Review Article

Annals of Clinical and Medical Case Reports

Anti-Sperm Antibody Positivity Results A Decrease In Sperm Penetration Rate Both In Vivo And In Vitro: A Systematic Review And Meta-Analysis

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Conceptualization: LS, LY, YL, YJH, NTX, LW. Data curation: LS, LY, FYM, DYY, HF, DMM, ZY. Methodology: LS, LY. Investigation: LS, LY. Writing-review: LS, LY. Validation: DYY, HF, DMM. Review: NTX, LW.

Received Date: 10 Oct 2023
Accepted Date: 26 Oct2023
Published Date: 01 Nov 2023

Citation:
Shuai Liu. Anti-Sperm Antibody Positivity Results A Decrease In Sperm Penetration Rate Both In Vivo And In Vitro: A Systematic Review And Meta-Analysis. Annals of Clinical and Medical Case Reports 2023.

1. Abstract

1.1. Background: Anti-sperm antibodies (ASA) that bind to sperm have been associated with infertility, but most of the available studies have conflicting results.

1.2. Objectives: We carried out a meta-analysis to evaluate whether female/male ASA-positiveness would have an impact on sperm penetration through the cervical mucus in these patients.

1.3. Materials and Methods: A systematic search of the target literature was conducted using PubMed, EMBASE, and Cochrane Library. Review Manager 5.4 software was used to analyze data. Relative risk (RR) with the corresponding 95% confidence intervals (95% CIs) were implemented as a measure of effect size to assess the value of post-coital test (PCT) and sperm-cervical mucus penetration test (SCMPT) between ASA-positive patients and control groups.

1.4. Results: A meta-analysis of the negative rate of PCT was performed in 10 controlled studies. There was a significant association between ASA and PCT negative rate (RR = 1,63, 95% CI = 1.37 to 1.95, p <0.01). Another meta-analysis of the positive rate of SCMPT was performed in 8 controlled studies. There was a significant difference in the SCMPT positive rate (RR = 0.65, 95% CI = 0.56 to 0.77, p <0.01).

1.5. Discussion: Compared with the control group, the sperm penetration rate in the ASA positive group was lower. Clinicians working on reproductive health and infertility should be aware of this issue in order to evaluate and treat patients in order to improve their pregnancy rate. It is recommended that infertile couples undergo routine ASA testing and propose targeted treatment strategies to help improve the success rate of reproductive therapy.

1.6. Conclusions: Both in vivo and in vitro experiments reflected decreased sperm penetration through the cervical mucus in ASA-positive patients.

2. Keywords:
Anti-sperm antibodies; Sperm penetration; PCT; SCMPT; Infertility

3. Introduction

Anti-sperm antibody (ASA), which can bind to sperm, has been detected in serum and seminal plasma and has been linked to infertility in a number of studies[1,2]. High-titer anti-sperm antibodies are typically indicative of unsuccessful fertilization when they are discovered in seminal plasma. A high level of ASA is present in males with a clinical history of testicular torsion, testicular cancer, epididymitis, bilateral orchitis associated with extensive destruction of seminiferous tubules, semen infection, varicocele and genital infection, as well as inflammation brought on by vasectomy[3-5] . Just 1-2%[6] of fertile males have significant levels of ASA in their semen, compared to 5-15%[7] of infertile men. In 1922, S. R. Meaker was the first to note the occurrence of ASA in females[8]. According to reports, the ASA of women was frequently significantly correlated with that of their male spouses. In their study, [9] Witkin and Chaudhry analyzed data from more than 600 couples and found that 12.4% of men had sperm surface antibodies and their wives had anti-sperm antibodies in their serum. A study showed that 29.6% of 459 infertile women had been detected ASA in serum[10]. Women who had ASA found in their serum samples tended to have it found more frequently in their cervical mucus samples. Presence of ASA in female partner serum may also increase the risk of miscarriage[11]. The impairment of sperm function is associated with the presence of ASA in male and/or female partners. According to one study, sperm concentration and motility were both inversely linked with ASA[12]. According to MElstein [13], the rate of sperm passage was significantly decreased when the cervical mucus protein concentration surpassed 12.5ug/mg, particularly when antibodies were present. Human sperm treated with specific antibodies have a decreased ability to penetrate the cervical mucus and also develop sperm agglutination and immobilization [14]. ASA levels are associated with seminal leukocyte concentrations [13,15], which can produce reactive oxygen species (ROS) that lead to sperm dysfunction and sperm DNA damage, but a prospective study [12] did not reveal sperm DNA damage. ASA may also mediate sperm apoptosis, which leads to a decrease in sperm numbers [1]. Sperm function impairment and sperm deficiency may affect sperm's ability to pass through cervical mucus, which may affect fertilization and make it challenging for sperm to reach the vicinity of the oocyte and interfere the process of sperm and oocyte binding. The detection method of sperm through cervical mucus mainly includes postcoital tests (PCT) and the sperm-cervical mucus penetration technique (SCMPT). Therefore, the objective of this research is to gather factual information from widely quoted literature to demonstrate if ASA can impact the capacity of sperm to travel through cervical mucus.

4. Materials and methods

This systematic review was developed rested upon the recommendations from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statements[16]. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. The protocol is registered in the PROSPERO registry (CRD42022342206, http://www. crd.york.ac.uk/PROSPERO).

4.1 Search strategy

To find all pertinent studies without regard to language restrictions, we carried out an organized search across three accessible databases (PubMed, EMBASE, and Cochrane Library). In addition, pertinent supplementary studies found in the primary and event studies' reference lists were examined. The following search phrases were entered into PubMed: (((((((((antisperm antibodies) OR (ASA)) OR (sperm antibodies)) OR (spermatozoa antibodies)) OR (spermatozoon antibodies)) OR (immunological infertility)) OR (autoantibodies)) OR (immunoglobulins)) AND (((((((sperm-mucus interaction) OR (cervix mucus)) OR (mucus, cervix)) OR (cervical mucus)) OR (mucus, cervical)) OR (cervical mucus analysis)) OR (postcoital test)) OR (sperm-cervical mucus penetration test)). This same combination of words was used to search in Cochrane Library. The following search phrases were entered into EMBASE: ('sperm antibody'/exp OR 'antisperm antibodies':ti,ab OR 'sperm antibodies':ti,ab OR 'spermatozoa antibodies':ti,ab OR 'spermatozoon antibodies':ti,ab OR 'autoantibody'/exp OR 'autoantibodies':ti,ab OR 'immunoglobulin':ti,ab OR 'immunological infertility':ti,ab) AND ('uterine cervix mucus'/exp OR 'uterine cervical mucus':ti,ab OR 'sperm-mucus interaction':ti,ab OR 'cervical mucus analysis'/exp OR 'postcoital test':ti,ab OR 'sperm-cervical mucus penetration test':ti,ab).

4.2. Eligibility criteria

The inclusion criteria for this study were as follows: a) studies measuring ASA in infertile couples; b) studies reporting value of PCT or SCMPT; PCT: The average motile sperm count in cervical mucus was examined 6 to 12 hours after coitus under high (×400) magnification. An PCT-negative result is suggested that sperm have a strong ability to pass through cervical mucus SCMPT: This test is an in vitro test for sperm penetration into cervical mucus. Sperm were incubated for 30 minutes at 37°C, and then they were inspected under a microscope (50 to 100 ×). Based on the greatest distance that a minimum of five motile sperm could travel, penetration was calculated. SCMPT-positive result is suggested that sperm have a strong ability to pass through cervical mucus. c) observational studies (cross-sectional, case-control, or cohort). Included studies were required to meet all of the above criteria. We excluded articles a) utilized unspecified additional techniques to evaluate sperm penetration; b) that were case reports; c) the content of the study does not include ASA; d) that could not get meaningful data for this review even after we contacted the authors via e-mail.

4.3. Study selection, data extraction, and quality of evidence

Read the titles of each article that the database search turned up, searched

all research that might be included in this review, regardless of population size, source, or age, and examined the abstracts of pertinent articles on the correlation between the surveys. Two scholars looked over the included articles, gathered information that was relevant to the study's objectives and used consensus to settle disagreements. All closely related literature, meta-analysis, and review articles were also reviewed for their reference lists to identify additional published work not indexed by above-mentioned databases. A third reviewer dealt with disputes over whether a study should be included. The data collected were as follows: authors and publication year, type of study, country, sample size, age, PCT negative rate, and SCMPT positive rate. Other information was obtained by contacting authors via e-mail. We used Newcastle Ottawa Scale (NOS) [17] to evaluate the quality of included cohort and case-control studies. The highest score for NOS was 9 points. Studies with an NOS score between 5 and 7 and greater than 7 were considered "medium"-quality studies and "high"-quality studies, respectively. On the contrary, studies with NOS score lower than 5 points were considered "low"-quality studies. We also analyzed the impact of possible conflicts of interest and whether the research was ethically approved [18].

4.4. Statistical analysis

Statistical analyses were conducted using REVMAN Review Manager 5.4 software. To assess the efficacy of PCT and SCMPT between two groups, we utilized relative risk (RR) and the corresponding 95% confidence interval (CI) as a measure of effect magnitude. The X^2 test was applied to assess statistical significance, and a pooled effect was deemed significant when P <0 .05. The percentage of variability across studies attributable to heterogeneity was estimated using the I^2 test, which was considered to be a significant difference when P <0.05. Low, medium, and high degrees of heterogeneity were clarified by I^2 values of 25%, 50%, and 90%, respectively. Due to excessive heterogeneity the random effects model was used to merge data. Subgroup and sensitivity analyses were also carried out to investigate the sources of heterogeneity between studies. We observed the funnel plot to see if there was any publication bias.

5. Results

5.1 Included studies

The search strategy identified a total of 1025 studies, of which 37 studies with titles and abstracts met the inclusion criteria. After excluded 21 studies those used different outcome variables or had no ASA testing data, there were finally 16 articles included in the scope of the analysis (Figure 1). Two studies conducted in Japan [19-20]; 5 studies in America [21-25]; 1 study in Italy[26]; 2 studies in German[27-28]; 3 studies in England[13, 29-30]; 1 study in France[31]; 1 study in Malaysia [32]; and 1 study in Sweden [33]. ASA detection methods include TAT[21-22,25,28,32-33], SIT[19,21-22,33], IBT[20,24,31], MAR[26-27] and immunolabeling[28,30,32]. Some studies[20,23-24,26-27,30-31] tested ASA from semen samples of men, some[13,21-22,25,32] from cervical mucus samples of women, and others[19,21-22,25,28-29,33] from serum samples of one spouse. In addition, some studies[19-20,23-24,26-28,30,32-33] assessed sperm penetration at cervical mucus using an in vivo assay - PCT, while others[13,21-22,25,27-29,31,33] used an in vitro assay - SCMPT.

5.2 Sperm penetration rate of ASA-positive patients

According to studies, the sperm penetration rate of ASA-positive patients was lower. For most studies, there were significant differences in characteristics between ASA-positive and ASA-negative patients (Table 1). The PCT-negative rate of ASA-positive patients ranged from 50.0% to 100.0%, while the negative patients were only 13.0% to 75.0%. The SCMPT positive rate of ASA-positive patients ranged from 2.9% to 68.8%, compared with 55.3%-95.0% of negative patients.

Table 1: Characteristics of the controlled studies on sperm penetration through the cervical mucus in ASA-positive and ASA-negative men in the systematic review

No.	Author (Year)	Country	Study design	Quality score	Conflict of interest	Ethicscommittee approval	Group	ASA detection method	ASA titer cutoff	ASA sample source	ASA samplegender	Sample size		PCT negative rate		P	SCMP semi-quantitative analysis		P	SCMPT positive rate		P
												ASA (+)	ASA (-)	ASA (+)	ASA (-)		ASA (+)	ASA (-)		ASA (+)	ASA (-)	
1	Shibahara et al. (2007)	Japan	Cohort	9	No	Yes	ASA(+) vs ASA (-)	SIT	2	serum	female	31	137	24(77.4%)	28(20.4%)	<0.00001						
							>SI50 vs <SI50	SIT	50	serum	female	10	21	10(100%)	14(66.7%)	0.04						
2	Menget al. (1989)	American	Cohort	8	No	Yes	ASA(+) vs ASA (-)	TAT	16	serum	male/female	142	707				2.4±0.51	3.5±0.03				
							ASA(+) vs ASA (-)	SIT	4	serum + CM	male/female	63	786				2.1±0.51	3.4±0.03				

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3	Menge et al. (1982)	American	Cohort	9	No	Yes	ASA(+) vs ASA (-)	SIT	4	CM	female	136	323							35(25.7%)	190 (58.8%)	
							ASA(+) vs ASA (-)	SIT/TAT	4/16	serum	female	139	354							39 (28.1%)	141 (55.6%)	
							ASA(+) vs ASA (-)	SIT/TAT	4/16	serum	male	103	419							50 (48.5%)	375 (89.5%)	
4	Gilbert et al. (1986)	American	Cohort	9	No	Yes	ASA(+) vs ASA (-)	-	-	semen	male	22	37	20(90.9%)	17(45.9%)	0.0004						
5	Barbonetti et al. (2019)	Italy	Case control	7	No	Yes	ASA(+) vs ASA (-)	MAR	50%	semen	male	103	17	553.4%)	3(17%)							
6	Check et al. (1991)	American	Cohort	8	No	Yes	ASA(+) vs ASA (-)	IBT	50%	semen	male	13	46	9(69.2%)	6(13.0%)							
7	Eggert-Kruse et al. (1991)	German	Cohort	8	No	Yes	ASA(+) vs ASA (-)	MAR	30%	semen	male	28	162	22(78.6%)	79(48.8%)							
							ASA(+) vs ASA (-)	MAR	30%	semen	male	17	173	13(76.5%)	88(50.9%)							
							ASA(+) vs ASA (-)	MAR	30%	semen	male	24	139				1.67 ± 0.92	1.71 ± 0.96	0.81	13(54.2%)	78(56.1%)	
							ASA(+) vs ASA (-)	MAR	30%	semen	male	24	139				1.63 ± 0.88	1.81 ± 0.92	0.36	13(54.2%)	85(61.2%)	
							ASA(+) vs ASA (-)	MAR	30%	semen	male	14	149				1.14 ± 0.86	1.76 ± 0.94	0.02	4(28.6%)	87 (58.4%)	
							ASA (+) vs SA(-)	MAR	30%	semen	male	14	149				1.14 ±0.66	1.84 ±0.91	5.5e-3	4(28.6%)	94 (63.1%)	
8	Ingerslev et al. (1980)	American	Cohort	9	No	Yes	ASA(+) vs ASA (-)	TAT	116	CM	female	2	19							1(50%)	12(63.2%)	
							ASA(+) vs ASA (-)	TAT	116	serum	female	14	7							8(57.1%)	5(71.4%)	

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9	Eggert-Kruse et al. (1989)	German	Cohort	9	No	Yes	ASA(+) vs ASA (-)	TAT	18	serum	male	34	199	18(52.9%)	87(43.7%)						110(55.3%)	
							ASA(+) vs ASA (-)	TAT	18	serum	female	42	193	24(57.1%)	82(42.5%)					16(47.1%)		
							ASA(+) vs ASA (-)	TAT	18	serum	male	34	199								125(64.8%)	
							ASA(+) vs ASA (-)	TAT	18	serum	female	42	193							24(57.1%)		
							ASA(+) vs ASA (-)	TAT	18	serum	male	34	199							19(55.9%)	116(58.3%)	
							ASA(+) vs ASA (-)	TAT	18	serum	female	42	193							23(54.8%)	139(72.0%)	<0.05
							ASA(+) vs ASA (-)	Immunolabelling	15%	serum	male	50	138	25(50.0%)	58(42.0%)							
							ASA(+) vs ASA (-)	Immunolabelling	15%	serum	female	42	154	21(50.0%)	66(42.9%)							
							ASA(+) vs ASA (-)	Immunolabelling	15%	serum	male	50	138							29(58.0%)	87(63.0%)	
							ASA(+) vs ASA (-)	Immunolabelling	15%	serum	female	42	154							19(45.2%)	80(51.9%)	
							ASA(+) vs ASA (-)	Immunolabelling	15%	serum	male	50	138							34(68.0%)	89(64.5%)	
							ASA(+) vs ASA (-)	Immunolabelling	15%	serum	female	42	154							16(38.1%)	89(57.8%)	<0.05
10	Koriyama etal. (2013)	Japan	Cohort	8	No	Yes	ASA(+) vs ASA (-)	IBT	20%	semen	male	11	140	6(54.5%)	29(20.7%)	0.020						
							ASA(+) vs ASA (-)	IBT	50%	semen	male	8	143	4(50.0%)	31(21.7%)	0.084						
11	Morgan et al. (1977)	England	Cohort	9	No	Yes	ASA(+) vs ASA (-)	-	-	serum	male	32	12							9(28.1%)	9(75.0%)	
							ASA(+) vs ASA (-)	-	-	serum	male	32	12							10(31.2%)	11(91.7%)	
12	Wall et al. (1974)	England	Cohort	7	No	Yes	ASA(+) vs ASA (-)	Immunolabelling	-	semen	male	45	70	26(57.8%)	25(35.7%)							
13	Almeida et al. (1986)	France	Cohort	8	No	Yes	ASA(+) vs ASA (-)	IBT	60%	semen	male	35	32							1(2.9%)	23(71.9%)	
14	Wong (1978)	Malaysia	Cohort	7	No	Yes	ASA(+) vs ASA (-)	SIT	-	CM	female	26	14	22(84.6%)	6(42.9%)							
							ASA(+) vs ASA (-)	TAT	-	CM	female	20	20	13(65.0%)	15(75.0%)							
							ASA(+) vs ASA (-)	Immunolabelling	-	CM	female	21	19	15(71.4%)	13(68.4%)							

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15	Fjallbrant et al. (1968)	Sweden	Cohort	9	No	Yes	ASA(+) vs ASA(-)	TAT	32-64	serum	male	16	20				0.38 ±0.62	1.35 ± 0.59	1.4 e-4	5(31.3%)	19(95.0%)	
							ASA(+) vs ASA(-)	TAT	32-64	serum	male	16	20							6(37.5%)	18(90.0%)	
							ASA(+) vs ASA(-)	TAT	32-64	serum	male	14	8	11(78.6%)	4(50.0%)		0.50± 0.73	1.60 ± 0.68	2.3 e-4			
							ASA(+) vs ASA(-)	SIT	6h	serum	male	16	20							6(37.5%)	18(90.0%)	
							ASA(+) vs ASA(-)	SIT	6h	serum	male	16	20				0.50 ±0.73	1.25 ± 0.64	3.3 e-3	5(31.3%)	19(95.0%)	
							ASA(+) vs ASA(-)	SIT	6h	serum	male	13	9	11(84.6%)	4(44.4%)							
16	Elstein et al. (1970)	England	Cohort	6	No	Yes	ASA(+) vs ASA(-)	-	-	CM	female	16	3				0.38 ±0.62	1.70 ± 0.57	9.3 e-6	11(68.8%)	2(66.7%)	

ASA, anti-sperm antibodies; SIT, sperm immobilization test; TAT, tray agglutination test; MAR, mixed antiglobulin reaction; IBT, immunobead binding test; CM, cervical mucus; PCT, postcoital test; SCMPT, sperm-cervical mucus penetration technique.

5.3 Meta-analysis

Meta-analysis of the negative rate of PCT was performed in 10 controlled studies [19-20,23-24,26-28,30,32-33]. Because of the differences in research methods within each study, we divided it into 19 sub-analysis. The ASA positive group consisted of 550 men, and the ASA negative group consisted of 1,700 people. The results showed that there was a

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Meta-analysis of the positive rate of SCMPT was performed in 8 controlled studies [13,22,25,27-29,31,33]. Because of the differences in research methods within each study, we divided it into 25 sub-analysis. The ASA positive group consisted of 985 men, and the ASA negative group consisted of 3,205 people. There was a significant difference in the SCMPT positive rate (RR = 0.65, 95% CI = 0.56 to 0.77, p <0.01) with a high degree of heterogeneity between studies ($I^2 = 71\%$, $p < 0.01$) (Figure 3) and SCMPT semi-quantitative analysis between the two groups (SMD = -2.24, 95% CI = -4.28 to -0.19, p = 0.03) ($I^2 = 99\%$, $p < 0.01$) (Figure 4). Fjallbrant et al[33] showed that different ASA and SCMPT assays produced differential results in the two groups (Supplementary Figure 1 A). Eggert-Kruse et al[27] found that different sources of cervical mucus may have a certain impact on the results (Supplementary Figure 1B).

Figure 1. Flow diagram

3.4 Heterogeneity analysis

Heterogeneity analysis includes subgroup analysis and sensitivity analysis. We conducted subgroup analysis on the results of PCT and SCMPT, and divided ASA patients into male and female groups according to their gender (Figure 2-3);

divided into serum group, semen group and CM group according to the source of ASA samples (Figure 5-6), and divided them into five subgroups including TAT, SIT, MAR, IBT and immunolabeling according to ASA detection methods (Figure 7-8). The results indicated that gender and sample source grouping factors were not sources of heterogeneity, but we found that male factors (PCT, RR=1.71; SCMPT, RR=0.61) were more likely than female factors (PCT, RR=1.47; SCMPT, RR=0.72) to cause a decrease in sperm penetration, and this difference was also reflected in the results of the semen and CM groupings. The detection methods of ASA can be regarded as a source of heterogeneity in the results of both PCT and SCMPT, the most sensitive method in PCT is IBT (RR=3.05), while the RR value of TAT is only 1.19. We also performed a sensitivity analysis of the PCT and SCMPT results and found no significant source of heterogeneity among studies.

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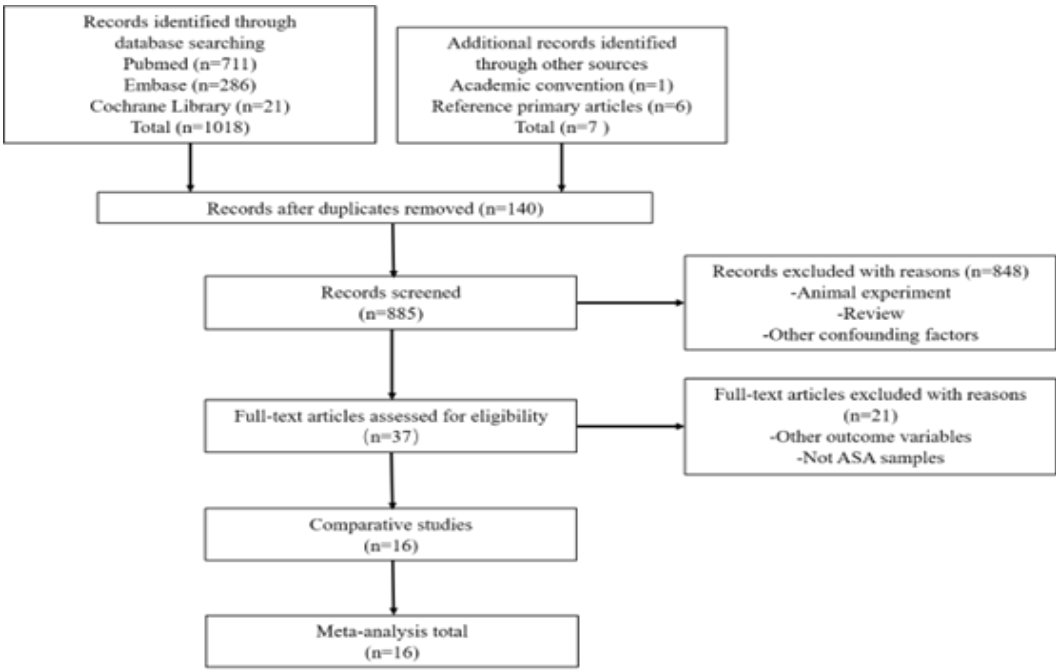

Figure 1. Flow diagram

Figure 2. Results of the meta-analysis for the PCT negative rate

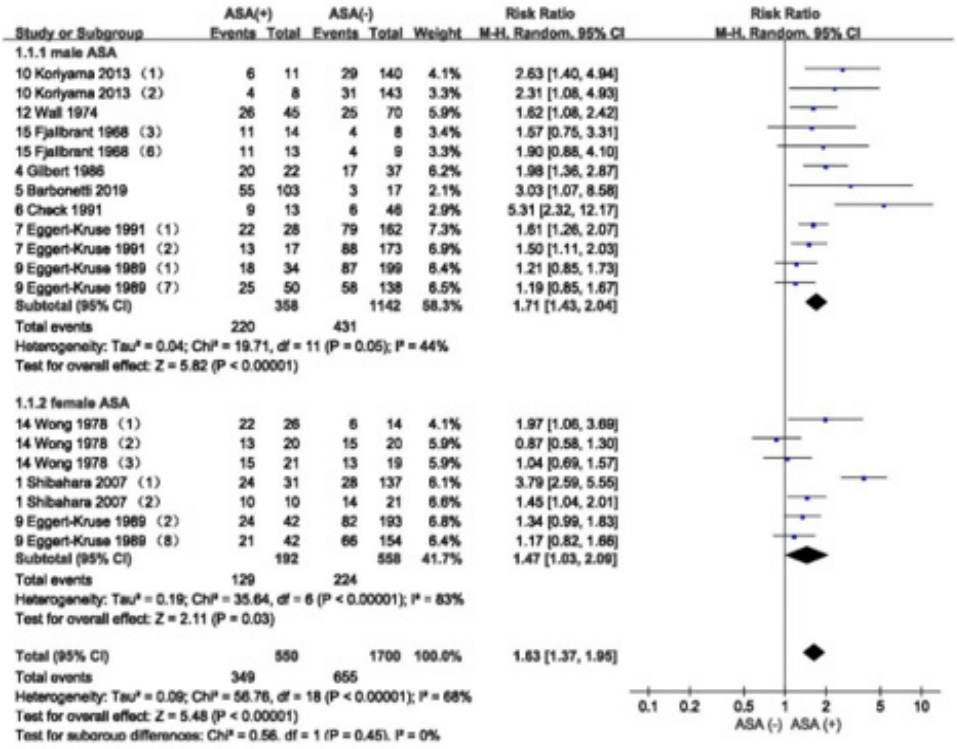

Figure 3. Results of the meta-analysis for the SCMPT positive rate

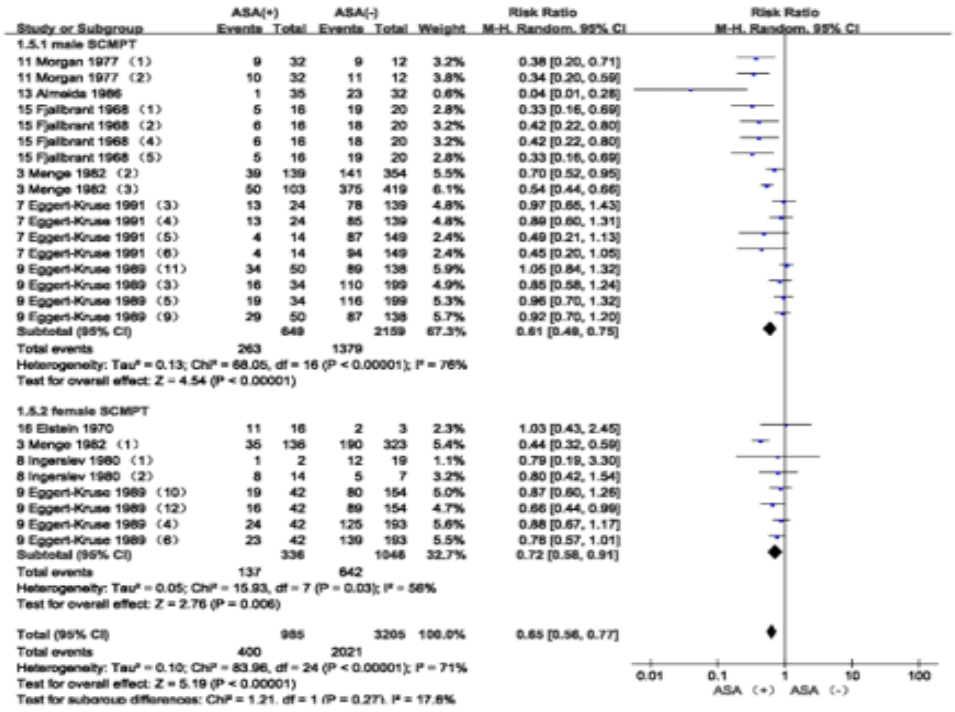

Figure 4. Results of the meta-analysis for the SCMPT semi-quantitative analysis

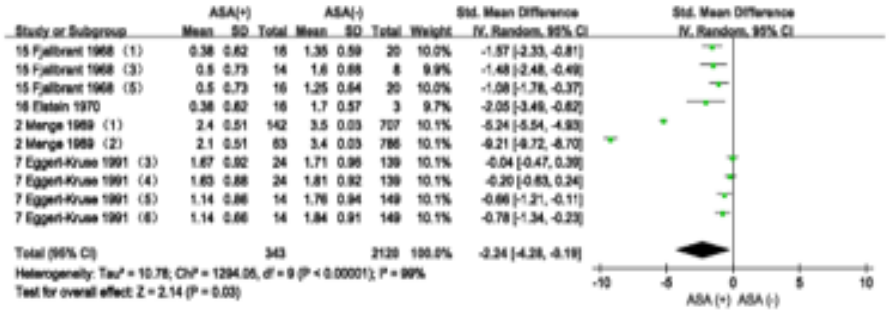

Figure 5. Results of the source of ASA samples subgroup analysis for the PCT negative rate

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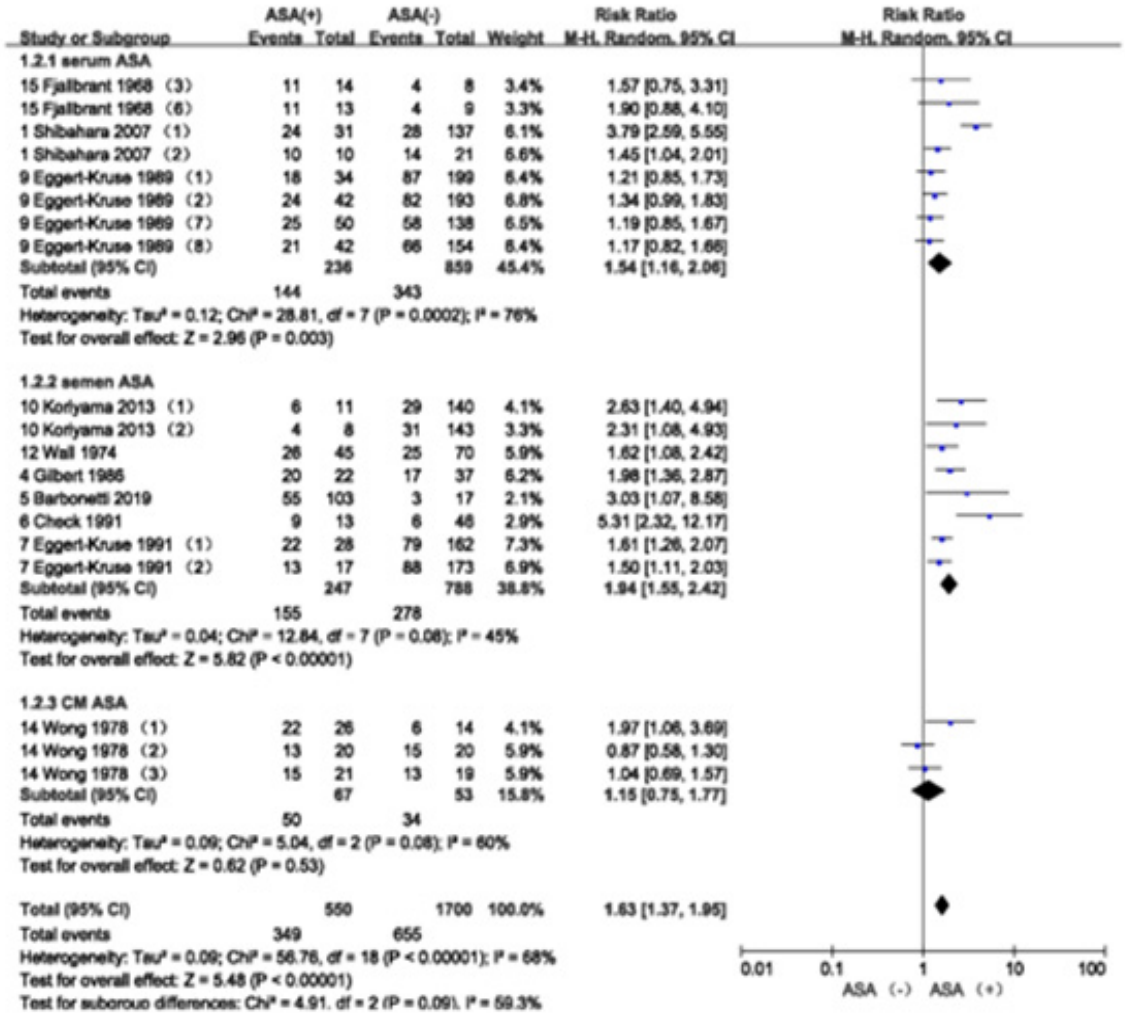

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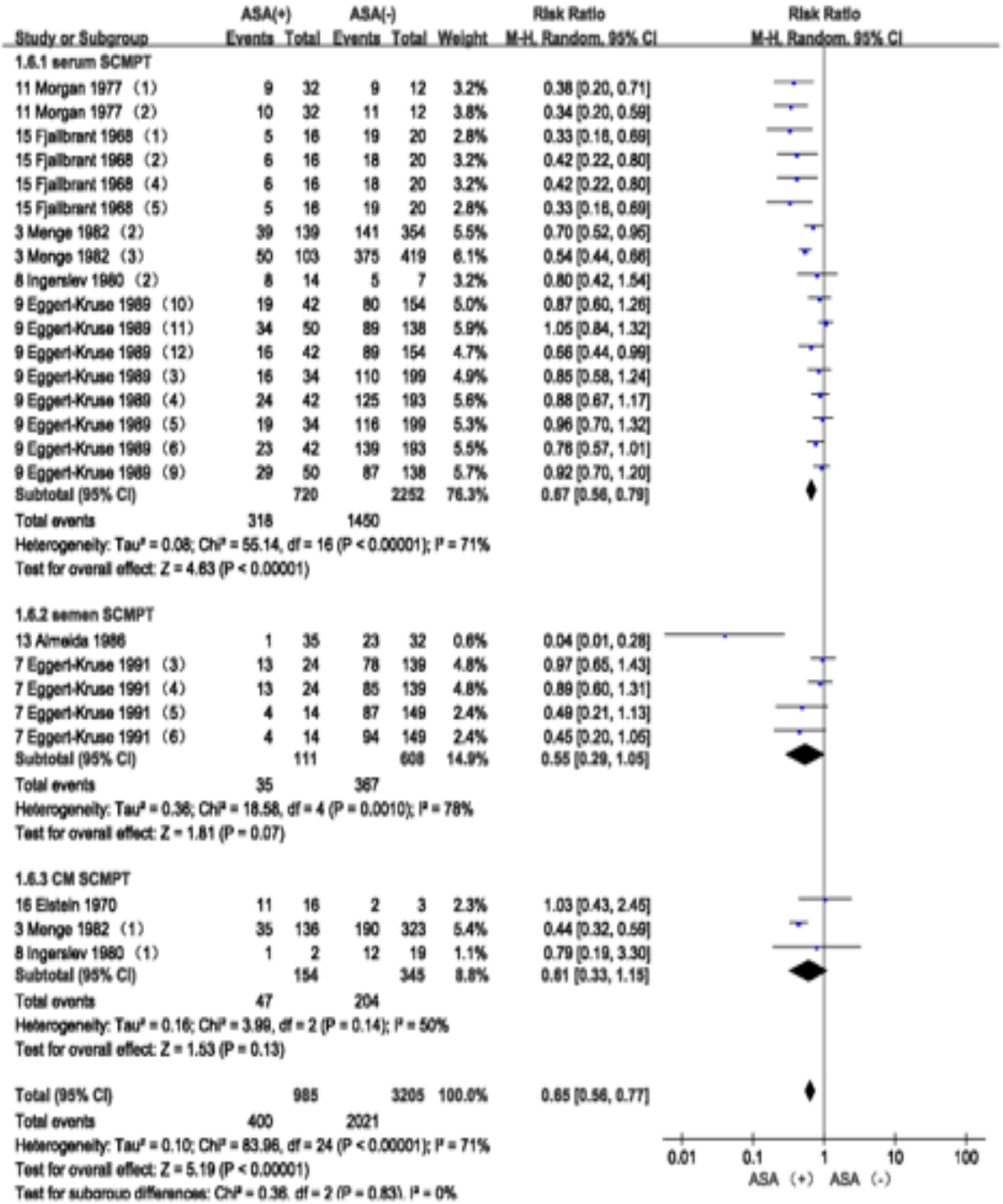
Figure 6. Results of the source of ASA samples subgroup analysis for the SCMPT positive rate



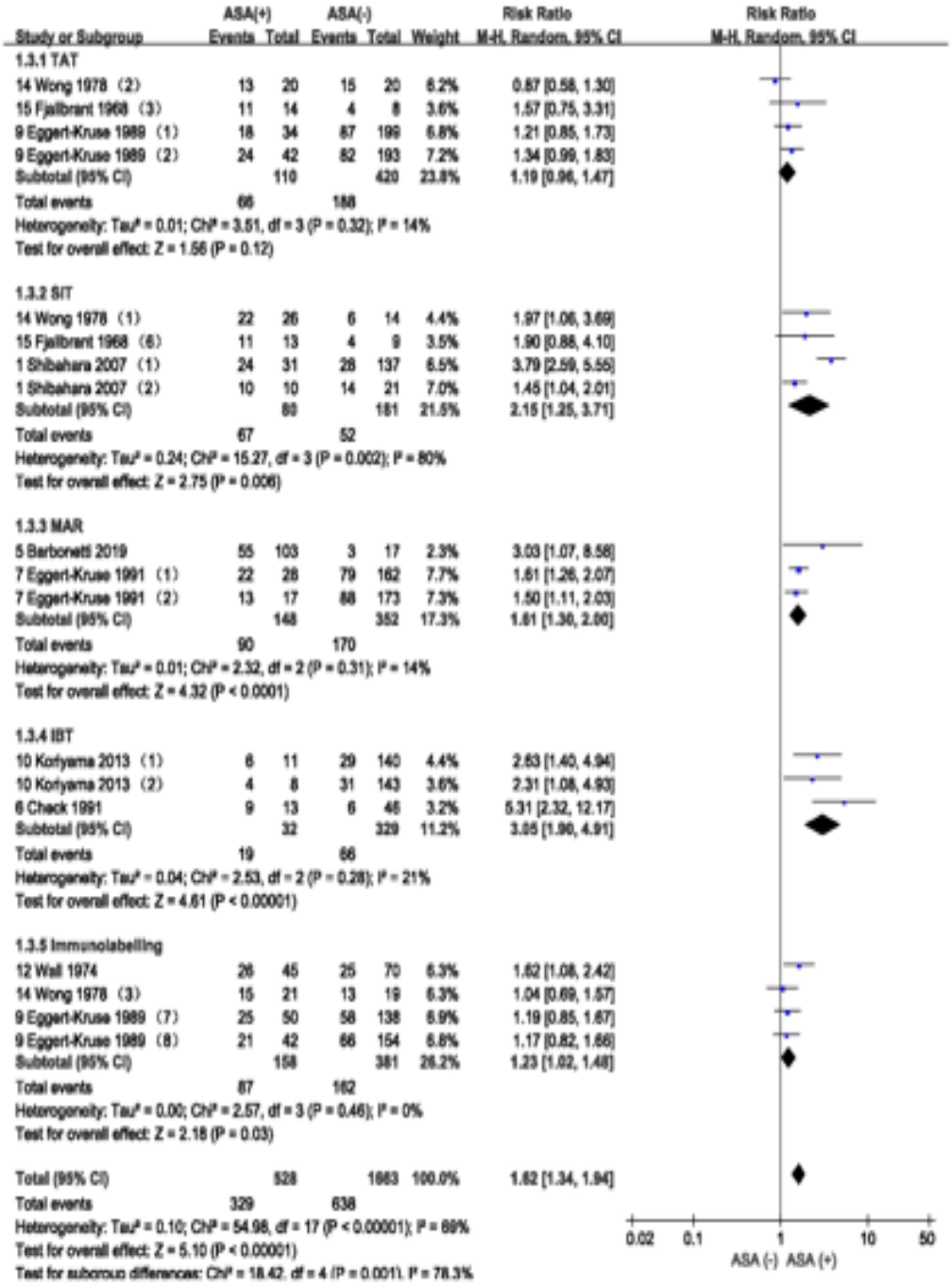


Figure 7. Results of the ASA detection methods subgroup analysis for the PCT negative rate

3.5 Publication bias

The funnel plot showed that the graph is symmetrical, which indicated that there was no publication bias in our meta-analysis (Figure 9 A-B). The quality scores of included cohort studies ranged from 6 to 9.

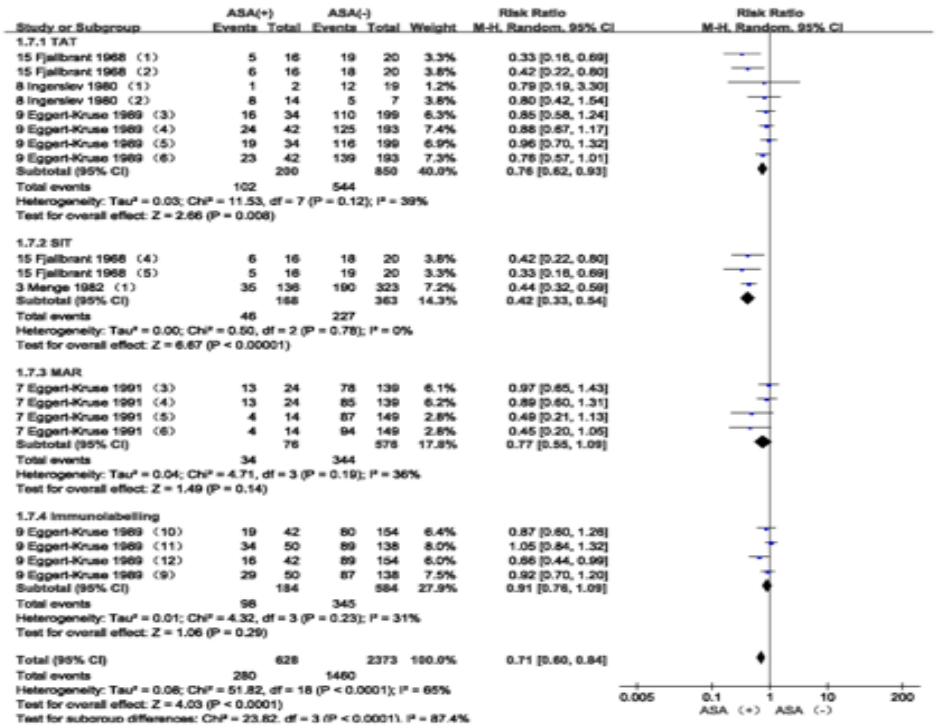
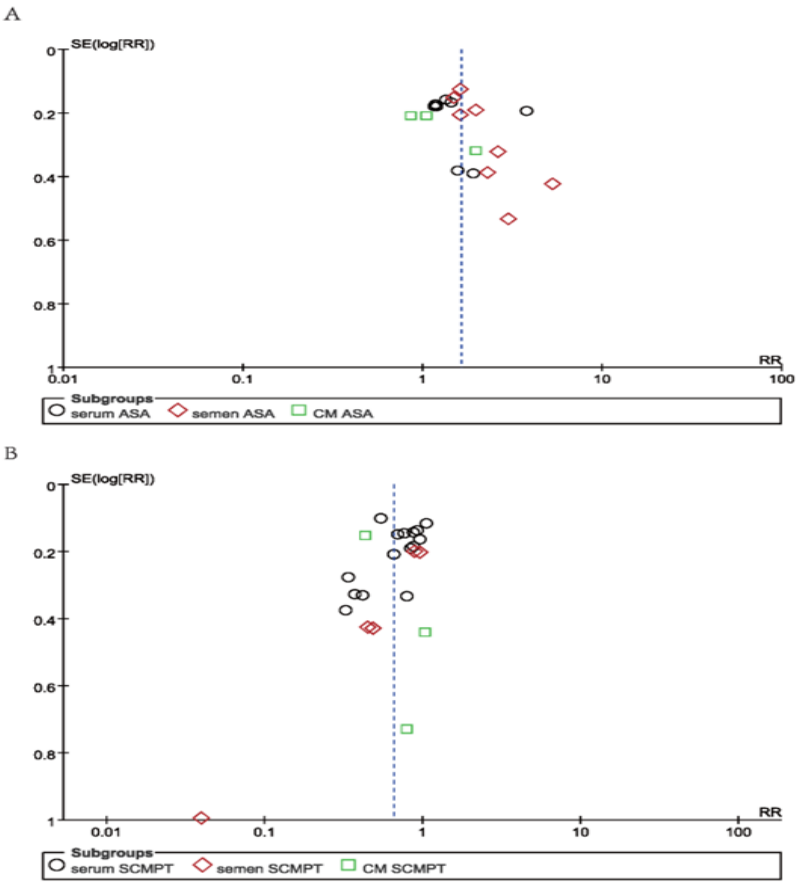


Figure 8. Results of the ASA detection methods subgroup analysis for the SCMPT positive rate

Figure 9. Funnel plot of the controlled studies



(Table 2). The only case-control study scored 7 points (Supplementary Table 1). Analysis of the methodological quality of the studies performed using NOS indicated moderate to high quality, which is expected in observational studies. All studies received ethical approval, and there was no conflict of interest between the authors.

Table 2: The quality of included cohort studies performed using NOS

Study	Selection				Comparability of cohorts on the basis of the design or analysis	Outcome			Scores
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study		Assessment of outcome	Was follow up long enough for outcomes to occur	Adequate of follow up of cohorts	
Shibahara et al.	★	★	★	★	★★	★	★	★	9
Menge et al.	★	★	★		★★	★	★	★	8
Menge et al.	★	★	★	★	★★	★	★	★	9
Gilbert et al.	★	★	★	★	★★	★	★	★	9
Check et al.	★	★	★	★	★	★	★	★	8
Eggert-Kruse et al.	★	★	★		★★	★	★	★	8
Ingerslev et al.	★	★	★	★	★★	★	★	★	9
Eggert-Kruse et al.	★	★	★	★	★★	★	★	★	9
Koriyama et al.	★	★	★	★	★	★	★	★	8
Morgan et al.	★	★	★	★	★★	★	★	★	9
Wall et al.	★	★	★		★	★	★	★	7
Almeida et al.	★	★	★	★	★	★	★	★	8
Wong	★	★	★	★		★	★	★	7
Fjallbrant et al.	★	★	★	★	★★	★	★	★	9
Elstein et al.	★	★	★			★	★	★	6

4 Discussion

This systematic review is the first to assess sperm penetration in ASA-positive couples using PCT and SCMPT as outcome variables. Although ASA antibodies and their effects on infertility are not novel, most of the available studies have conflicting results, and methods to assess sperm penetration are not uniform, prompting us to conduct this systematic review. The results of the meta-analysis confirmed that ASA positivity was associated with a decrease in sperm penetration rate, both in vivo and in vitro.

Our findings are of great value and can provide new clinical ideas for professionals dealing with reproductive health. There are several theories on the mechanisms driving the decline in cervical mucus permeability, some of which we list here. Antibodies directed against sperm components have shown to exert detrimental effects on different pre- and post-fertilization events[34]. Anti-sperm antibodies can affect sperm concentration, liquefaction, transport, sperm motility and viability, gamete

interaction and also early embryonic development, implantation and fetal development[35-38]. Moreover, ASA may alter sperm plasma membrane functional integrity, sperm capacitation, sperm binding and penetration of the zona pellucida (ZP)[39-42]. Other ASA may act as opsonins, facilitating the recognition and destruction of sperm by phagocytes or may evoke the complement cascade that leads to sperm lysis [43]. We focused primarily on the reduced capacity of sperm to penetrate cervical mucus in individuals who are ASA positive in the body, which may result in a reduction in the rate of conception. Sperm agglutination or fixation in cervical mucus may result from ASA in female cervical mucus [44]. The ASA will bond to the sperm in the male body, giving rise to what appears to be a normal sperm count, but the sperm may not function as intended. Moreover, the combination sticks to the protein network of the cervical mucus, making it challenging to pass through the cervix's mucus [33]. There have also been studies that found more ASA in men with decreased sperm forward motility [45], which may affect sperm passing through cervical mucus [32]. We think that ASA-positive patients may have compromised fertility due to reduced ability to pass cervical mucus.

We used subgroup analysis and sensitivity analysis to explore the source of heterogeneity, different detection methods of ASA may be one source of heterogeneity, and we noticed that even for the same assay, different cutoff values for antibody titers lead to differences in results, suggesting that more prospective studies may be needed in the future to control for the confounding variables of ASA detection method and titer threshold to further validate the experimental results. The 16 controlled studies we included were performed in different regions, 15 from developed countries and 1 from developing country. The sample sizes of different studies varied greatly, with the most [22] contained 522 samples and the least [13] were only 19 samples. Besides, the heterogeneity between studies may come from factors such as regional and cultural differences, age of participants, and the sample size. This study has some limitations. First, due to the limited number of controlled studies on sperm penetration in ASA-positive patients, this analysis did not include a sufficient number of studies and included studies at earlier times. Second, some studies did not have complete data information. Third, differences in the control group may not be representative of the general population. The last limitation was the high heterogeneity of research. We recommend further research based on the relevant criteria of region, sample size, rigorous statistical analysis and research design. In addition, future studies should consider gender differences in the source of ASA antibodies and differences in detection methods when interpreting the results.

An important feature of this review was the inclusion of articles using PCT and SCMPT as outcome variables. To protect patient privacy, sperm penetration was assessed by observational studies; therefore, we attempted to obtain a variety of relevant case-control, cohort, and cross-sectional studies. The lack of data and diversity of studies requires careful and differentiated examination. Data were carefully examined to minimize risk of bias. Two validated methods were used to assess risk of bias and quality, namely funnel plots and NOS scales. Compared with the control group, the sperm penetration rate was lower in the ASA-positive group. Clinicians working in reproductive health and infertility should be aware of this issue in order to assess and treat patients to improve patient pregnancy rates. Routine ASA antibody testing is recommended for infertile couples. Semen anti-sperm antibodies are not related to pregnancy rates after IVF or ICSI, suggesting that both forms of ART remain viable options for infertile couples with semen ASA[47-48] . For ASA-positive patients, artificial insemination and assisted reproduction can get past the problem of sperm traveling through cervical mucus, allowing more sperm to reach the oocyte and increasing the likelihood of fertilization. Also, since partners who test positive for ASA in male blood and semen are more likely to have the substance in cervical mucus, which is comparable to raising the amount of ASA exposed to sperm, assisted reproductive technology can prevent this negative effect. Therefore, providing couples with ASA screening and suggesting treatment strategies can help improve the success rate of reproductive therapy. In conclusion, both in vivo and in vitro experiments reflected decreased sperm penetration through the cervical mucus in ASA-positive patients, so it is advised that ASA screening for couples be added to the routine exam.

Funding

Shandong Provincial Medical and Health Science and Technology Development Plan(202205030189);Science and Technology Program of Jinan Municipal Health Commission(2022-2-193).

Reference

1. Elstein M , Macdonald R R . The relation of cervical mucus proteins to sperm penetrability[J]. BJOG: An International Journal of Obstetrics & Gynaecology, 1970.
2. WILSON L. Sperm agglutinins in human semen and blood. *Proc Soc Exp Biol Med*. 1954; 85(4): 652-655.
3. Heidenreich A, Bonfig R, Wilbert DM, Strohmaier WL, Engelmann UH. Risk factors for antisperm antibodies in infertile men. *Am J Reprod Immunol*. 1994; 31(2-3): 69-76.
4. Mazumdar S, Levine AS. Antisperm antibodies: etiology, pathogenesis, diagnosis, and treatment. *Fertil Steril*. 1998; 70(5): 799-810.
5. Alexander NJ, Anderson DJ. Vasectomy: consequences of autoimmunity to sperm antigens. *Fertil Steril*. 1979; 32(3): 253-260.
6. Sinisi AA, Di Finizio B, Pasquali D, Scurini C, D'Apuzzo A, Bellastella A. Prevalence of antisperm antibodies by SpermMARtest in subjects undergoing a routine sperm analysis for infertility. *Int J Androl*. 1993; 16(5): 311-314.
7. Bohring C, Krause W. Immune infertility: towards a better understanding of sperm (auto)-immunity. The value of proteomic analysis. Hum Reprod. 2003; 18(5): 915-924.
8. Meaker S: Some aspects of the problem of sterility. Boston Med Surg J 1922; 187: 535–539.
9. Anderson DJ, Hill JA. Cell-mediated immunity in infertility. Am J Reprod Immunol Microbiol. 1988; 17(1): 22-30.
10. Menge AC, Medley NE, Mangione CM, Dietrich JW. The incidence

and influence of antisperm antibodies in infertile human couples on sperm-cervical mucus interactions and subsequent fertility. *Fertil Steril*. 1982; 38(4): 439-446.

11. Putowski L, Darmochwal-Kolarz D, Rolinski J, Oleszczuk J, Jakowicki J. The immunological profile of infertile women after repeated IVF failure (preliminary study). *Eur J Obstet Gynecol Reprod Biol*. 2004; 112(2): 192-196.
12. Zini A, Phillips S, Lefebvre J, et al. Anti-sperm antibodies are not associated with sperm DNA damage: a prospective study of infertile men. *J Reprod Immunol*. 2010; 85(2): 205-208.
13. Elstein M, Macdonald RR. The relation of cervical mucus proteins to sperm penetrability. J Obstet Gynaecol Br Commonw. 1970; 77(12): 1123-1126.
14. Menge AC, Black CS. Effects of antisera on human sperm penetration of zona-free hamster ova. *Fertil Steril*. 1979; 32(2): 214-221.
15. Eggert-Kruse W, Neuer A, Clussmann C, et al. Seminal antibodies to human 60kd heat shock protein (HSP 60) in male partners of subfertile couples. *Hum Reprod*. 2002; 17(3): 726-735.
16. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *J Clin Epidemiol*. 2009; 62(10): e1-e34.
17. Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality if nonrandomized studies in meta-analyses. URL: http://www.ohri.ca/programs/clinical_epidemiology/oxford.htm
18. Elia N, von Elm E, Chatagner A, et al. How do authors of systematic reviews deal with research malpractice and misconduct in original studies? A cross-sectional analysis of systematic reviews and survey of their authors. BMJ open 2016; 6: e010442.
19. Shibahara H, Shiraishi Y, Hirano Y, Kasumi H, Koyama K, Suzuki M. Relationship between level of serum sperm immobilizing antibody and its inhibitory effect on sperm migration through cervical mucus in immunologically infertile women. *Am J Reprod Immunol*. 2007; 57(2): 142-146.
20. Koriyama J, Shibahara H, Ikeda T, Hirano Y, Suzuki T, Suzuki M. Toward standardization of the cut-off value for the direct immunobead test using the postcoital test in immunologically infertile males. *Reprod Med Biol*. 2012; 12(1): 21-25.
21. Menge AC, Beitner O. Interrelationships among semen characteristics, antisperm antibodies, and cervical mucus penetration assays in infertile human couples. *Fertil Steril*. 1989; 51(3): 486-492.
22. Menge AC, Medley NE, Mangione CM, Dietrich JW. The incidence and influence of antisperm antibodies in infertile human couples on sperm-cervical mucus interactions and subsequent fertility. *Fertil Steril*. 1982; 38(4): 439-446.
23. Haas GG Jr. The inhibitory effect of sperm-associated immunoglobulins on cervical mucus penetration [published correction appears in Fertil Steril 1986 Dec; 46(6): 1172]. *Fertil Steril*. 1986; 46(2): 334-337.
24. Check JH, Bollendorf A. Effect of antisperm antibodies on postcoital results and effect of intrauterine insemination on pregnancy outcome. *Arch Androl*. 1992; 28(1): 25-31.
25. Ingerslev HJ. Spermagglutinating antibodies and sperm penetration of cervical mucus from infertile women with spermagglutinating antibodies in serum. *Fertil Steril*. 1980; 34(6): 561-568.
26. Barbonetti A, Castellini C, D'Andrea S, et al. Prevalence of anti-sperm antibodies and relationship of degree of sperm auto-immunization to semen parameters and post-coital test outcome: a retrospective analysis of over 10 000 men. *Hum Reprod*. 2019; 34(5): 834-841.
27. Eggert-Kruse W, Hofsäss A, Haury E, Tilgen W, Gerhard I, Runnebaum B. Relationship between local anti-sperm antibodies and sperm-mucus interaction in vitro and in vivo. *Hum Reprod*. 1991; 6(2): 267-276.
28. Eggert-Kruse W, Christmann M, Gerhard I, Pohl S, Klinga K, Runnebaum B. Circulating antisperm antibodies and fertility prognosis: a prospective study. *Hum Reprod*. 1989; 4(5): 513-520.
29. Morgan H, Stedronska J, Hendry WF, Chamberlain GF, Dewhurst CJ. Sperm/cervical-mucus crossed hostility testing and antisperm antibodies in the husband. *Lancet*. 1977; 1(8024): 1228-1230.
30. Wall JR, Harrison RF, Stedronska J, Lessof MH. Antibodies against spermatozoa in infertile women with poorly invading spermatozoa on postcoital tests. *Am J Obstet Gynecol*. 1975; 121(2): 198-201.
31. Elstein M, Macdonald RR. The relation of cervical mucus proteins to sperm penetrability. *J Obstet Gynaecol Br Commonw*. 1970; 77(12): 1123-1126.
32. De Almeida M, Soumah A, Jouannet P. Incidence of sperm-associated immunoglobulins in infertile men with suspected autoimmunity to sperm. *Int J Androl*. 1986; 9(5): 321-330.
33. Wong WP. Sperm antibodies in the cervical mucus and sera and postcoital tests in infertile women. *Eur J Obstet Gynecol Reprod Biol*. 1978; 8(6): 363-367.
34. Vazquez-Levin MH, Marín-Briggiler CI, Veaute C. Antisperm antibodies: invaluable tools toward the identification of sperm proteins involved in fertilization. *Am J Reprod Immunol*. 2014; 72(2): 206-218.
35. Bronson RA. Antisperm antibodies: a critical evaluation and clinical guidelines. *J Reprod Immunol*. 1999; 45(2): 159-183.
36. Ohl DA, Naz RK. Infertility due to antisperm antibodies. *Urology*. 1995; 46(4): 591-602.
37. Chiu WW, Chamley LW. Clinical associations and mechanisms of action of antisperm antibodies. *Fertil Steril*. 2004; 82(3): 529-535.
38. Cui D, Han G, Shang Y, et al. Antisperm antibodies in infertile men and their effect on semen parameters: a systematic review and meta-analysis. *Clin Chim Acta*. 2015; 444: 29-36.
39. Rossato M, Galeazzi C, Ferigo M, Foresta C. Antisperm antibodies modify plasma membrane functional integrity and inhibit osmosensitive calcium influx in human sperm. *Hum Reprod*. 2004; 19(8): 1816-1820.
40. Shibahara H, Shigeta M, Inoue M, et al. Diversity of the blocking effects of antisperm antibodies on fertilization in human and mouse. *Hum Reprod*. 1996; 11(12): 2595-2599.
41. Nakagawa K, Yamano S, Kamada M, et al. Sperm-immobilizing

antibodies suppress an increase in the plasma membrane fluidity of human spermatozoa. *Fertil Steril*. 2004; 82 Suppl 3: 1054-1058.

42. Mahony MC, Blackmore PF, Bronson RA, Alexander NJ. Inhibition of human sperm-zona pellucida tight binding in the presence of antisperm antibody positive polyclonal patient sera. *J Reprod Immunol*. 1991; 19(3): 287-301.
43. Bronson RA, Cooper GW, Rosenfeld DL. Correlation between regional specificity of antisperm antibodies to the spermatozoan surface and complement-mediated sperm immobilization. *Am J Reprod Immunol (1980)*. 1982; 2(4): 222-224.
44. Franklin RR, Dukes CD. Antispermatozoal antibody and unexplained infertility. *Am J Obstet Gynecol*. 1964; 89: 6-9.
45. MacLeod J, Wang Y. Male fertility potential in terms of semen quality: a review of the past, a study of the present. *Fertil Steril*. 1979; 31(2): 103-116.
46. Kremer J, Jager S. The significance of antisperm antibodies for sperm-cervical mucus interaction. *Hum Reprod*. 1992; 7(6): 781-784.
47. Zini A, Fahmy N, Belzile E, Ciampi A, Al-Hathal N, Kotb A. Antisperm antibodies are not associated with pregnancy rates after IVF and ICSI: systematic review and meta-analysis. *Hum Reprod*. 2011; 26(6): 1288-1295.
48. Zini A, Lefebvre J, Kornitzer G, et al. Anti-sperm antibody levels are not related to fertilization or pregnancy rates after IVF or IVF/ICSI. *J Reprod Immunol*. 2011; 88(1): 80-84.