

# Effects of antisperm antibodies post vasectomy reversal on pregnancy rates

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**Summary** *Objective: To investigate the correlation between antisperm antibodies (ASAs), pregnancy rates, and the method of conception following vasectomy reversal. This is particularly relevant as patients undergoing vasectomy reversal often express concerns about the potential inhibitory effects of ASAs on achieving pregnancy. Additionally, the American Urological Association guidelines for vasectomy emphasize the need for further research to address this question.*

*Patient and Methods: We conducted a retrospective analysis involving chart reviews and phone interviews with individuals who underwent vasectomy reversal at our institution between May 2015 and April 2023. Patients who underwent vasectomy reversal for reasons other than fertility, as well as those lacking postoperative semen analysis with ASA data, were excluded. We classified patients based on low (below 50%) or high (50% or above) ASA levels determined by their initial postoperative semen analysis. The primary outcome measured was the pregnancy rate, including details on the method of conception.*

*Results: A total of 145 patients were subjected to chart review. The median age at the time of surgery was 43 years, with a median obstruction interval of 7.7 years. The median age of their partners was 29 years. The majority (80%) of patients underwent bilateral vasovasostomy. Among them, 60 patients (41.4%) exhibited low (< 50%) ASA levels, while 85 (58.6%) had high (≥ 50%) ASA levels. Follow-up phone interviews were completed by 48 patients. Among them, the 19 men with low ASA levels, 13 (68.4%) achieved pregnancy, with 6 (31.6%) experiencing spontaneous conception. For the 29 men with high ASA levels, 21 (72.4%) achieved pregnancy, including 11 (38%) through spontaneous conception. The p-value from Fisher's exact test was 0.2.*

*Conclusions: Our findings suggest that ASA levels do not show a significant association with either the pregnancy rate or the method of conception following vasectomy reversal.*

**KEY WORDS:** Antisperm antibody; Conception; Vasectomy.

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## INTRODUCTION

Vasectomy is conducted around 500,000 times annually in the United States, being a secure and efficient method of permanent male contraception (1). About 6% of men

opting for vasectomy subsequently expresses a desire for additional pregnancies (2). For these individuals, there are four choices for expanding their families: vasectomy reversal, sperm extraction with *in-vitro* fertilization (IVF), donor sperm insemination, and adoption. Various factors, including parental age, female factor infertility, desired number of children, and cost, play a role in deciding between these options. Approximately 30,000 patients annually choose vasectomy reversal (3). Among those considering vasectomy reversal, a key question is the impact of seminal *antisperm antibody* (ASA) levels on postoperative pregnancy rates. Seminal ASAs in the general infertility population can cause immunologic infertility by affecting various sperm-related processes. However, it remains unclear if seminal ASAs also result in infertility after vasectomy reversal (4, 5). Despite the presumed high seminal ASA levels in individuals who have undergone vasectomy reversal, the pregnancy rates are substantial, with about 73% achieving pregnancy (6). Given these considerations, the clinical significance of seminal ASA levels after vasectomy reversal warrants further investigation (7, 8). Previous research on this topic, conducted in the 1980s, predates advancements in microscopic vasectomy reversal techniques and relies on outdated testing methods for seminal ASA levels (9). The 2015 *American Urologic Association* (AUA) vasectomy guidelines highlight the need for additional research on the impact of antisperm antibodies and their influence on fertility rates after vasectomy reversal (10, 11). Our laboratory routinely conducts IgG ASA testing on all semen samples with progressive motile sperm concentrations of ≥ 2M/ml, including post-vasectomy reversal samples. This presents a unique opportunity for us to assess the relationship between seminal ASA levels following vasectomy reversal and pregnancy rates, as well as the methods of conception. Through retrospective chart reviews and phone interviews of patients who underwent vasectomy reversal at our institution, we aim to explore the potential association between seminal ASA levels and pregnancy rates, methods of conception, and semen analysis parameters. Our hypothesis was that seminal ASA levels were not correlated with pregnancy rates or methods of conception after vasectomy reversal.

## PATIENT AND METHODS

In this study, we enrolled consecutive patients who underwent vasectomy reversal at our institution between May 2012 and April 2020, under the care of two surgeons. Patients undergoing vasectomy reversal for pain or those lacking postoperative semen analysis with ASA were excluded from the study. All procedures performed in this study complied with institutional and/or national research council ethical standards as well as the 1964 Declaration of Helsinki and its subsequent amendments or similar ethical standards. Protocols and written informed consent for all participants were approved by the *Research Ethics Committee of Thumbay University Hospital* (affiliated to *Gulf Medical University, REC #: 21/2015*).

### Data collection

To gather comprehensive data, we conducted a chart review encompassing patient and partner demographics (such as age, number of prior pregnancies, and obstruction interval at the time of vasectomy reversal), details about the surgical technique, intraoperative vasal fluid quality, postoperative complications, and parameters from follow-up semen analyses. Chart review and phone interviews were the primary methods employed. Patients meeting the inclusion criteria were invited to participate in a phone interview, and notifications were sent via SMS to alert them about an expected call from the study team. The study team made up to three attempts to contact each patient for the phone interview. During the interview, a standardized script was utilized, addressing inquiries about pregnancies post-vasectomy reversal and the methods of conception.

### ASA testing and semen analysis

Standard semen analysis, including volume, concentration, motility, total motile sperm count, and strict morphology, was performed on all samples using *World Health Organization* (WHO) reference techniques and appropriate ranges based on the year of the semen analysis. Additionally, IgG Sperm MAR ASA testing was routinely conducted on all semen samples with progressive motile sperm concentrations of  $\geq 2\text{M/ml}$ , a technique first described in 1992 (12). Our laboratory, in accordance with WHO 5<sup>th</sup> edition reference range for ASA levels, performed IgG ASA testing on semen samples with a 50% cut-off to categorize low (below 50%) and high ( $\geq 50\%$ ) ASA levels. Furthermore, we evaluated seminal ASA levels as a continuous variable to explore if an alternative cut-off could more accurately predict pregnancy rates.

### Outcomes

Our primary objectives centered on assessing the pregnancy rate and the method of conception, including details such as intercourse, *intrauterine insemination* (IUI), IVF, and other methods like donor insemination, as reported by the patients during the phone interviews. Secondary outcomes focused on the semen analysis parameters obtained from the initial postoperative semen analysis.

### Statistical analyses

Statistical analyses were conducted using descriptive statistics to evaluate demographic and clinical characteristics of

patients meeting inclusion criteria, comparing those who completed phone interviews to the overall cohort. The Wilcoxon signed-rank test was employed to assess differences in semen analysis parameters between individuals with low and high seminal ASA levels. To analyse pregnancy rates and methods of conception among men with different seminal ASA levels, Fisher's exact test was utilized. Considering the potential confounding effect of the time interval since vasectomy on pregnancy outcomes, the relationship between seminal ASA levels and obstructive interval was evaluated using the Wilcoxon signed-rank test. Additionally, a logistic model was employed to explore whether an alternative seminal ASA level cut-off, apart from the conventional 50%, could provide better predictive value for pregnancy rates. Sensitivity analyses were incorporated to address two decisions made in our primary analyses. Firstly, we conducted a sensitivity analysis to determine the impact of using continuous seminal ASA levels instead of dichotomized low and high levels. Secondly, to assess the influence of using the last postoperative semen analysis (rather than the first) on our findings, we reanalysed the primary outcomes using the last postoperative semen analysis for patients with multiple postoperative analyses. All statistical analyses were carried out using SAS version 9.4 (*SAS Institute Inc.*), and a p-value below 0.05 was considered statistically significant.

**Table 1.**

*Demographics and clinical characteristics for studied patients.*

		Entire cohort (n = 145)	Phone interview cohort (n = 48)
<b>Patient</b>			
Age at time of procedure (years)	Median (IQR)	43 (35-48)	43 (34-49)
# of prior pregnancies	Median (IQR)	2 (1-3)	2 (1-3)
<b>Partner</b>			
Age at time of procedure (years)	Median (IQR)	29 (26-36)	29 (25-37)
Obstruction interval (months)		7.7 (4.1 - 11.4)	8.2 (4.5- 12)
Procedure			
Bilateral WV	n (%)	116 (80%)	36 (75%)
Formal two-layer/Formal two-layer	n (%)	29 (20%)	12 (25%)
Formal two-layer/Modified two-layer	n (%)	4 (2.7%)	2 (4.16%)
Formal two-layer/unknown	n (%)	1 (0.7%)	0
Modified two-layer/Formal two-layer	n (%)	5 (3.4%)	3 (6.25%)
Modified two-layer/Modified two-layer	n (%)	102 (70.3%)	31 (64.5%)
Modified two-layer/Unknown	n (%)	1 (0.7%)	1 (2%)
Unknown/Unknown	n (%)	1 (0.7%)	0
W/VE	n (%)	26 (17.9%)	8 (16.6%)
Formal two-layer	n (%)	8 (31%)	2 (27%)
Modified two-layer	n (%)	18 (68%)	6 (74%)
Bilateral VE	n (%)	1 (0.7%)	0
Unilateral WV	n (%)	3 (2%)	3 (4.16%)
Formal two-layer	n (%)	2 (67%)	2 (67%)
Modified two-layer	n (%)	1 (33%)	1 (33%)
Intraoperative vasal fluid quality			
Right side			
Clear/watery	n (%)	24 (16.6%)	12 (25%)
cloudy/opaque/opalescent/milky	n (%)	80 (55.1%)	23 (47.9%)
thick/yellow/toothpaste/creamy/pasty	n (%)	18 (12.5%)	7 (14.6%)
scant/paucity	n (%)	8 (5.5%)	2 (4.4%)
Missing	n (%)	15 (10.3%)	4 (8.1%)

Motility			
Motile	n (%)	43 (29.64%)	17 (35.4%)
Nonmotile	n (%)	50 (34.36%)	14 (29.2%)
Missing	n (%)	52 (36%)	17 (35.3%)
Left side			
Clear/watery	n (%)	22 (15.2%)	8 (16.6%)
cloudy/opaque/opalescent/milky	n (%)	80 (55.2%)	24 (50%)
thick/yellow/toothpaste/creamy/pasty	n (%)	20 (13.8%)	9 (18.75%)
scant/paucity	n (%)	9 (6.2%)	2 (4.2%)
Missing	n (%)	14 (9.6%)	5 (10.45%)
Motility			
Motile	n (%)	30 (21%)	15 (31.2%)
Nonmotile	n (%)	35 (24%)	15 (31.2%)
Missing	n (%)	80 (55%)	18 (37.6%)
Postoperative complications			
None	n (%)	137 (94.4%)	44(91.6%)
Infection	n (%)	4 (2.8%)	2 (4.2%)
Other	n (%)	4 (2.8%)	2 (4.2%)
Follow up semen analysis parameters			
Number of follow up SAs			
1 <sup>st</sup>	n (%)	89 (61%)	27 (56.3%)
2 <sup>nd</sup>	n (%)	34 (24%)	15 (31.2%)
3 <sup>rd</sup>	n (%)	22 (15%)	6 (12.5%)
First semen analysis (SA) values			
Time to first SA (weeks)	Median (IQR)	11 (8.1-20)	10 (8-19)
Concentration (M/ml)	Median (IQR)	33 (13-70)	32 (10-69)
Total motility (%)	Median (IQR)	38 (18-47)	39 (11-41)
Total motile sperm count (M/ejaculate)	Median (IQR)	27 (9-64)	21 (7- 57)
Normal morphology (%)	Median (IQR)	7 (4-9)	8 (6-11)
Antisperm antibody (%)	Median (IQR)	48 (13-88)	46 (11- 87)
< 50%	n (%)	60 (42%)	20(41.66%)
≥ 50%	n (%)	84 (58%)	28 (59.34%)

## RESULTS

After applying the specified inclusion and exclusion criteria, we identified a cohort comprising 145 patients who underwent vasectomy reversal at our institution between May 2012 and April 2020. The median age at the time of surgery for this cohort was 43 years, with a median obstruction interval of 7.7 years. The median age of their partners was 29 years. Among the 145 patients, 116 (80 %) underwent bilateral vasovasostomy, 24 (16.6%) underwent a combination of vasovasostomy and vasoepididymostomy, 3 (2%) underwent unilateral vasovasostomy, and 2 (1.4%) underwent bilateral vasoepididymostomy (Table 1). Forty eight (33%) participated in a phone interview. The demographic and clinical characteristics, including obstruction interval, vasectomy reversal technique, intraoperative vasal fluid quality, and postoperative complications, were comparable between the overall cohort of 145 patients and the subgroup of 48 patients who completed the phone interview. Of the 145 patients, 60 had low seminal ASA levels (< 50%), while 85 had high seminal ASA (≥ 50%) levels. There were no statistically significant differences in obstruction interval, vasectomy reversal technique, or semen analysis parameters

(e.g., concentration, total motility, total motile sperm count, strict morphology) between the low and high seminal ASA groups (Table 2). Of the 48 patients who completed the phone interview, 19 (39.6%) had low seminal ASA levels, and 29 (60.4%) had high seminal ASA levels (Table 3). The median time from vasectomy reversal to the date of completing the phone interview was 8.2 years for the low seminal ASA group and 7.9 years for the high seminal ASA group. Among the men with low seminal ASA levels, 13 (68.4%) achieved a pregnancy, with 9 (47.3%) having spontaneous pregnancies and 4 (21.1%) using IVF. Among the men with high seminal ASA levels, 22 (75.8%) achieved a pregnancy, with 12 (41.4%) having spontaneous pregnancies, 3 (10.4%) using intrauterine insemination (IUI), and 7 (24%) using IVF. The Fisher's exact test p-value for differences in pregnancy rates and methods of conception was 0.2. No statistically significant relationship was found between obstruction interval and pregnancy rates (Wilcoxon test p-value = 0.5) (Table 3). To assess whether a different seminal ASA level cut-off could better predict pregnancy rates, we employed a logistic model to examine the relationship between seminal ASA levels as a continuous variable and pregnancy rates. The analysis revealed no association between seminal ASA levels and pregnancy rates, with a p-value of 0.98. In sensitivity analyses, our findings remained consistent when using continuous seminal ASA levels instead of dichotomized levels (Wilcoxon p-value 0.97). Among the 79 patients with multiple postoperative semen analyses, 17 (22%) experienced changes in their seminal ASA categories across analyses. Specifically, 9 (53%) patients shifted from the low to the high seminal ASA group, 7 (41%) shifted from the high to the low seminal ASA group, and 1 (6%) changed across groups in both directions.

Furthermore, our results were unchanged when using seminal ASA levels from the last postoperative semen analyses instead of those from the first postoperative semen analysis (Signed rank test, p-value = 0.5).

**Table 2.** Vasectomy reversal technique, obstruction interval, and follow up semen analysis parameters for studied patients.

		Low ASA levels (< 50%), N = 60	High ASA levels (≥ 50%), N = 85	p-value
Obstruction interval (years)	median (IQR)	7.1 (3.5-12)	7.7 (5.2-10.2)	0.31
Technique of vasectomy reversal				
Bilateral W	n (%)	50 (83.33%)	67 (78.8%)	0.72
W/VE	n (%)	9 (15%)	16 (18.8%)	
Unilateral W	n (%)	1 (1.67%)	2 (2.4%)	
Follow up semen analysis parameters				
Number of follow up SAs				
1 <sup>st</sup>	n (%)	43 (71.66%)	46 (54.1%)	0.06
2 <sup>nd</sup>	n (%)	10 (16.67%)	24(28.2%)	
3 <sup>rd</sup>	n (%)	7 (11.67%)	15 (17.7%)	
First semen analysis values				
Time to first SA (weeks)	median (IQR)	9 (7.6-19)	10 (6-15)	> 0.9
Concentration (M/ml)	median (IQR)	36 (12-74)	32 (12-57)	0.31
Total motility (%)	median (IQR)	38 (19-46)	39 (21-42)	0.73
Total motile sperm count (M/ejaculate)	median (IQR)	32 (5.1-76)	26 (8.8-64)	> 0.9
Normal morphology (%)	median (IQR)	6 (5-9)	8 (4-11)	0.74

**Table 3.**  
Pregnancy rates and methods of conception for studied patients, based on the first postoperative semen analysis.

		Low ASA levels ( $< 50\%$ ), N = 19	High ASA levels ( $\geq 50\%$ ), N = 29	p-value
Obstruction interval (years)	median (IQR)	8 (3.0-14)	8.0 (6.0-11)	0.7
Time from vasectomy reversal to first ASA measurement (weeks)	median (IQR)	7.9 (7.2-15)	10(8.1-11)	0.4
Time from vasectomy reversal to phone interview (years)	median (IQR)	10.3 (5.2-14.7)	9.6 (3.1-11.6)	0.2
Postoperative pregnancy				
Yes	n (%)	13 (68.4%)	21 (72.4%)	0.2
Spontaneous	n (%)	11 (58%)	11 (38%)	
IVF	n (%)	2 (10.4%)	7 (24%)	
IUI	n (%)	0 (0%)	3 (10.4%)	
No	n (%)	6 (31.6%)	8 (27.6%)	

## DISCUSSION

We have three main findings. Firstly, there is no association between seminal ASA levels after vasectomy reversal and pregnancy rates or methods of conception. Secondly, men with low and high seminal ASA levels showed no differences in postoperative semen analysis parameters. Thirdly, we were unable to identify a specific cut-off level for postoperative seminal ASA levels that strongly correlates with pregnancy rates. Overall, these findings indicate that seminal ASA levels are not linked to pregnancy rates, the method of conception, or semen analysis parameters following vasectomy reversal. These insights can enhance the counselling of patients before and after undergoing vasectomy reversal.

Our discovery that seminal ASA levels after vasectomy reversal are not associated with pregnancy rates or methods of conception contrasts with earlier studies from the 1980s (10, 13). In particular, *Thomas et al.* (13) found no association between serum or seminal ASA titres and pregnancy rates in 35 men who underwent vasectomy reversal at a single centre with at least 1 year of follow-up. *Parslow et al.* (10) in their evaluation of 130 men at two canters, observed that higher preoperative serum ASA titres were linked to lower pregnancy rates, while postoperative seminal ASA titres were not associated with pregnancy rates after at least 1 year of follow-up. *Belker et al.*, cited by *Nam et al.* (14), in their prospective study of patients who had undergone vasovasostomy, found that 66% of those who achieved pregnancy had no measurable serum ASA levels, while 71% of those unable to achieve pregnancy had measurable serum ASA levels. These studies suggested that patients with serum ASA levels exceeding 2 million per millilitre might require IVF to achieve pregnancy, irrespective of seminal ASA levels. Our study had longer follow-up than studies previously mentioned and used the modern day IgG SpermMAR technique to measure seminal ASAs, as recommended by the WHO laboratory manual (12). Despite these earlier findings, our study highlights that seminal ASA levels following vasectomy reversal are not correlated with decreased pregnancy rates. This information can offer reassurance to patients seeking to have children after a vasectomy. For healthcare providers, our results can serve

as valuable insights when counselling patients before and after vasectomy reversal. Furthermore, we observed no significant differences in postoperative semen analysis parameters between men with low and high ASA levels. The impact of seminal ASA on semen analysis parameters in the general male infertility population has shown mixed findings. Some studies have reported associations between elevated seminal ASA levels and increased sperm agglutination, (15, 16) decreased sperm concentration, (17, 18) and reduced sperm motility (4, 16-18). However, the influence of seminal ASA levels on semen analysis parameters within the vasectomy reversal patient population has not been thoroughly evaluated. In our study, there was no statistically significant distinction in semen

analysis parameters, including concentration, total motility, total motile sperm count, and strict morphology, between men with low and high seminal ASA levels, using either the first or the last postoperative semen analysis. These findings underscore the necessity of establishing specific semen analysis reference values for vasectomy reversal patients, enabling more accurate counselling of postoperative patients on their likelihood of spontaneous conception (19).

Finally, we were unable to identify a specific cut-off level for postoperative seminal ASA levels strongly associated with pregnancy rates. The 2010 WHO laboratory manual recommended ASA testing as a routine component of semen analyses with a 50% cut-off to categorize low and high ASA levels. However, limited evidence supports this 50% cut-off, and the 2010 WHO laboratory manual acknowledges it as a "consensus" threshold value. The 2021 WHO laboratory manual discussed the limited evidence behind ASA reference values and cautioned against over interpreting ASA values as causative of subfertility. Despite efforts using a logistic model to find a more effective seminal ASA cut-off for predicting pregnancy rates after vasectomy reversal, we were unable to identify a cut-off that reliably predicted a couple's likelihood of achieving pregnancy (20). Our study comes with several limitations. Firstly, the data were retrospectively obtained from a single institution, potentially limiting the generalizability of our observations to other settings. Nevertheless, our study presents a contemporary analysis compared to earlier studies from the 1980s, featuring a longer follow-up and the use of modern, WHO-recommended laboratory techniques for measuring ASA levels. Secondly, approximately one-third of the included patients participated in a phone interview, introducing the potential for selection bias. However, the demographic and clinical characteristics of the interviewed men were similar to those of the overall cohort. Thirdly, postoperative semen analyses were conducted at varying time points after surgery, with some patients undergoing multiple analyses. We chose to utilize the first postoperative semen analysis to categorize patients as having low or high seminal ASA levels. Although 22% of the cohort exhibited movement across seminal ASA groups over time, our sensitivity analysis

using the last postoperative semen analysis did not alter our findings. Fourthly, since our laboratory routinely performs ASA testing on semen samples with progressive motile sperm concentrations of  $\geq 2$  million per millilitre, there may be a selection bias as patients with severe oligoasthenospermia were excluded from our analysis. However, we identified 17 samples with sperm concentrations  $< 2$  million per millilitre and available seminal ASA levels, as ASA testing on semen samples was conducted at the laboratory's discretion for all samples. Additionally, men with progressive motile sperm concentrations  $< 2$  million per millilitre are likely to require IVF to achieve pregnancy, irrespective of seminal ASA levels. Notwithstanding these limitations, our study holds significant implications for both patients and healthcare providers. For individuals seeking to conceive after a vasectomy, our findings provide assurance that high seminal ASA levels after vasectomy reversal are not linked to decreased pregnancy rates.

## CONCLUSIONS

Postoperative seminal ASA levels are not associated with pregnancy rates, methods of conception, or semen analysis parameters after vasectomy reversal surgeries. Accordingly we highly recommend pre- and post-operative patient counselling in the context of vasectomy reversal.

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