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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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.

Annals of Clinical and Medical Case Reports

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 'spermmucus 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 PCTnegative 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 $100 \times$). 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 X² 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 I² test, which was considered to be a significant difference when P <0.05. Low, medium, and high degrees of heterogeneity were clarified by I² 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[13,21-22,25,28] 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

N	Auth	Cou	Study	Quality	Conflict	Ethicscom	Caura	ASA	ASA	ASA sam	ASA sam	Sampl size		PCT rate	negative	р	SCMP semi-qua titative a		P	SCMPT j	positive	Р
0.	or (Year)	ntry	design	score	interest	approval	Group	method	cutoff	ple sou rce	plegen der	AS A (+)	AS A (-)	ASA (+)	ASA (-)	r	ASA (+)	ASA (-)	r	ASA (+)	ASA (-)	r
,	Shib ahara et	Jap	Cohort	9	No	v	ASA(+) vs ASA (-)	SIT	2	serum	fem ale	31	137	24(7 7.4 %)	28(2 0.4 %)	<0. 00 01						
I	al. (2007)	an	Conort	9	NO	Yes	>SI50 vs <si50< td=""><td>SIT</td><td>50</td><td>serum</td><td>female</td><td>10</td><td>21</td><td>10(1 00 %)</td><td>14(6 6.7 %)</td><td>0.0 4</td><td></td><td></td><td></td><td></td><td></td><td></td></si50<>	SIT	50	serum	female	10	21	10(1 00 %)	14(6 6.7 %)	0.0 4						
2	Menge	Am eri	Cohort	8	No	Yes	ASA(+) vs ASA (-) ASA(+)	TAT	16	serum	male/ female	142	707				2.4± 0.51	3.5±				
2	et al. (1989)	can	Conort	0	INO	ies	ASA(+) vs ASA (-)	SIT	4	serum + CM	male/ female	63	786				2.1± 0.51	3.4± 0.03				

																						I
							ASA(+)													35(2	190	
							vs ASA	SIT	4	СМ	female	136	323							5.7	(58.	
	Menge	Am					(-) ASA(+)													%) 39	8%) 141	
3	et al.	eri	Cohort	9	No	Yes	vs ASA	SIT/TAT	4/16	serum	female	139	354							(28.	(55.	
5	(1982)	can	Conort		110	105		SIL		Jorum	Tennane	137	551									
	(1702)	can					(-) ASA(+)													1%) 50	<u>6%)</u> 375	
							vs ASA	SIT/TAT	4/16	serum	male	103	419							(48.	(89.	
	Gilbert	Am					(-) ASA(+)							20(9	17(4					5%)	5%)	
4	et al.	eri	Cohort	9	No	Yes	vs ASA	-	-	semen	male	22	37	0.9	5.9	0.0						
	(1986) Barbon	can					(-)							%)	%)	004						
			Cas				ASA(+)															
5	etti	Ita	eco	7	No	Yes	vs ASA	MAR	50%	semen	male	103	17	553.	3(17							
	et al.	ly	ntrol				(-)							4%)	%)							
	(2019) Chečk	Am					ASA(+)							9(6								
6	et al.	eri	Cohort	8	No	Yes	vs ASA	IBT	50%	semen	male	13	46	9.	6(13.							
	(1991)	can					(-) ASA(+)							2%) 22(7	0%)							
								2445	2007			20	1/2		79(4							
							vs ASA	MAR	30%	semen	male	28	162	8.6	8.8							
							(-) ASA(+)							%) 13(7	%) 88(5							
							vs ASA	MAR	30%	semen	male	17	173	6.5	0.9							
							(-) ASA(+)							%)	%)		1.67	1.71			78(5	
	Eggert						vs ASA	MAR	30%	semen	male	24	139				±	±	0.	13(5	6.1	
	-Kruse	Ger							5070	Semen	maie	2.	,				0.92		81	4.2%)		
7	et al.	man	Cohort	8	No	Yes	(-) ASA(+)										1.63	0.96 1.81	0.	13(54.	%) 85(6	
	(1991)						vs ASA	MAR	30%	semen	male	24	139				±	±	36	2%)	1.2	
							(-) ASA(+)										0.88 1.14	0.92 1.76		4(%) 87	
							vs ASA	MAR	30%	semen	male	14	149				±	±	0.	28.	(58.	
							(-) ASA										0.86	0.94 1.84	02	<u>6%)</u> 4(<u>4%)</u> 94	
																			5.5			
							(+) vs	MAR	30%	semen	male	14	149				±0.	±0.	e-3	28.	(63.	
							SA(-) ASA(+)										66	91		6%) 1(50	1%) 12(6	
	Ingers	Am					vs ASA	TAT	116	СМ	female	2	19								3.2	
8	lev	eri	Cohort	9	No	Yes	(-) ASA(+)													%)	%)	
	et al.	can					vs ASA	TAT	116	serum	female	14	7							8(57.	5(71	
	(1980)								110	Serum	Tennare	14	,							1%)	.4%)	
						1	L (-)	1														_

														102-	0.77		 	 		I
							ASA(+)	TAT	10			24	100	18(5	87(4					
							vs ASA	TAT	18	serum	male	34	199	2.9	3.7					
							(-) ASA(+)							%) 24(5	%) 82(4					
							vs ASA	TAT	18	serum	female	42	193	7.1	2.5					
							(-) ASA(+)							%)	%)				110(
							vs ASA	TAT	18	serum	male	34	199					16(4	55.	
																		7.1%)		
							(-) ASA(+)											24(5	<u>3%)</u> 125(
							vs AS	TAT	18	serum	female	42	193					7.1%)	64.	
							A(-) ASA(+)												8%) 116	
							vs ASA	TAT	18	serum	male	34	199					19(5	(58.	
							(-) ASA(+)											5.9%)	3%) 139	
	Eggert-							TAT	10		C 1	12	102					23(5		<0.
	Kruse	Ger					vs ASA	TAT	18	serum	female	42	193					4.8%)	(72.	05
9	et al.	man	Cohort	9	No	Yes	(-) ASA(+)	Immuno						25(5	58(4				0%)	
	(1989)						vs ASA		15%	serum	male	50	138	0.0	2.0					
	()						(-) ASA(+)	labelling						%) 21(5	%) 66(4					
							v s	Immuno	15%	serum	female	42	154	0.0	2.9					
								labelling	1570	serum	Temate	42	134	%)	%)					
							ASA(-) ASA(+)	Immuno						70)	70)			29(5	87(6	
							vs ASA	labelling	15%	serum	male	50	138					8.0%)	3.0	
							(-) ASA(+)												%) 80(5	
							vs ASA	Immuno	15%	serum	female	42	154					19(4	1.9	
								labelling										5.2%)		
							(-) ASA(+)	Immuno										34(6	%) 89(6	
							vs ASA	labelling	15%	serum	male	50	138					8.0%)	4.5	
							(-) ASA(+)												%) 89(5	
							vs ASA	Immuno	15%	serum	female	42	154					16(3	7.8	<0.
							(-)	labelling										8.1%)	%)	05
							ASA(+)							6(54	29(2					
	Koriya						vs ASA	IBT	20%	semen	male	11	140	.5%)	0.7	0.020				
10	ma etal.	Jap	Cohort	8	No	Yes	(-)							.576)	%)					
	(2013)	an					ASA(+)							4(50	31(2					
							vs ASA	IBT	50%	semen	male	8	143	.0%)	1.7	0.084				
_							(-) ASA(+)								%)			9(28	9(75	
	Morgan						vs ASA	-	-	serum	male	32	12							
11	et al.	Eng	Cohort	9	No	Yes	(-) ASA(+)											.1%)	.0%) 11(9	
	(1977)	land					vs ASA	_	-	serum	male	32	12					1.2	1.7	
								-		serum	maie	52	12					%)	%)	
	Wall	Eng					(-) ASA(+)	Immuno						26(5	25(3			70)	20)	
12	et al.	land	Cohort	7	No	Yes	vs ASA	labelling	-	semen	male	45	70	7.8	5.7					
	(1974) Almei	and					(-) ASA(+)	moening						%)	%)				23(7	
13	da et al.	Fra	Cohort	8	No	Yes	vs ASA	IBT	60%	semen	male	35	32					1(2.	1.9	
	(1986)	nce																9%)	%)	
	(1700)						(-) ASA(+)							22(8	6(42					
							vs ASA	SIT	-	СМ	female	26	14	4.6	.9%)					
		Mal					(-) ASA(+)							%) 13(6	15(7					
14	Wong	ay	Cohort	7	No	Yes	vs ASA	TAT	-	СМ	female	20	20	5.0	5.0					
	(1978)	sia					(-) ASA(+)							%) 15(7	%) 13(6					
								Immuno												
							vs ASA	labelling	-	CM	female	21	19	1.4	8.4					
							(-)	_						%)	%)					

							ASA(+) vs ASA (-) ASA(+)	TAT	32-64	serum	male	16	20			0.38 ±0.62	1.35 ± 0.59	1.4 e-4	5(31 .3%)	19(9 5.0 <u>%)</u> 18(9
							ASA(+) vs ASA (-) ASA(+)	TAT	32-64	serum	male	16	20	11/7		 	1.60		6(37 .5%)	0.0
15	Fjallb rantet	Swe	Cohort	9	No	Yes	ASA(+) vs ASA (-) ASA(+)	TAT	32-64	serum	male	14	8	11(7 8.6 %)	4(50 .0%)	0.50± 0.73	1.60 ± 0.68	2.3 e-4		19/0
	al. (1968)	den					ASA(+) vs ASA (-) ASA(+)	SIT	6h	serum	male	16	20				1.25		6(37 .5%)	18(9 0.0 <u>%)</u> 19(9
							ASA(+) vs ASA (-) ASA(+)	SIT	6h	serum	male	16	20			0.50 ±0.73	1.25 ± 0.64	3.3 e-3	5(31 .3%)	5.0 %)
							ASA(+) vs ASA (-)	SIT	6h	serum	male	13	9	11(8 4.6 %)	4(44 .4%)					
16	Elstein et al. (1970)	Eng la nd	Cohort	6	No	Yes	ASA(+) vs ASA (-)	-	-	СМ	female	16	3			0.38 ±0.62	1.70 ± 0.57	9.3 e-6	11(6 8.8 %)	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

5.3 Meta-analysis

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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 (I² = 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) (I² = 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.

Annals of Clinical and Medical Case Reports

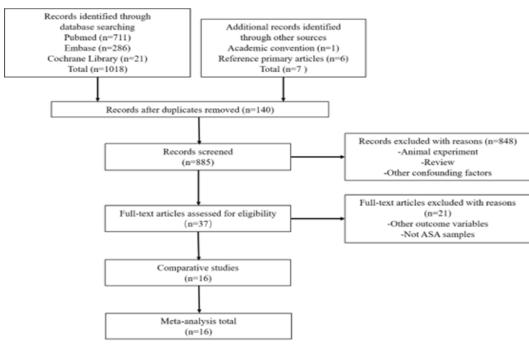


Figure 1. Flow diagram

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

	ASA(ASA(Risk Ratio	Risk Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H. Random, 95% Cl	M-H, Random, 95% Cl
1.1.1 male ASA							
10 Koriyama 2013 (1)	6	11	29	140	4.1%	2.63 [1.40, 4.94]	
10 Koriyama 2013 (2)	4	8	31	143	3.3%	2.31 [1.08, 4.93]	
12 Wall 1974	26	45	25	70	5.9%	1.62 [1.08, 2.42]	
15 Fjallbrant 1968 (3)	11	14	4	8	3.4%	1.57 [0.75, 3.31]	
15 Fjallbrant 1968 (6)	11	13	4	9	3.3%	1.90 [0.88, 4.10]	
4 Gilbert 1986	20	22	17	37	6.2%	1.98 [1.36, 2.87]	
5 Barbonetti 2019	55	103	3	17	2.1%	3.03 [1.07, 8.58]	
6 Check 1991	9	13	6	48	2.9%	5.31 [2.32, 12.17]	
Eggert-Kruse 1991 (1)	22	28	79	162	7.3%	1.61 [1.26, 2.07]	-
7 Eggert-Kruse 1991 (2)	13	17	88	173	6.9%	1.50 [1.11, 2.03]	
Eggert-Kruse 1989 (1)	18	34	87	199	6.4%	1.21 [0.85, 1.73]	+
Eggert-Kruse 1989 (7)	25	50	58	138	6.5%	1.19 [0.85, 1.67]	
Subtotal (95% CI)		358		1142	58.3%	1.71 [1.43, 2.04]	•
Total events	220		431				
1.1.2 female ASA							1.0.0
14 Wong 1978 (1)	22	26	6	14	4.1%	1.97 [1.06, 3.69]	
14 Wong 1978 (1)	22 13	26 20	6 15	14 20	4.1%	1.97 [1.05, 3.69] 0.87 [0.58, 1.30]	
14 Wong 1978 (1) 14 Wong 1978 (2)							- <u>+</u>
14 Wong 1978 (1) 14 Wong 1978 (2) 14 Wong 1978 (3)	13	20	15	20	5.9%	0.87 [0.58, 1.30]	
14 Wong 1978 (1) 14 Wong 1978 (2) 14 Wong 1978 (3) 1 Shibahara 2007 (1)	13 15	20 21 31 10	15 13	20 19	5.9% 5.9%	0.87 [0.58, 1.30] 1.04 [0.69, 1.57]	Ť
14 Wong 1978 (1) 14 Wong 1978 (2) 14 Wong 1978 (3) 1 Shibahara 2007 (1) 1 Shibahara 2007 (2) 9 Eggert-Kruse 1969 (2)	13 15 24 10 24	20 21 31 10 42	15 13 28 14 82	20 19 137 21 193	5.9% 5.9% 6.1% 6.6% 6.8%	0.87 [0.58, 1.30] 1.04 [0.69, 1.57] 3.79 [2.59, 5.55] 1.45 [1.04, 2.01] 1.34 [0.99, 1.83]	
14 Wong 1978 (1) 14 Wong 1978 (2) 14 Wong 1978 (3) 1 Shibahara 2007 (1) 1 Shibahara 2007 (2) 9 Eggert-Kruse 1989 (2) 9 Eggert-Kruse 1989 (8)	13 15 24 10	20 21 31 10 42 42	15 13 28 14	20 19 137 21 193 154	5.9% 5.9% 6.1% 6.8% 6.8% 6.4%	0.87 [0.58, 1.30] 1.04 [0.69, 1.57] 3.79 [2.59, 5.55] 1.45 [1.04, 2.01] 1.34 [0.99, 1.83] 1.17 [0.82, 1.66]	Ť
14 Wong 1978 (1) 14 Wong 1978 (2) 14 Wong 1978 (3) 15 Nibahara 2007 (1) 1 Shibahara 2007 (2) 9 Eggert-Kruse 1989 (2) 9 Eggert-Kruse 1989 (8) Subtetal (95% CI)	13 15 24 10 24 21	20 21 31 10 42	15 13 28 14 82	20 19 137 21 193	5.9% 5.9% 6.1% 6.6% 6.8%	0.87 [0.58, 1.30] 1.04 [0.69, 1.57] 3.79 [2.59, 5.55] 1.45 [1.04, 2.01] 1.34 [0.99, 1.83]	Ť
1.1.2 female ASA 14 Wong 1978 (1) 14 Wong 1978 (2) 14 Wong 1978 (3) 15 Nibahara 2007 (1) 1 Shibahara 2007 (2) 9 Eggert-Kruse 1989 (2) 9 Eggert-Kruse 1989 (8) Subtotal (95% Cf) Total events	13 15 24 10 24 21 129	20 21 31 10 42 42 192	15 13 28 14 82 65 224	20 19 137 21 193 154 558	5.9% 5.9% 6.1% 6.8% 6.8% 6.4% 41.7%	0.87 [0.58, 1.30] 1.04 [0.69, 1.57] 3.79 [2.59, 5.55] 1.45 [1.04, 2.01] 1.34 [0.99, 1.83] 1.17 [0.82, 1.66]	Ť
14 Wong 1978 (1) 14 Wong 1978 (2) 14 Wong 1978 (3) 1 Shibahara 2007 (1) 1 Shibahara 2007 (2) 9 Eggert-Kruse 1989 (2) 9 Eggert-Kruse 1989 (8) Subtotal (95% CI) Total events Heterogeneity: Tau ^a = 0.19;	13 15 24 10 24 21 129 Chi ^p = 35.6	20 21 31 10 42 42 192 4, df =	15 13 28 14 82 65 224	20 19 137 21 193 154 558	5.9% 5.9% 6.1% 6.8% 6.8% 6.4% 41.7%	0.87 [0.58, 1.30] 1.04 [0.69, 1.57] 3.79 [2.59, 5.55] 1.45 [1.04, 2.01] 1.34 [0.99, 1.83] 1.17 [0.82, 1.66]	Ť
14 Wong 1978 (1) 14 Wong 1978 (2) 14 Wong 1978 (3) 15 Nibahara 2007 (1) 1 Shibahara 2007 (2) 9 Eggert-Kruse 1989 (2) 9 Eggert-Kruse 1989 (8) Subtetal (95% CI)	13 15 24 10 24 21 129 Chi ^p = 35.6	20 21 31 10 42 42 192 4, df =	15 13 28 14 82 65 224	20 19 137 21 193 154 558 00001);	5.9% 5.9% 6.1% 6.8% 6.8% 6.4% 41.7%	0.87 [0.58, 1.30] 1.04 [0.69, 1.57] 3.79 [2.59, 5.55] 1.45 [1.04, 2.01] 1.34 [0.99, 1.83] 1.17 [0.82, 1.66]	Ť
14 Wong 1978 (1) 14 Wong 1978 (2) 14 Wong 1978 (2) 14 Wong 1978 (3) 15 Nibahara 2007 (1) 1 Shibahara 2007 (2) 9 Eggert-Kruse 1989 (2) 9 Eggert-Kruse 1989 (2) 9 Eggert-Kruse 1989 (8) Subtetsal (95% Cl) Total events Heterogeneity: Tau ^a = 0.19; Test for overall effect: Z = 2. Total (95% Cl)	13 15 24 10 24 21 129 Chi ^p = 35.6	20 21 31 10 42 192 4, df = 3)	15 13 28 14 82 65 224	20 19 137 21 193 154 558 00001);	5.9% 5.9% 6.1% 6.8% 6.8% 6.4% 41.7%	0.87 [0.58, 1.30] 1.04 [0.69, 1.57] 3.79 [2.59, 5.55] 1.45 [1.04, 2.01] 1.34 [0.99, 1.83] 1.17 [0.82, 1.66] 1.47 [1.03, 2.09]	Ť
14 Wong 1978 (1) 14 Wong 1978 (2) 14 Wong 1978 (2) 14 Wong 1978 (3) 15 Nibahara 2007 (1) 1 Shibahara 2007 (2) 9 Eggent-Kruse 1989 (2) 9 Eggent-Kruse 1989 (8) Subtetal (95% CI) Total events Heterogeneity: Tau ⁴ = 0.19; Test for overall effect: Z = 2.	13 15 24 10 24 21 129 Ch ^p = 35.6 11 (P = 0.0 349	20 21 31 10 42 42 192 4, df = 3) 550	15 13 28 14 82 66 224 6 (P < 0.0	20 19 137 21 193 154 558 00001); 1700	5.9% 5.9% 6.1% 6.8% 6.8% 6.8% 41.7% P = 83%	0.87 [0.58, 1.30] 1.04 [0.68, 1.57] 3.79 [2.59, 5.55] 1.45 [1.04, 2.01] 1.34 [0.99, 1.83] 1.17 [0.82, 1.66] 1.47 [1.03, 2.09]	+
14 Wong 1978 (1) 14 Wong 1978 (2) 14 Wong 1978 (2) 14 Wong 1978 (3) 15 Shibahara 2007 (1) 15 Shibahara 2007 (2) 9 Eggert-Kruse 1989 (2) 9 Eggert-Kruse 1989 (8) Subtotal (95% CI) Total events Feat for overall effect: Z = 2. Fotal (95% CI) Total events	13 15 24 10 24 21 129 Ch ^p = 35.6 11 (P = 0.0 349 Ch ^p = 56.7	20 21 31 10 42 42 192 4, df = 3) 550 8, df =	15 13 28 14 82 66 224 6 (P < 0.0	20 19 137 21 193 154 558 00001); 1700	5.9% 5.9% 6.1% 6.8% 6.8% 6.8% 41.7% P = 83%	0.87 [0.58, 1.30] 1.04 [0.68, 1.57] 3.79 [2.59, 5.55] 1.45 [1.04, 2.01] 1.34 [0.99, 1.83] 1.17 [0.82, 1.66] 1.47 [1.03, 2.09]	Ť

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

ī

	ASA(+)	ASA(-)		Risk Ratio		Risk Ratio	
itudy or Subgroup	Evente	Total	Events	Total	Weight	M-H. Random, 95% Cl		M-H. Random, 95% Cl	
.5.1 male SCMPT									
1 Morgan 1977 (1)	9	32	9	12	3.2%	0.38 [0.20, 0.71]			
1 Morgan 1977 (2)	10	32	11	12	3.8%	0.34 [0.20, 0.59]			
3 Almoida 1986	1	35	23	32	0.6%	0.04 [0.01, 0.28]			
5 Fjallbrant 1968 (1)	5	16	19	20	2.8%	0.33 [0.16, 0.69]			
5 Fjalbrant 1968 (2)	6	16	18	20	3.2%	0.42 [0.22, 0.80]			
5 Fjalbrant 1968 (4)	6	16	18	20	3.2%	0.42 [0.22, 0.80]			
5 Fjalbrant 1968 (5)	5	16	19	20	2.8%	0.33 [0.16, 0.69]			
Menge 1982 (2)	39	139	141	354	5.5%	0.70 [0.52, 0.95]		-	
Menge 1982 (3)	50	103	375	419	6.1%	0.54 [0.44, 0.66]		-	
Eggert-Nruse 1991 (3)	13	24	78	139	4.8%	0.97 [0.65, 1.43]		+	
Eggert-Kruse 1991 (4)	13	24	85	139	4.8%	0.89 [0.60, 1.31]		+	
Eggert-Kruse 1991 (5)	4	14	87	149	2.4%	0.49 [0.21, 1.13]			
Eggert-Kruse 1991 (6)	4	14	94	149	2.4%	0.45 [0.20, 1.05]			
Eggert-Kruse 1989 (11)	34	50	89	138	5.9%	1.05 [0.84, 1.32]		+	
Eggert-Kruse 1989 (3)	16	34	110	199	4.9%	0.85 [0.58, 1.24]		-+	
Eggert-Kruse 1989 (5)	19	34	116	199	5.3%	0.96 [0.70, 1.32]		+	
Eggert-Kruse 1989 (9)	29	50	87	138	5.7%	0.92 [0.70, 1.20]		.+	
ubtotal (95% CI)		649		2159	67.3%	0.61 [0.49, 0.75]		•	
otal events	263		1379						
eterogeneity: Tau ^a = 0.13; C	chi# = 68.05.	df = 16	5 (P < 0.0	0001);	1* = 76%				
est for overall effect: Z = 4.5	4 (P < 0.000	01)							
.5.2 female SCMPT									
6 Elstein 1970	11	16	2	3	2.3%	1.03 [0.43, 2.45]		-	
Menge 1982 (1)	36	136	190	323	5.4%	0.44 [0.32, 0.59]		~	
Ingerslev 1980 (1)	1	2	12	19	1.1%	0.79 [0.19, 3.30]			
Ingenslev 1980 (2)	8	14	5	7	3.2%	0.80 [0.42, 1.54]			
Eggert-Kruse 1989 (10)	19	42	80	154	5.0%	0.87 [0.60, 1.26]		-+	
Eggert-Kruse 1989 (12)	16	42	89	154	4.7%	0.66 [0.44, 0.99]		-	
Eggen-Kruse 1989 (4)	24	42	125	193	5.6%	0.88 [0.67, 1.17]		-+	
Eggert-Kruse 1989 (6)	23	42	139	193	5.5%	0.78 [0.57, 1.01]			
ubtotal (96% CI)		336		1048	32.7%	0.72 [0.58, 0.91]		•	
otal eventa	137		642						
isterogeneity: Tau ^e = 0.05; C est for overall effect: Z = 2.7			(P = 0.03	l); I# = 1	56%				
otal (95% Ci)		985		3205	100.0%	0.65 [0.56, 0.77]		•	
otal events	400		2021						
leterogeneity: Tau ^a = 0.10; 0	hP = 83.96.	df = 2	4 (P < 0.0	00011	P = 71%		0.01	0.1 1 10	1

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

		(+)A8		,	(SA(-)			Std. Mean Difference		Std. Mean Ofference	
Study or Subgroup	Mean	50	Total	Mean	50	Total	Weight.	IV, Random, 95% Ci		IV. Random, 95% Cl	
15 Fjalbrant 1968 (1)	0.38	0.62	18	1.35	0.59	20	10.0%	-1.87 [-2.33, -0.81]			
15 Fjellbrent 1968 (3)	0.5	0.73	14	1.6	0.88		9.9%	-1.48 [-2.48, -0.49]			
15 Fjølbrant 1968 (5)	0.5	6,73	16	1.25	0.64	- 20	10.0%	-1.08 [-1.78, -0.37]		-	
16 Elatain 1970	0.36	0.62	16	1.7	0.57	3	9.7%	-2.05 [-3.49, -0.62]			
2 Merge 1989 (1)	2.4	0.51	142	3.5	0.03	707	10.1%	-5.24 [-5.54, -4.93]		•	
2 Marga 1989 (2)	2.1	0.51	63	3.4	0.05	786	10.1%	-9.21 [-9.72, -8.70]			
7 Eggert-Kruee 1991 (3)	1.67	0.92	24	1.71	0.96	139	10.1%	-0.04 [-0.47, 0.39]		+	
7 Eggert-Kruse 1991 (4)	1.63	0.88	24	1.81	0.82	139	10.1%	-0.20 [-0.63, 0.24]		-	
7 Eggert-Kruse 1991 (5)	1.14	0.86	14	1.76	0.94	149	10.1%	-0.66 [-1.21, -0.11]		~	
7 Eggeri-Kruse 1991 (6)	1.14	0.66	14	1.84	0.91	149	10.1%	-0.78 [-1.34, -0.23]		-	
Total (95% CI)			343			2120	100.0%	-2.24 [-4.28, -0.19]		+	
Heleropeneity: Tau* = 10.78;	Chi? = 1	294.0	6. đ = 1	10-10	0000	1); P = 1	10%		+		÷.
Test for overall effect Z = 2.1									-10	-5 0 5 ASA (+) ASA (-)	10
										How (*) How (r)	

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

	ASA(+		ASA(Risk Ratio	Risk Ratio
Study or Subgroup	Events	Total	Eventa	Total	Weight	M-H. Random, 95% Cl	M-H. Random. 95% Cl
1.2.1 serum ASA							
15 Fjallbrant 1968 (3)	11	14	4	8	3.4%	1.57 [0.75, 3.31]	
15 Fjalbrant 1968 (6)	11	13	4	9	3.3%	1.90 [0.88, 4.10]	
1 Shibahara 2007 (1)	24	31	28	137	6.1%	3.79 [2.59, 5.55]	-
1 Shibahara 2007 (2)	10	10	14	21	6.6%	1.45 [1.04, 2.01]	-
9 Eggert-Kruse 1989 (1)	18	34	87	199	6.4%	1.21 [0.85, 1.73]	+
Eggert-Kruse 1989 (2)	24	42	82	193	6.8%	1.34 [0.99, 1.83]	-
9 Eggert-Kruse 1989 (7)	25	50	58	138	6.5%	1.19 [0.85, 1.67]	-
Eggert-Kruse 1989 (8)	21	42	66	154	6.4%	1.17 [0.82, 1.66]	+-
Subtotal (95% CI)		236		859	45.4%	1.54 [1.16, 2.06]	•
Total events	144		343				
Heterogeneity: Tau* = 0.12; (Chi# = 28.81	. df = 7	7 (P = 0.0	0002); 1	- 76%		
Test for overall effect: Z = 2.0	96 (P = 0.00	33)					
1.2.2 semen ASA							
10 Korlyama 2013 (1)	6	11	29	140	4.1%	2.63 [1.40, 4.94]	
10 Korlyama 2013 (2)	4	8	31	143	3.3%	2.31 [1.08, 4.93]	
12 Wall 1974	26	45	25	70	5.9%	1.62 [1.08, 2.42]	
4 Gilbert 1985	20	22	17	37	6.2%	1.98 [1.36, 2.87]	-
5 Barbonetti 2019	55	103	3	17	2.1%	3.03 [1.07, 8.58]	
8 Check 1991	9	13	6	48	2.9%	5.31 [2.32, 12.17]	
7 Eggert-Kruse 1991 (1)	22	28	79	162	7.3%	1.61 [1.26, 2.07]	-
7 Eggert-Kruse 1991 (2)	13	17	88	173	6.9%	1.50 [1.11, 2.03]	-
Subtotal (95% CI)		247		788	38.8%	1.94 [1.55, 2.42]	•
Total events	155		278				00.50
Heterogeneity: Tau ^a = 0.04; (. et = 1		8): P -	45%		
Test for overall effect: Z = 5.							
1.2.3 CM ASA							
14 Wong 1978 (1)	22	26	6	14	4.1%	1.97 [1.06, 3.69]	
4 Wong 1978 (2)	13	20	15	20	5.9%	0.87 [0.58, 1.30]	-
14 Wong 1978 (3)	15	21	13	19	5.9%	1.04 (0.69, 1.57)	+
Subtotal (95% CI)		67		53	15.8%	1.15 [0.75, 1.77]	•
Total events	50		34				
Heterogeneity: Tau ^a = 0.09; (df = 2		12 P = 1	60%		
Test for overall effect: Z = 0.0							
Total (95% CI)		550		1700	100.0%	1.63 [1.37, 1.95]	•
Total events	349		655				
Heterogeneity: Tau* = 0.09; (de		00001	Y P = 68%		
Test for overall effect: Z = 5.4			10 10 - 00		A 00 %		0.01 0.1 1 10 10
TORY OF OAD BUILDER TO A BUILDE			2 (P = 0				ASA (-) ASA (+)

Annals of Clinical and Medical Case Reports

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

	ASA(+)	ASA(-)		Risk Ratio	Risk Ratio
Study or Subgroup	Events Tota	Events T	otal Weight	M-H, Random, 95% Cl	M-H. Random. 95% Cl
1.6.1 serum SCMPT					
11 Morgan 1977 (1)	9 32	9	12 3.2%	0.38 [0.20, 0.71]	
11 Morgan 1977 (2)	10 32	11	12 3.8%	0.34 [0.20, 0.59]	
15 Fjallbrant 1968 (1)	5 16	19	20 2.8%	0.33 [0.16, 0.69]	
15 Fjallbrant 1968 (2)	6 16	18	20 3.2%	0.42 [0.22, 0.80]	
15 Fjallbrant 1968 (4)	6 16	18	20 3.2%	0.42 [0.22, 0.80]	
15 Fjallbrant 1968 (5)	5 16	19	20 2.8%	0.33 [0.18, 0.69]	
3 Menge 1982 (2)	39 139	141	354 5.5%	0.70 [0.52, 0.95]	-
3 Mongo 1982 (3)	50 103	375	419 6.1%	0.54 [0.44, 0.66]	* I
8 Ingenslev 1980 (2)	8 14	5	7 3.2%	0.80 [0.42, 1.54]	-
9 Eggert-Kruse 1989 (10)	19 42	80	154 5.0%	0.87 [0.60, 1.26]	+
9 Eggert-Kruse 1989 (11)	34 50		138 5.9%	1.05 [0.84, 1.32]	+
9 Eppert-Kruse 1989 (12)	16 42		154 4.7%	0.66 [0.44, 0.99]	-
9 Eggert-Kruse 1989 (3)	16 34		199 4.9%	0.85 [0.58, 1.24]	+
9 Eggert-Kruse 1989 (4)	24 42		193 5.6%	0.88 [0.67, 1.17]	+
9 Eggert-Kruse 1989 (5)	19 34		199 5.3%	0.96 [0.70, 1.32]	+
9 Eggert-Kruse 1989 (6)	23 42		193 5.5%	0.76 [0.57, 1.01]	-
9 Eggen-Kruse 1989 (9)	29 50		138 5.7%		1
Subtotal (95% Cl)	20 00		252 76.3%	0.92 [0.70, 1.20] 0.67 [0.56, 0.79]	•
, , ,	318	1450	202 10.3%	ares farsa' arial	•
Total events Heterogeneity: Tau ^a = 0.08; C			041- II - 74N		
		16 (P < 0.00	001); P = 71%		
Test for overall effect: Z = 4.63	s (P < 0.00001)				
1.6.2 semen SCMPT					
13 Almeida 1986	1 35	23	32 0.6%	0.04 [0.01, 0.28]	
7 Eggert-Kruse 1991 (3)	13 24	78	139 4.8%	0.97 [0.65, 1.43]	+
7 Eggert-Kruse 1991 (4)	13 24	85	139 4.8%	0.89 [0.60, 1.31]	-
7 Eggert-Kruse 1991 (5)	4 14	87	149 2.4%	0.49 [0.21, 1.13]	
7 Eggert-Kruse 1991 (6)	4 14	94	149 2.4%	0.45 [0.20, 1.05]	
Subtotal (95% CI)	111		608 14.9%	0.55 [0.29, 1.05]	•
Total events	35	367			_
Heterogeneity: Tau ² = 0.38; C	h ² = 18.58, df =	4 (P = 0.001)	01: IP = 78%		
Test for overall effect: Z = 1.8					
1.6.3 CM SCMPT					
			3	100 00 40 0 40	
16 Elstein 1970	11 16	_	3 2.3%	1.03 [0.43, 2.45]	-
3 Menge 1982 (1)	35 136		323 5.4%	0.44 [0.32, 0.59]	
B Ingerslev 1980 (1)	1 2		19 1.1%	0.79 [0.19, 3.30]	
Subtotal (95% CI)	154		345 8.8%	0.61 [0.33, 1.15]	
Total events	47	204			
Heterogeneity: Tau ^a = 0.16; C		(P = 0.14); F	= 50%		
Test for overall effect: Z = 1.53	3 (P = 0.13)				
Total (95% CI)	985	3	205 100.0%	0.65 [0.56, 0.77]	•
Total events	400	2021			
leterogeneity: Tau ^a = 0.10; C			001): P = 71%		1. 1. 1 1. 1 1
Test for overall effect: Z = 5.11					0.01 0.1 1 10 100
Fest for subgroup differences:			0-04		ASA (+) ASA (-)

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		484/3		Risk Ratio	Risk	Setter.
Study or Subgroup	ASA(+) Events Tota	ASA(-) I Evente Total	Weight	M-H, Random, 95% C		
1.3.1 TAT	Evenue 100	a synta losa	meight	MHT, Patricotti, 22 % G	M-O, MADA	20, 223 M
14 Wong 1978 (2)	13 2	0 15 20	6.2%	0.87 [0.58, 1.30]	_	-
15 Fjalbrant 1968 (3)	11 1			1.57 [0.75, 3.31]	_	
9 Eggert-Kruse 1989 (1)	18 3			1.21 [0.85, 1.73]	-	-
9 Eggert-Kruse 1989 (2)	24 4			1.34 [0.99, 1.83]		-
Subtotal (95% CI)	11			1.19 [0.96, 1.47]		
Total events	66	188	22.011	run fernet mert		•
Heterogeneity: Tau [#] = 0.01; 0			LAN.			
Test for overall effect: Z = 1.8		3 (r = 0.82), r =	14.35			
rescior overall endoc 2 = 1.0	90 (P = 0.12)					
1.3.2 SIT						
14 Wong 1978 (1)	22 2	6 6 14	4.4%	1.97 [1.06, 3.69]		
15 Fjellbrent 1968 (6)	11 1			1.90 [0.88, 4.10]	+	-
1 Shibahara 2007 (1)	24 3			3.79 [2.59, 5.55]		-
1 Shibahara 2007 (2)	10 1		7.0%	1.45 [1.04, 2.01]		
Subtotal (95% CI)			21.5%	2.15 [1.25, 3.71]		•
Total events	67	52		with frank and d		•
Heterogeneity: Tau ^a = 0.24; 0			- 80%			
Test for overall effect: Z = 2.7		- 3 (P = 0.002); P	- 00%			
Test for Overall effect, 2 2.1	() - 0.000)					
1.3.3 MAR						
5 Barborwiti 2019	55 10	3 3 17	2.3%	3.03 [1.07, 8.58]		
7 Eggert-Kruse 1991 (1)	22 2			1.61 [1.26, 2.07]		*
7 Eggert-Kruse 1991 (2)	13 1			1.50 [1.11, 2.03]		-
Subtotal (95% Ci)	14		17.3%	1.61 [1.30, 2.00]		٠
Total events	90	170				-
Heterogeneity: Tau ² = 0.01; 0			14%			
Test for overall effect: Z = 4.3						
1.3.4 IBT						
10 Koriyama 2013 (1)	6 1	1 29 140	4.4%	2.63 [1.40, 4.94]		_
10 Koriyama 2013 (2)	4	8 31 143	3.6%	2.31 [1.08, 4.93]		
6 Check 1991	9 1	3 6 45	3.2%	5.31 [2.32, 12.17]		-
Subtotal (95% Ci)	3	2 329	11.2%	3.05 [1.90, 4.91]		•
Total events	19	66				
Heterogeneity: Tau ^a = 0.04; 0	ChP = 2.53, df =	2 (P = 0.28); P =	21%			
Test for overall effect: Z = 4.6	\$1 (P < 0.00001)				
1.3.5 Immunolabelling						
12 Wall 1974	26 4	5 25 70	6.3%	1.62 [1.08, 2.42]		
14 Wong 1978 (3)	15 2	1 13 19	6.3%	1.04 [0.69, 1.57]	-	-
9 Eggert-Kruse 1989 (7)	25 5	0 58 138	6.9%	1.19 (0.85, 1.67)	1	-
9 Eggert-Kruse 1989 (8)	21 4			1.17 [0.82, 1.66]	-	-
Subtotal (95% CI)	15	8 381	26.2%	1.23 [1.02, 1.48]	1	•
Total events	87	162				
Heterogeneity: Tau* = 0.00; 0		3 (P = 0.46); P =	0%			
Test for overall effect: Z = 2.1	18 (P = 0.03)					
Total INSK CO.			440.00	4 40 14 04 4 0 17		•
Total (95% Ci)	52		100.0%	1.62 [1.34, 1.94]		•
Total events	329	638				
Heterogeneity: Tau ^a = 0.10; 0			χ P = 697	•	0.02 0.1 1	10 50
Test for overall effect: Z = 5.1					ASA (-)	ASA (+)
Test for subcroup differences	C CAP = 18.42.	ar = 4 (P = 0.001)	P = 78.3	•		

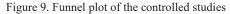
Figure 7. Results of the ASA detection methods subgroup analysis for the PCT negative rate

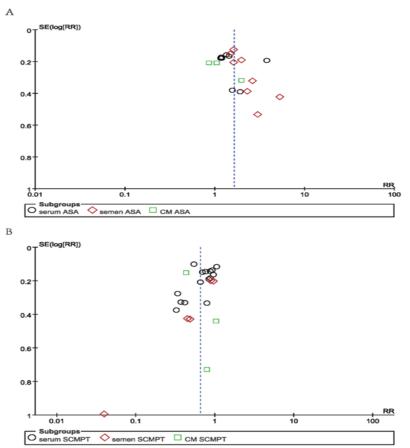
3.5 Publication bias

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.

1.7.1 TAT 15 Fjalbrart 1968 (1) 15 Fjalbrart 1968 (2) 8 Ingenske 1960 (2) 9 Egpert-Kruse 1969 (3) 9 Egpert-Kruse 1969 (3) 9 Egpert-Kruse 1969 (4) 9 Egpert-Kruse 1969 (5) 9 Egpert-Kruse 1969 (6) Subtrati (95% CI)	5 6 1 8 16 24 19 23 102 1.53, 0.008 6 5 36 46 0.000	16 16 14 34 42 200 cf' = 7 (3) 16 18 136 158 tf = 2 (P	19 18 5 110 125 116 139 544 P=0.12 18 19 190 227	20 20 199 193 199 193 850 2); I ^a = : 20 20 323 363	3.3% 3.8% 1.2% 3.8% 6.5% 7.4% 6.9% 40.0% 33% 3.3% 7.2% 14.3%	MHL Random. 93% C 0.33 (p.16, 0.69) 0.42 (p.22, 0.80) 0.79 (p.16, 3.30) 0.86 (p.42, 1.54) 0.86 (p.65, 1.24) 0.86 (p.65, 1.24) 0.86 (p.65, 1.24) 0.86 (p.65, 1.24) 0.86 (p.65, 1.24) 0.86 (p.65, 1.24) 0.96 (p.70, 1.32) 0.76 (p.62, 0.93) 0.76 (p.62, 0.93) 0.42 (p.22, 0.80) 0.33 (p.16, 0.68) 0.44 (p.32, 0.59) 0.42 (p.33, 0.54)	M-	H. Random. 92% Cl	
15 Fjallbrant 1988 (1) 15 Fjallbrant 1988 (2) 8 Ingenslev 1980 (2) 8 Ingenslev 1980 (2) 9 Eggert-Kruse 1980 (3) 9 Eggert-Kruse 1980 (4) 9 Eggert-Kruse 1980 (4) 15 Fjallbrant 1988 (4) 15 Fjallbrant 1988 (4) 15 Fjallbrant 1988 (4) 15 Fjallbrant 1988 (5) 3 Mange 1982 (1) 8 Mange 1985 (1) 17 Eggert-Kruse 1991 (3) 7 Eggert-Kruse 1991 (4)	6 1 8 16 24 19 23 102 1.53, 0.008 6 5 35 46 0.50, 0 0.000	16 2 14 34 42 200 df = 7 (3) 16 16 16 136 168 188 tf = 2 (P	18 12 5 110 125 116 139 544 P=0.12 18 19 190 227	20 19 7 199 193 850 2); I* = : 20 20 323 363	3.8% 1.2% 3.8% 6.3% 7.4% 6.9% 7.3% 40,0% 33% 3.8% 3.8% 3.3% 7.2% 14.3%	0.42 [0.22, 0.80] 0.79 [0.18, 3.30] 0.80 [0.42, 1.54] 0.86 [0.38, 1.24] 0.86 [0.57, 1.01] 0.96 [0.70, 1.32] 0.76 [0.57, 1.01] 0.76 [0.62, 0.93] 0.42 [0.22, 0.80] 0.33 [0.16, 0.89]		•	
15 Fjalboart 1968 (1) 8 Ingensiv 1960 (1) 8 Ingensiv 1980 (2) 9 Egpert-Kruse 1980 (3) 9 Egpert-Kruse 1980 (4) 9 Egpert-Kruse 1980 (4) 9 Egpert-Kruse 1980 (5) 9 Egpert-Kruse 1980 (5) 9 Egpert-Kruse 1980 (6) 15 Fjallbrant 1988 (5) 15 Fjallbrant 1988 (5) 16 Fraiberge 1982 (1) 9 Extended 1985 (2) 17.3 MAR 7 Egpert-Kruse 1991 (3) 7 Egpert-Kruse 1991 (5)	6 1 8 16 24 19 23 102 1.53, 0.008 6 5 35 46 0.50, 0 0.000	16 2 14 34 42 200 df = 7 (3) 16 16 16 136 168 188 tf = 2 (P	18 12 5 110 125 116 139 544 P=0.12 18 19 190 227	20 19 7 199 193 850 2); I* = : 20 20 323 363	3.8% 1.2% 3.8% 6.3% 7.4% 6.9% 7.3% 40,0% 33% 3.8% 3.8% 3.3% 7.2% 14.3%	0.42 [0.22, 0.80] 0.79 [0.18, 3.30] 0.80 [0.42, 1.54] 0.86 [0.38, 1.24] 0.86 [0.57, 1.01] 0.96 [0.70, 1.32] 0.76 [0.57, 1.01] 0.76 [0.62, 0.93] 0.42 [0.22, 0.80] 0.33 [0.16, 0.89]		•	
8 ingensive 1980 (1) 8 ingensive 1980 (2) 9 Eggent-Kruse 1989 (2) 9 Eggent-Kruse 1989 (4) 9 Eggent-Kruse 1989 (4) 9 Eggent-Kruse 1989 (4) 9 Eggent-Kruse 1988 (5) 10 Eggent-Kruse 1988 (5) 10 Eggent-Kruse 1988 (4) 15 Fjallbaret 1988 (4) 15 Fjallbaret 1988 (5) 3 Menge 1982 (1) Subtotal (95% CI) Total eventa Heterogeneity: Tau" = 0.00; Chi" = 0 Total eventa Heterogeneity: Tau" = 0.00; Chi" = 0 Total eventa 1.7.3 MAR F Eggent-Kruse 1991 (3) F Eggent-Kruse 1991 (5)	1 8 16 24 19 23 102 1.53, 0.008 6 5 35 46 0.000	2 14 34 42 290 df = 7 (8) 16 16 16 188 188 tf = 2 (P	12 5 110 125 116 139 544 P=0.12 18 19 190 227	19 7 199 193 850 2); * = ; 20 20 323 363	1.2% 3.8% 6.3% 7.4% 6.9% 40.0% 33% 3.8% 3.3% 7.2% 14.3%	0.79 [0.19, 330] 0.80 [0.42, 1.54] 0.85 [0.58, 1.24] 0.85 [0.58, 1.24] 0.86 [0.71, 137] 0.96 [0.71, 132] 0.76 [0.57, 1.01] 0.76 [0.57, 1.01] 0.76 [0.52, 0.83] 0.42 [0.22, 0.80] 0.33 [0.16, 0.68]		+++++++++++++++++++++++++++++++++++++++	
8 Ingentiev 1980 (2) 9 Egopt-Kouse 1989 (3) 9 Egopt-Kouse 1989 (4) 9 Egopt-Kouse 1989 (4) 9 Egopt-Kouse 1989 (5) 9 Egopt-Kouse 1989 (5) 9 Egopt-Kouse 1989 (5) 15 Fjallbrant 1988 (4) 15 Fjallbrant 1988 (4) 15 Fjallbrant 1988 (5) 16 Marge 1982 (1) 10 Marge 1983 (2) 10 Tobil events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Tobil events 1.7.3 MAR 7 Egopt-Kouse 1991 (3) 7 Egopt-Kouse 1991 (5)	8 16 24 19 23 102 1.53, 0.008 6 5 35 46 0.000	14 34 42 290 df = 7 (3) 16 16 16 16 16 16 16 16 16 16 16 16 16	5 110 125 116 139 544 P = 0.12 18 19 190 227	7 199 193 199 193 850 2); l ^a = ; 20 20 323 363	3.8% 6.3% 7.4% 6.9% 7.3% 40.0% 33% 3.8% 3.8% 3.3% 7.2% 14.3%	0.80 [9.42, 15-6] 0.85 [9.85, 124] 0.85 [9.85, 124] 0.86 [9.67, 1.17] 0.96 [9.70, 132] 0.76 [9.62, 0.93] 0.76 [9.62, 0.93] 0.42 [9.22, 0.80] 0.33 [9.16, 0.89]		•	
Eggent-Kruse 1989 (3) Eggent-Kruse 1989 (4) Eggent-Kruse 1989 (5) Eggent-Kruse 1989 (6) Subtotal (95% CI) Total events Heterogeneity: Tau ² = 0.03; Ch ² = 1 Total events Fighbark 1988 (4) ST Fjalbark 1988 (4) ST Fjalbark 1988 (5) Statust (85% CI) Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Heterogeneity: Tau ⁴ = 0.00; Ch ² = 0 Total events Total events	16 24 19 23 102 1.53, 0.008 6 5 35 46 5,50,60 0.000	34 42 34 42 200 df = 7 { 8} 16 166 136 168 136 168 tf = 2 (P	110 125 116 139 544 P=0.12 18 19 190 227	199 193 199 193 850 193 850 20 20 20 323 363	6.3% 7.4% 6.9% 7.3% 40.0% 38% 3.8% 3.3% 7.2% 14.3%	0.85 [0.58, 1.24] 0.88 [0.87, 1.17] 0.96 [0.57, 1.01] 0.76 [0.52, 0.93] 0.76 [0.62, 0.93] 0.42 [0.22, 0.80] 0.33 [0.18, 0.68] 0.44 [0.32, 0.58]		+++++++++++++++++++++++++++++++++++++++	
b Eggent-Kouse 1989 (4) 9 Eggent-Kouse 1989 (5) 9 Eggent-Kouse 1989 (6) 9 Eggent-Kouse 1989 (6) 9 Eggent-Kouse 1988 (6) 16 Fjallbrant 1988 (4) 15 Fjallbrant 1988 (4) 15 Fjallbrant 1988 (4) 15 Fjallbrant 1988 (5) 16 Mange 1982 (1) 10 Mange 1982 (1) 10 Mange 1982 (1) 10 Mange 1982 (1) 10 Tobil events Heterogeneity: Tau" = 0.00; Chi ^a = 0 Total events Heterogeneity: Tau" = 0.00; Chi ^a = 0 1.7.3 MAR 7 Eggent-Kouse 1991 (3) 7 Eggent-Kouse 1991 (5)	24 19 23 102 1.53, 0.008 6 5 35 46 5,50,6 0.000	42 34 42 290 df = 7 { 3} 16 168 136 168 tf = 2 (P	125 116 139 544 P = 0.12 18 19 190 227	193 199 193 850 2); * = ; 20 20 323 363	7.4% 6.9% 7.3% 40.0% 39% 3.8% 3.8% 3.3% 7.2% 14.3%	0.88 [0.87, 1.17] 0.96 [0.57, 1.01] 0.76 [0.57, 1.01] 0.76 [0.62, 0.93] 0.42 [0.22, 0.80] 0.33 [0.18, 0.68] 0.44 [0.32, 0.58]		+++++++++++++++++++++++++++++++++++++++	
B Eggen+K-use 1988 (5) Biggen+K-use 1988 (6) Subtotal (95% CI) Total events Heterogeneity: Tau ^a = 0.03; Ch ^a = 1 Test for overall effect: Z = 2.66 (P = 1.7.2 SiT 5 Fjallbarnt 1988 (4) 15 Fjallbarnt 1988 (4) 15 Fjallbarnt 1988 (5) Subtotal (95% CI) Total events Heterogeneity: Tau ^a = 0.00; Ch ^a = 0 Test for overall effect Z = 6.67 (P < 1.7.3 MAR F Eggen+K-use 1991 (3) F Eggen+K-use 1991 (5)	19 23 102 1.53, 0.008 6 5 35 46 0.50, 0 0.000	34 42 290 df = 7 { 3} 16 18 136 158 ff = 2 (P	116 139 544 P = 0.12 18 19 190 227	199 193 850 2); I* = ; 20 20 323 363	6.9% 7.3% 40.0% 38% 3.8% 3.3% 7.2% 14.3%	0.96 [0.70, 1.32] 0.76 [0.57, 1.01] 0.76 [0.62, 0.93] 0.42 [0.22, 0.80] 0.33 [0.16, 0.69] 0.44 [0.32, 0.59]		+++++++++++++++++++++++++++++++++++++++	
P Egpen-House 1985 (6) Subtotal (9% C) Total events Heaterogeneity: Tau* = 0.03; Ch* = 1 Fest for overall effect: Z = 2.66 (P = 1.7.2 SIT 15 Fjallbrard 1988 (4) 15 Fjallbrard 1988 (5) 3 Menge 1982 (1) Subtotal (9% C) Total events Heaterogeneity: Tau* = 0.00; Ch* = 0 Total events Heaterogeneity: Tau* = 0.00; Ch* = 0 Total events 1.7.3 MAR 7 Egpen-Kouse 1991 (3) 7 Egpen-Kouse 1991 (5)	23 102 1.53, 0.008 6 5 35 46 0.50, 0 0.000	42 200 df = 7 (3) 16 136 156 ff = 2 (P	139 544 P = 0.12 18 19 190 227	193 850 2); I ^a = : 20 20 323 363	7.3% 40.0% 38% 3.8% 3.3% 7.2% 14.3%	0.76 [0.57, 1.01] 0.76 [0.62, 0.93] 0.42 [0.22, 0.80] 0.33 [0.16, 0.69] 0.44 [0.32, 0.59]		Ŧ	
Bubicital (95% CI) Total events Heterogeneity: Tau* = 0.03; Ch* = 1 Test for overall effect: Z = 2.68 (P = 1.7.2 SIT 5 Fjallbrart 1968 (4) 15 Fjallbrart 1968 (4) 15 Fjallbrart 1968 (5) Subtotal (95% CI) Total events Heterogeneity: Tau* = 0.00; Ch* = 0 Test for overall effect Z = 6.67 (P < 1.7.3 MAR F Eggen+Kruse 1991 (3) F Eggen+Kruse 1991 (4)	102 1.53, 0.008 6 5 35 46 0.50, d 0.000	200 df = 7 (3) 16 18 136 168 tf = 2 (P	544 P = 0.12 18 19 190 227	850 2); I ^a = ; 20 323 363	40.0% 39% 3.8% 3.3% 7.2% 14.3%	0.76 (0.62, 0.93) 0.42 (0.22, 0.80) 0.33 (0.18, 0.69) 0.44 (0.32, 0.59)		• •	
Total events Total events Test for overall effect: Z = 2.66 (P = 1.7.2 SIT 15 Fjallbrant 1968 (4) 15 Fjallbrant 1968 (5) 16 Menge 1982 (1) 16 Menge 1982 (1) 16 Menge 1982 (1) 16 Menge 1985 (2) Total events Heterogeneity: Tau" = 0.00; Chi ^a = 0 Total events 1.7.3 MAR 7 Eggen-Kouse 1991 (3) 7 Eggen-Kouse 1991 (5)	1.53, 0.008 6 5 35 46 0.000	df = 7 (8) 16 18 136 168 ff = 2 (P	P = 0.12 18 19 190 227	20 20 323 363	39% 3.8% 3.3% 7.2% 14.3%	0.42 [0.22, 0.80] 0.33 [0.16, 0.69] 0.44 [0.32, 0.59]			
Heterogeneity: Tau" = 0.03; Chi" = 1 Teat for overall effect: Z = 2.66 (P = 1.7.2 SIT 5 Fjallbrart 1968 (4) 15 Fjallbrart 1968 (5) 3 Merge 1982 (1) Subtotal (95% Cf) Todal events Heterogeneity: Tau" = 0.00; Chi" = 0 Teat for overall effect: Z = 6.67 (P < 1.7.3 MAR 1/2 Ggort-Kruse 1991 (3) 7 Ggort-Kruse 1991 (4)	1.53, 0.008 6 5 35 46 0.000	16 18 136 158 158	P = 0.12 18 19 190 227	20 20 323 363	3.8% 3.3% 7.2% 14.3%	0.33 [0.16, 0.69] 0.44 [0.32, 0.59]		Ŧ	
Test for overall effect: Z = 2.66 (P = 1.7.2 SIT 5 Fjallbaret 1966 (4) 15 Fjallbaret 1968 (5) 3 Mange 1962 (1) Subtotal (95% CI) Total eventa Helerogeneity: Tau" = 0.00; Chi" = 0 Total for overall effect: Z = 6.67 (P < 1.7.3 MAR 7 Eggent-Kruse 1991 (3) 7 Eggent-Kruse 1991 (4)	0.008 6 5 35 46 0.000	16 18 136 158 158	18 19 190 227	20 20 323 363	3.8% 3.3% 7.2% 14.3%	0.33 [0.16, 0.69] 0.44 [0.32, 0.59]		Ŧ	
15 Fjallbant 1968 (4) 15 Fjallbant 1968 (5) Skenge 1822 (1) Subtotal (95% CI) Total events Heterogeneity: Tav* 0.00; Chi* = 0 Test for overall effect: Z = 0.67 (P < 1.7.3 MAR 7 Eggen+Kruse 1991 (3) 7 Eggen+Kruse 1991 (4)	5 35 46 0.000	16 136 168 # = 2 (P	19 190 227	20 323 363	3.3% 7.2% 14.3%	0.33 [0.16, 0.69] 0.44 [0.32, 0.59]		Ŧ	
15 Fjalbuart 1988 (S) 3 Marge 1982 (1) Subtotal (95% CI) Total eventa Itelerogeneity: Tau" = 0.00; Chi ^p = 0 Total for overall effect. Z = 0.67 (P < 1.7.3 MAR 7 Eggent-Kruse 1991 (3) 7 Eggent-Kruse 1991 (4)	5 35 46 0.000	16 136 168 # = 2 (P	19 190 227	20 323 363	3.3% 7.2% 14.3%	0.33 [0.16, 0.69] 0.44 [0.32, 0.59]		•	
3 Marga 1982 (1) Subtotal (95% CI) Total events Heterogeneity: Tau ^a = 0.00; Ch ^a = 0 Test for overall effect: 2 = 0.00; Ch ^a = 0 Test for overall effect: 2 = 0.00; Ch ^a = 0 Test for overall effect: 4 7 Eggen+Kruse 1991 (3) 7 Eggen+Kruse 1991 (5)	35 46 50, d 0.000	136 168 # = 2 (P	190	323 363	7.2%	0.44 [0.32, 0.59]		•	
Bubtolist (195%, CI) Total events Heterogeneity: Tau" = 0.00; Chi" = 0 Total for oversill officit Z = 0.07; Chi" = 0 1.7.3 MAR 7 Eggent-Kruse 1991 (3) 7 Eggent-Kruse 1991 (4)	46 0.50, d 0.000	166 ff = 2 (P	227	363	14.3%			•	
Total events Heterogeneity: Tau* = 0.00; Chi* = 0 Test for overall effect: Z = 6.67 (P < 1.7.3 MAR 7 Eggert-Kruse 1991 (3) 7 Eggert-Kruse 1991 (4) 7 Eggert-Kruse 1991 (5)	0.000	# = 2 (P				una (una, una)		•	
Helerogeneity: Tau* = 0.00; Chi* = 0 Test for overall effect: Z = 6.67 (P < 1.7.3 MAR 7 Eggert-Kruse 1991 (3) 7 Eggert-Kruse 1991 (4) 7 Eggert-Kruse 1991 (5)	0.000			: I* = 0 ⁴	%				
Test for overall effect: Z = 6.67 (P < 1.7.3 MAR 7 Eggent-Kruse 1991 (3) 7 Eggent-Kruse 1991 (4) 7 Eggent-Kruse 1991 (5)	0.000		- 0.70)		~				
7 Eggert-Knuse 1991 (3) 7 Eggert-Knuse 1991 (4) 7 Eggert-Knuse 1991 (5)									
7 Eggert-Knuse 1991 (3) 7 Eggert-Knuse 1991 (4) 7 Eggert-Knuse 1991 (5)									
7 Eggerl-Kruse 1991 (4) 7 Eggerl-Kruse 1991 (5)									
7 Eggert-Kruse 1991 (5)	13	24	78	139	6.1%	0.97 [0.65, 1.43]			
	13	24	85 87	139	6.2%	0.89 [0.60, 1.31]			
CEGDell-Munee 1881 (/6)	1	14	87	149	2.8%	0.49 [0.21, 1.13]			
Subtotal (95% CI)	•	14	94	149	2.8%	0.45 [0.20, 1.05] 0.77 [0.55, 1.09]		•	
Total events	34		344			and farmer road		•	
Heterogeneity: Tau ^a = 0.04; Chi ^a = 4		4f = 3.0P		P = 3	6%				
Test for overall effect: Z = 1.49 (P =			- 0.183						
1.7.4 Immunolabelling									
Eggert-Rruse 1989 (10)	19	42	80	154	6.4%	0.87 [0.60, 1.26]		+	
9 Eggert-Kruse 1989 (11)	34	50	89	138	8.0%	1.05 [0.84, 1.32]		÷	
9 Eggert-Kruse 1989 (12)	16	42	89	154	6.0%	0.66 [0.44, 0.99]		-	
9 Eggert-Kruse 1989 (9)	29	50	87	138	7.5%	0.92 [0.70, 1.20]			
Subtotal (95% CI)		184		584	27.9%	0.91 [0.76, 1.09]		•	
Total events	98		345						
Heterogeneity: Tau ^a = 0.01; Chi ^a = 4 Test for overall effect: Z = 1.06 (P =			= 0.23)	; P = 3	1%				
Total (95% CI)		628		2373	100.0%	0.71 [0.60, 0.84]		•	
	280		1460						
Heterogeneity: Tau* = 0.06; Chi* = 5		df = 18		0011:1	° = 65%				-
Test for overall effect: Z = 4.03 (P <							0.005 0.1	1 10 A (+) ASA (-)	20

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





Annals of Clinical and Medical Case Reports

(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

	Selection					Outcome			
Study	Representa- tiveness of the exposed cohort	Selection of the non- exposed cohort	Ascertain- ment 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	Assess- ment of outcome	Was follow up long enough for outcomes to occur	Adequate of follow up of cohorts	Scores
Shibahara	*	*	*	*	**	*	*	*	9
et al.	~	-	-	-	~ ~	-	-	-	
Menge et al.	*	*	*		**	*	*	*	8
Menge	*	*	*	*	**	*	*	*	9
et al.									
Gilbert et al.	*	*	*	*	**	*	*	*	9
Check et al.	*	*	*	*	*	*	*	*	8
Eggert-									
Kruse et al.	*	*	*		**	*	*	*	8
Ingerslev	*	*	*	*	**	*	*	*	9
et al.	~	~	~	-	~ ~	-	~	-	
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 ASApositive 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 preand post-fertilization events[34]. Anti-sperm antibodies can affect sperm concentration, liquefaction, transport, sperm motility and viability, gamete

Annals of Clinical and Medical Case Reports

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.

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