ORIGINAL ARTICLE

Assisted Reproductive Technology

Success rates of *in vitro* fertilization versus intracytoplasmic sperm injection in men with serum anti-sperm antibodies: a consecutive cohort study

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Antisperm antibodies (ASAs) are assumed to be a possible causative factor for male infertility, with ASAs detected in 5%–15% of infertile men but in only 1%–2% of fertile ones. It remains unclear whether ASAs have an adverse effect on the outcome of *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI). This study investigated differences in the rates of fertilization, pregnancy, and live births associated with serum ASA-positive and ASA-negative men following IVF or ICSI. Five hundred and fifty-four consecutive infertile couples undergoing IVF (*n* = 399) or ICSI (*n* = 155) were included. The two-sample two-sided *t*-test and Chi-square or Fisher's exact test was used for statistical analysis. Lower rates of fertilization (41.7% *vs* 54.8%, *P* = 0.03), good embryos (18.9% *vs* 35.2%, *P* = 0.00), pregnancy (38.5% *vs* 59.4%, *P* = 0.00), and live births (25.8% *vs* 42.5%, *P* = 0.00) were observed in men of the IVF group with a positive serum ASA than in those with a negative ASA. ASA positivity/negativity correlated with pregnancy rates (*P* = 0.021, odds ratio [OR]: 0.630, 95% confidence interval [CI]: 0.425–0.932) and live birth rates (*P* = 0.010, OR: 1.409, 95% CI: 1.084–1.831) after controlling for the female serum follicle-stimulating hormone level and the couple's ages at IVF. Women coupled with ASA-positive men had lower live birth rates with IVF than with ICSI (25.8% and 47.4%, respectively; *P* = 0.07). Women coupled with ASA-positive men had lower rates of pregnancy and live births following IVF than those coupled with ASA-negative men but had a similar outcome with ICSI.

INTRODUCTION

Antisperm antibodies (ASAs) are assumed to be a possible causative factor for male infertility, with ASAs detected in 5%–15% of infertile men but in only 1%–2% of fertile ones.[1] In infertile men, ASAs may be detected in seminal plasma and serum and on the surface of spermatozoa. Developmental abnormalities of the formation of the blood–testis barrier and traumatic disruption of this barrier can lead to the formation of ASAs in men.[2–4] Typically, high levels of ASAs are found in men with a history of testicular trauma, varicocele, mumps orchitis, spinal cord injury, congenital absence of the vas, and vasectomy. Other conditions associated with ASAs include biopsy or malignancy of the testis, a history of cryptorchidism, prostatitis, sexually transmitted diseases, and idiopathic conditions.[5,6]

ASAs are believed to have an adverse effect on male fertility by (1) reducing the sperm output, sperm motility, and agglutination of sperm; (2) impairing the ability of sperm to penetrate the cervical mucus; (3) interfering with fertility by inducing sperm injury caused by complement and/or phagocytic cells in the female genital tract; and (4) impairing sperm–egg interaction, acrosome reaction, and binding to the zona pellucida.[7,8]

However, the relationship between the presence of ASAs in men and infertility continues to be disputed, and it has been unclear in the existing literature whether ASAs have an adverse effect on the outcome of *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Studies on the fertilization rate following IVF have reported contradictory results, with some studies showing a detrimental effect of ASAs on the fertilization rate[9,10] and others showing no detrimental effect.[11–13] In contrast, the studies on ASAs and the pregnancy rate following ICSI have mostly shown that ASAs do not affect pregnancy rates after ICSI.[14–16] The aim of our study was to investigate the rates of fertilization, pregnancy, and live births of infertile couples with a serum ASA-positive or ASA-negative male partner who underwent a cycle of IVF or ICSI.

PATIENTS AND METHODS

Patient population

This consecutive cohort study was approved by the Institutional Review Board of the Provincial Hospital Affiliated to Shandong University,

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Jinan, China. All patients were counseled, and signed consent form was approved by the local Ethics Committee.

This study included a cohort of consecutive infertile couples undergoing a cycle of IVF or ICSI in the Center for Reproductive Medicine, Provincial Hospital Affiliated to Shandong University, Jinan, China from May 2013 to December 2014. They visited the center for an infertility evaluation and had a minimum of 1 year of unprotected intercourse. All male patients underwent a careful medical history interview, physical examination, and semen analyses. They were required to have clinical potential risk factors for the development of ASAs, including a history of varicocele, varicocele repair, testicular trauma, testicular infection, and inguinal hernia repair.

Data collection and analysis of serum ASA levels

The following demographic data were collected: male and female ages, female serum follicle-stimulating hormone (FSH) level, sperm parameters, number of eggs retrieved, number of good embryos, fertilization rate, good embryo rate, pregnancy rate, and live birth rate. The choice of fertilization method was based on the diagnosis of infertility. The IVF group mainly consisted of couples with a female factor of infertility. The criterion for performing ICSI was a total sperm count <1 000 000 after gradient centrifugation.[16]

Semen samples were collected by masturbation on the day of oocyte retrieval and assessed according to the World Health Organization (WHO) guidelines.[17] Serum ASA levels were measured by using an enzyme-linked immunosorbent assay (ELISA) test kit (EIA-1826; ELISA, USA) according to the manufacturer's instruction. An ASA level of <75 IU was considered negative, whereas an ASA level of >75 IU was considered positive.

IVF or ICSI procedure

IVF or ICSI was performed using a standardized ovarian stimulation regimen, oocyte retrieval, and fertilization, followed by a planned transfer of up to 2–3-day embryos, as recommended by the American Society of Reproductive Medicine and Chinese guidelines.[16] The outcomes of IVF/ICSI were documented after the embryo fertilization day. Clinical pregnancy was defined by the presence of a gestational sac with heartbeat, as observed by ultrasonography at 5–7 weeks after embryo transfer (ET). Live birth was defined as delivery of any viable newborn at 28 weeks or more of gestation after ET. Miscarriage was defined as any spontaneous interruption of clinical pregnancy. Ectopic pregnancy was defined as a pregnancy that developed outside of the uterine cavity.

Statistical analyses

Descriptive statistics were presented as the mean and standard deviation to summarize continuous variables and as counts and percentages to summarize categorical variables. Bivariate analyses were performed to compare group differences (ASA-positive men *vs* ASA-negative men) with the two-sample two-sided *t*-test to determine differences in continuous variables and with the Chi-square or Fisher's exact test to determine differences in categorical variables. Multivariable logistic regression models were used to evaluate the binary outcomes and group differences, after controlling for confounders of interest. Outcomes such as miscarriage rate did not have a sufficient number of events to warrant a multivariable model. SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Among 554 couples, 554 cycles (399 IVF and 155 ICSI) were included and assessed. Fifty-eight men were positive for serum ASAs, including 15 men with varicocele, 11 men with postvaricocelectomy, and 1 man with a history of orchitis. The other 496 men were negative for serum ASAs (controls), including 92 men with varicocele, 57 men with postvaricocelectomy, 2 men with a history of orchitis, 1 man with inguinal hernia repair, 1 man with cryptorchidism repair, and 1 man with hypospadias repair.

The prevalence of ASAs was 10.5% (58/554) among all patients, 9.8% (39/399) among IVF cycles, and 12.3% (19/155) among ICSI cycles. There was no association between a history of male varicocele, varicocele repair, or previous orchitis and ASA development as shown in **Table 1**. Only men with ASA negativity had a history of inguinal hernia repair, cryptorchidism repair, or hypospadias repair, and the population of men with ASA negativity was larger than that of those with ASA positivity. Overall, no men reported a history of testicular trauma.

Demographic data of men coupled with women who underwent the 399 IVF and 155 ICSI cycles are displayed in **Table 2**. In 9.8% (39/399) of men with IVF and 12.3% (19/155) with ICSI, serum ASAs were positive. For all endpoints and treatment groups, only the first cycle from each couple was included. No differences were detected in female age, male age, and the serum FSH level of women between the serum ASA-positive and ASA-negative subgroups of the IVF or ICSI groups. Data concerning sperm density, progressive motility, normal strict sperm morphology, number of metaphases II, and 2-pronuclei oocytes were also comparable between the subgroups.

Within the IVF groups, 39 men with ASA positivity were coupled with women who had 15 pregnancies (7 miscarriages and 8 live births) and 399 men with ASA negativity were coupled with women who had 214 pregnancies (59 miscarriages, 2 ectopic pregnancies, and 153 live births). The rates of fertilization (41.7% ± 23.4% *vs* 54.8% ± 29.9%, $P = 0.03$) and good embryos (18.9% ± 12.6% *vs* 35.2% ± 22.6%, $P = 0.00$) were lower in couples with an ASA-positive male partner than in those with an ASA-negative male partner (**Figure 1**). Lower rates of achieving pregnancy (38.5% *vs* 59.4%, $P = 0.01$) and live births (20.5% *vs* 42.5%, $P = 0.01$) were seen in the ASA-positive group than in the ASA-negative group. Statistical analysis with binary logistic analysis showed that the variable of ASA positivity/negativity correlated with pregnancy ($P = 0.021$, odds ratio [OR]: 0.630, 95% confidence interval [CI]: 0.425–0.932) and live births ($P = 0.010$, OR: 1.409, 95% CI: 1.084–1.831) after controlling female age, male age, and the serum FSH level of women.

Table 1: Relationship between antisperm antibody status and male risk factors

Variables	Total (n=554)	ASA positive (n=58)	ASA negative (n=496)	P
Varicocele, *n* (%)	107 (19.3)	15 (25.9)	92 (18.5)	0.246
Varicocele repair, *n* (%)	68 (12.3)	11 (19.0)	57 (11.5)	0.153
Previous orchitis, *n* (%)	3 (0.6)	1 (1.7)	2 (0.4)	0.725
Inguinal hernia repair (*n*)	1	0	1	NA
Cryptorchidism repair (*n*)	1	0	1	NA
Hypospadias repair (*n*)	1	0	1	NA

ASA: antisperm antibody; NA: not available

Table 2: Comparison of men with serum antisperm antibody positivity and those with antisperm antibody negativity

Variables	ASA positive	IVF ASA negative	P	ASA positive	ICSI ASA negative	P
Cycles included (*n*)	39	360		19	136	
Female age (year), mean±s.d. (range)	32.1±4.7 (21–40)	31.2±4.6 (20–43)	0.26	32.2±5.8 (23–41)	30.6±4.6 (21–42)	0.17
Female FSH (IU l^{-1}), mean±s.d. (range)	7.5±3.4 (3.9–19.1)	7.3±3.0 (1.5–33)	0.68	9.3±5.3 (4.0–16.8)	6.9±5.4 (1.3–29.5)	0.13
Male age (year), mean±s.d. (range)	32.7±5.0 (24–40)	33.0±4.9 (23–50)	0.72	32.8±6.1 (22–43)	33.2±6.1 (23–53)	0.81
Sperm concentration (10^6 ml^{-1}), mean±s.d. (range)	78.1±40.7 (23.6–192.9)	78.5±44.0 (5–230.5)	0.95	11.9±15.8 (0–46.3)	10.7±19.1 (0–89.1)	0.79
Progressive sperm motility (%), mean±s.d. (range)	59.8±17.7 (25.3–94.9)	59.6±15.1 (5–95)	0.96	20.1±16.5 (0–47)	21.1±25.0 (0–72)	0.87
Normal strict sperm morphology (%), mean±s.d. (range)	2.9±1.3 (0.9–6.3)	2.8±1.4 (0.3–9.1)	0.69	1.5±1.2 (0–3.6)	1.0±1.5 (0–6)	0.20
Oocyte retrieved (*n*), mean±s.d.	14.4±6.0 (6–24)	13.7±6.5 (3–46)	0.63	11.0±9.7 (4–45)	12.2±9.9 (2–62)	0.63
2 pronuclei (*n*), mean±s.d. (range)	5.5±3.8 (1–14)	6.8±3.8 (1–19)	0.11	5.8±3.8 (1–13)	7.3±5.4 (0–24)	0.25
Good embryo (*n*), mean±s.d. (range)	3.0±2.4 (0–9)	4.7±3.5 (0–17)	0.02	3.5±2.4 (0–8)	3.6±3.9 (0–19)	0.86
Embryo transferred (*n*), mean±s.d. (range)	2.0±0.2 (1–2)	1.9±0.3 (1–2)	0.37	2.0±0.7 (1–3)	1.8±1.0 (1–3)	0.44

IVF: *in vitro* fertilization; ICSI: intracytoplasmic sperm injection; FSH: follicle-stimulating hormone; s.d.: standard deviation; ASAs: antisperm antibodies
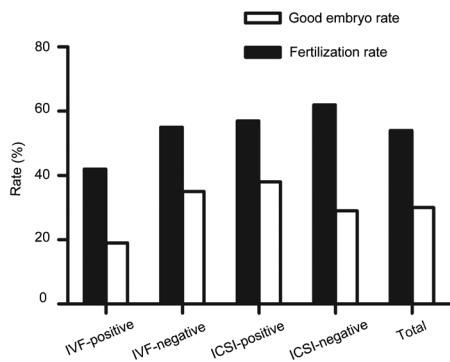
Figure 1: Outcomes of fertility and good embryo rates.

Among the ICSI cycles, 19 men with ASA positivity were coupled with women who had 10 pregnancies (1 miscarriage and 9 live births) and 156 men with ASA negativity were coupled with women who had 84 pregnancies (23 miscarriages, 1 ectopic pregnancy, and 60 live births). No remarkable difference was observed in the rates of fertilization or good embryos between the groups, and the same was true for the rates of pregnancy and live births.

Regarding outcomes, the rates of pregnancy and live birth were not remarkably different between the ASA-negative group and ASA-positive group. Although an increasing trend was observed between ICSI (47.4%) and IVF (20.5%) in the ASA-positive group, it was not statistically meaningful (**Table 3**).

DISCUSSION

In this study, 10.5% of all men were ASA positive, which is comparable with the 9.5% prevalence of ASAs in men that was reported in a similar study conducted in the United States.[17] Data on the frequency of ASAs in infertile men have shown high variability (from 7% to 44%), depending on the method of evaluation.[18] This variability may be also associated with the tool used to detect ASAs and the study sample size. No meaningful association was found between the presence of ASA and a history of varicocele, varicocele repair, or orchitis. The reason for this may be the fact that, despite the long list of risk factors related to the development of ASAs, most cases were idiopathic and all infertile men were at risk of ASAs.[19]

In IVF and ICSI cycles, serum ASA positivity was associated with the outcomes following IVF but not ICSI. In IVF group, couples with

ASA-positive male partners had lower rates of pregnancy and live births than couples with ASA-negative male partners. The combined ORs for not achieving pregnancy and live birth using IVF in the presence of positive serum ASAs were 2.345 (95% CI: 1.190–4.622) and 2.864 (95% CI: 1.281–6.405), respectively. The study also indicated that couples with ASA-positive male partners had lower rates of fertilization and good embryos than couples with ASA-negative male partners. However, we cannot make similar conclusions regarding the effect of serum ASA positivity on ICSI outcomes based on these data, regardless of the comparison of ASA-positive men versus ASA-negative men in the ICSI group or IVF group.

ASAs may be produced when the blood–testis barrier is breached, allowing sperm antigens to induce an immune response. The adverse effects of ASAs on fertility are inferred from the reportedly higher prevalence of ASA positivity in infertile men than in fertile men.[2,3] Plausible mechanisms of this include the following: reducing sperm motility and oocyte binding by direct and indirect interactions that cause the release of cytokines, which impair function, and by reducing sperm cells, which penetrate the cervical mucus.[7,8] Studies on the correlation between ASAs and the outcome of IVF or ICSI have shown conflicting results. Similar to our study, numerous previous studies have shown that ASAs may adversely affect IVF and/or pregnancy rates. Previous researchers[8] studied 33 couples with male immunologic infertility and demonstrated that patients with a high positive mixed antiglobulin reaction (MAR) test result (≥90%) had lower fertilization and pregnancy rates than those with lower ASA titers. Acosta *et al.*[10] treated 29 direct immunobead test (dIBT)-positive men with IVF and found that the ASA-positive group achieved lower fertilization and pregnancy rates than the ASA-negative group. As such, these authors suggested that infertile couples with high ASA levels proceed to ICSI. In contrast to our results, however, other IVF studies have reported no remarkable differences in ASA-positive and ASA-negative populations, and that ASAs do not affect fertilization and pregnancy rates following IVF.[20] These differences are likely a result of the following: (1) the degree of sperm autoimmunization, which is the method used to detect ASAs and a particular subset of ASAs with clinical significance, as low-to-moderate antibody levels may not have a profound effect on fertility; and (2) the highly varying cutoff points of ASAs used in studies (between 10% and 80%).[21]

ASAs can be detected by various tests using spermatozoa, seminal plasma, serum mucus, or cervical mucus. Common tests used to detect ASAs, such as MAR and IBT, need to be performed by a specialist in

Table 3: Rate of pregnancy and live births following *in vitro* fertilization and intracytoplasmic sperm injection

Variables	Within IVF			Within ICSI			Between IVF/ICSI	
	Rate (%)	P	D (95% CI)	Rate (%)	P	D (95% CI)	P	D (95% CI)
Pregnancy								
ASA (positive)	38.5 (15/39)	NA	NA	52.6 (10/19)	NA	NA	0.46	0.6 (0.2–1.7)
ASA (negative)	61.1 (214/350)	0.02	0.4 (0.2–0.8)	61.8 (84/136)	0.61	0.7 (0.3–1.8)	0.71	0.9 (0.6–1.4)
Live birth								
ASA (positive)	20.5 (8/39)	NA	NA	47.4 (9/19)	NA	NA	0.07	0.3 (0.1–0.9)
ASA (negative)	42.5 (153/360)	0.01	0.4 (0.2–0.8)	44.1 (60/136)	0.98	1.1 (0.4–1.0)	0.82	0.9 (0.6–1.4)

ASA: antisperm antibody; CI: confidence interval; D: mean difference; ICSI: intracytoplasmic sperm injection; IVF: *in vitro* fertilization; NA: not available

the reproductive laboratory.[21,22] Because of consistent errors in the performance of the test for detecting ASAs in standardized samples, a high degree of variation in the results obtained from twenty laboratories was reported, with positive dIBT values ranging from 21% to 82%.[22] In addition, the direct MAR test and dIBT require processed sperm to bind to the test's immunobead, and this is impossible in men with severe oligospermia or lack of sperm motility.[17] For laboratories without this expertise, a simpler test that does not require sperm processing is needed. In our study, serum ASA levels were measured by ELISA, which is a simple, reliable, and highly reproducible method for detecting ASAs compared with other available ASA assessments. Men with serum ASA positivity (ASA level >75 IU) in our previous study had a titer of at least >50% according to the MAR test, defined by the WHO[23] as the best available clinical and predictive value of ASAs.[17] Therefore, ASA positivity detected by ELISA in this study may constitute a clinically meaningful test result.

Following the successful introduction of ICSI for the treatment of couples with other forms of male factor infertility, ICSI has become an alternative for managing couples affected by ASAs. Microinjection of such compromised sperm into the oocyte cytoplasm can minimize the inhibitory effects of ASAs on spermatozoa zonapellucida binding and other subsequent events of fertilization. It is likely to increase the fertilization rate of infertile men with ASA positivity to a level comparable to that of other infertility indications.[24,25] Numerous studies have consistently shown that ASAs do not affect fertilization and pregnancy rates following ICSI. Nagy *et al.*[14] performed an analysis of 55 ICSI cycles in 37 patients with high ASA-bound sperm levels (>80% MAR or dIBT result) and reported no difference in pregnancy rates between the ASA-positive and ASA-negative groups. Lahteenmaki *et al.*[26] treated 29 infertile ASA-positive men who were coupled with women who underwent ICSI of which 22 of them achieved a poor IVF rate. After ICSI, fertilization and cleavage rates for the ASA-positive group (79% and 89%, respectively) were similar to those of the ASA-negative group (68% and 93%, respectively). In our study, an increasing trend in the rates of pregnancy and live births was observed in ICSI compared with IVF among the ASA-positive subgroup, but this was not statistically meaningful because of the relatively small population of patients with ASA positivity in the ICSI and IVF groups. Future studies involving a larger cohort may be needed to determine if the trend of success reaches statistical relevance.

In the present study, the mean sperm concentration, percentage of progressive motility, and percentage of strict morphology were comparable between the ASA-positive and ASA-negative men in the IVF and ICSI groups. These data are not in line with those of some studies that revealed an inverse relationship between ASA levels and sperm parameters.[13,27] The reason for this may be that the selection process was based on the results of these men's sperm analysis, which determined the choice of IVF or ICSI.

CONCLUSIONS

The presence of ASAs may decrease the *in vitro* sperm–oocyte fertilization rate and good embryo rate, lowering the rates of pregnancy and live births following IVF treatments. ICSI seems able to overcome this problem, producing similar pregnancy and live birth rates in ASA-positive couples compared with ASA-negative couples. A trend in the increase of the rates of pregnancy and live births in IVF versus ICSI within the ASA-positive group was observed, but it was not a meaningful difference. Because of the rarity of immunological infertility, future studies should include a larger cohort to confirm the effect of ASAs on the outcomes following IVF and ICSI.

AUTHOR CONTRIBUTIONS

SML conceived and designed the study, acquired data, and played an important role in interpreting the results. XL and SLW contributed to data analysis, manuscript preparation, and constructive discussions. XLY, YZX, and LLH contributed to data acquisition. JLL and FFC contributed to manuscript preparation. ZJC conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

All authors declare no competing financial interests.

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