inclusion and exclusion criteria
(6) Case and control definitions
(7) Random selection of study participants
(8) Concurrent controls
(9) Exposure assessed prior to outcome measurement
(10) Exposure measures and assessment
(11) Blinding of exposure assessors
(12) Statistical analysis.