# The effects of isotypes and regional distribution of antisperm antibodies on semen parameters and fertilizing ability

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# 항정자항체가 정액성상 및 수정능력에 미치는 영향

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**방명걸 · 문신용**

=국문초록=

항정자항체의 종류 및 존재부위가 정액성상 및 수정능력에 미치는 영향을 조사하였다. 항정자항체의 종류 및 존재부위는 immunobead binding test에 의하여 시행하였으며, 정자의 수정능력은 투명대 제거 햄스터 난자 침입법에 의하여 시행하였다.

항정자항체는 정자수, 운동성 및 운동지수에 악영향을 끼쳤으며, 수정능력에도 악영향을 끼쳤다. 항정자항체의 존재부위에 따른 차이는 보이지 않았다. 항정자항체 IgG가 정자두부 혹은 정자미부에 존재할 경우 및 항정자항체 IgA가 정자미부에 존재할 경우 수정능력을 크게 감소시켰다.

Key Words: antisperm antibody, antisperm antibody isotype, binding site of antisperm antibody, fertilizing capacity, immunobead binding test, semen parameter, sperm penetration assay

## Introduction

It is well-established that antisperm antibodies (ASAs) are detectable in either the male or female partner in a significant proportion (approximately 10%) of infertile couples (Pattinson et al., 1987; Collins et al., 1993). Antibodies to sperm are most often detected in

couples with unexplained infertility; they may impair fertility by interfering with sperm cervical mucus penetration, with sperm transport in general, and, in particular, with penetration of the oocyte-surrounding layers, with the integrity of sperm cell membranes, with sperm capacitation and acrosome reaction, with zona pellucida binding and penetration, and with zygote development (Acosta et al., 1994).

There are three major types of ASAs in humans: immunoglobulin(Ig)G, IgA, and IgM; each can be directly bound to a patient's spermatozoa, as detected by the immunobead binding test (IBT). IBT has definite advantages over conventional ASA tests because it provides information on the regional distribution of antibodies on the sperm surface and simultaneously allows determination of the proportion of antibody-coated motile spermatozoa (Clarke, 1990).

Several studies have attempted to establish a correlation between ASAs and fertilization results *in vitro*; it is widely agreed that a high antibody titer and binding of antibody to the sperm head are the most powerful predictors of fertilization failure (Zouari et al., 1993; Yeh et al., 1995; Ford et al., 1996), and the prognosis is further worsened by other adverse semen parameters (Acosta et al., 1994; Lahteenmaki, 1993). The presence of surface-bound antibodies on motile spermatozoa, especially the IgA class, has been implicated in IVF impairment (Clarke et al., 1985). However, they have been few reports of the impact of Ig isotypes and the regional distribution of ASA on the human sperm surface on either fertilization results *in vitro* (Clarke et al., 1985; Witkin et al., 1992) or semen quality (Mathur et al., 1986; De Almeida et al., 1991).

To more clearly understand the influences of Ig isotypes and the regional distribution of ASA on the human sperm surface on both fertilizing capacity and semen parameters, we evaluated fertilizing capacity using sperm penetration assay (SPA); semen parameters were evaluated using different classes of antibody present in different regions of spermatozoa.

## MATERIALS AND METHODS

Sixty-seven of 355 males showed positive ASA on the sperm by IBT and were included in this study as an ASA-positive group. A group of 96 males with normal fertility was used for comparison.

### Semen Analysis

Semen samples were collected after a fixed 3-day period of abstinence and examined immediately after liquefaction. Sperm count and percentage motility were analyzed by a computer-aided sperm analysis system (CellTrak/S; Motion Analysis Corporation, Santa Rosa, CA). Sperm kinetics was manually determined from 0 (none) to 4 (excellent), following the subjective grading system; after analysis, the samples were divided for IBT and SPA.

**Direct Immunobead Binding Test**

The test for ASA in semen samples was performed as recommended by Clarke et al. (1985). Immunobeads were obtained from BIO-RAD Laboratories (Richmond, CA) and consisted of polyacrylamide beads of 5-10 ㎛ diameter with covalently-bound rabbit antibodies to human immunoglobulin classes (anti IgG, IgA, and IgM). The lyophilized immunobeads (50 ㎎) were reconstituted by adding 10 ㎖ of sterile phosphate buffered saline (PBS), and then stored at 4 ℃ for 4-6 weeks. Immediately before use, 200 ㎕ of each reagent was washed once in PBS containing 0.5% bovine serum albumin (BSA).

Sperm were washed twice with PBS containing 0.5% BSA, centrifuged at 650×*g* for 5 minutes and resuspended with the same buffer at a concentration of 5 to $10\times10^6$/㎖. A drop of washed immunobead reagent was mixed with an equal drop of washed sperm (5 ㎕), covered with a 22×22 mm coverslip, and incubated in a moist chamber for 10 minutes at room temperature. The preparation was observed under phase-contrast microscope at a magnification of ×400; a motile sperm was scored if one or more beads were bound to its surface, and on this basis, 400 were counted. A specimen in which 20% or more motile sperm showed positive binding was classified as positive results. On the basis of one or more positive IBT results for ASAs attached to their own motile spermatozoa, a total of 67 males were found to have ASA.

**Sperm Penetration Assay**

SPA was performed using the method of Chang et al. (1990) and Pang et al. (1993). After semen analysis, the sample was diluted with an equal volume of sterile TEST-Yolk buffer (TYB) and mixed well. The mixture was gradually cooled at 4 ℃ and the samples were incubated for 2 days at 4 ℃. The supernatant was then aspirated and added 3.0 ㎖ of 37 ℃ Ham's F-10 containing 0.3% human serum albumin (HSA). Sperm were washed twice with the same medium, centrifuged at 650×*g* for 5 minutes, resuspended with 0.5-1.0 ㎖ of Ham's F-10 containing 1.0% HSA and incubated at 37 ℃ for 1.5-2.0 hours.

Zona-free ova were obtained from superovulated hamsters. The cumulus mass was removed with PBS containing 0.1% hyaluronidase and the zona pellucida was digested with PBS containing 0.1% trypsin and 0.3% HSA. After digestion, approximately 30 zona-free ova were added to 1 ㎖ of $1\times10^6$ human motile sperm medium and incubated at 37 ℃ 5% $CO_2$ for 3.5 hours. The eggs were then prepared for examination. They were washed to remove unattached sperm, carefully flattened under a coverslip supported by four dots of paraffin wax, and fixed overnight with Carnoy fixative (methanol : glacial acetic acid = 3 :1) before staining with 0.25% acetic lacmoid. For scoring, a phase-contrast microscope at 1,000 magnification was used. A penetrated sperm was indicated by the presence of a swollen head associated with a tail and the number of these was recorded. Fertilizing capacity was defined as the number of penetrated sperm divided by the number of inseminated eggs.

**Statistical Evaluation**

For all statistical manipulations (T-test, Duncan multiple range test, correlation, and stepwise multiple regression analysis), an SPSS/PC$^+$ was used. Data were expressed as means or percentage means±SD.

## RESULTS

The locations of ASA-binding on the sperm surface are summarized in Table 1. The presence of ASAs in the sperm head, tail (mainly tail-tip), or head and tail together had significant negative effects on sperm count, percentage motility, sperm kinetics, and fertilizing capacity, compared with ASA negative semen ($P<0.05$). The distribution of ASAs was not, however, significantly different between those regions.

In patients with detectable ASAs on sperm head, sperm count, percentage motility, sperm kinetics and fertilizing capacity were significantly lower than in ASA-negative semen ($P<0.05$). When IgG, IgA and IgM were present on sperm tail, there were significant detrimental effects on semen parameters and fertilizing capacity, compared with ASA-negative semen ($P<0.05$); too few samples contained IgM, however, for the effects of this to be examined (Table 2).

The correlation coefficient between selected variables is shown in Table 3; there was high correlation between sperm count and IgG and IgA on sperm head, and between percentage motility and IgG, IgA, and IgM on sperm tail. Fertilizing capacity, moreover, correlated closely with IgG and IgA on sperm head or tail. There was, however, no significant correlation between IgM and fertilizing capacity.

Finally, we analyzed the effect of variables on fertilizing capacity (Table 4). Among these, using stepwise multiple regression analysis, ASA IgG to the sperm head or tail, and ASA IgA to the tail appeared to have a significantly detrimental effect on fertilizing capacity ($R^2=0.028402$, $P<0.0001$).

## DISCUSSION

Studies of reproductive failure in humans have provided considerable evidence to suggest the involvement of ASAs present in either sera or semen. How these is unclear, though it interfere with IVF-ET has been suggested that antibodies on different regions of the sperm surface may have different effects on fertilization and conception (Zouari et al., 1993; Yeh et al., 1995; Clarke et al., 1985; Ayvaliotis et al., 1985). In addition, different isotypes of ASA appear to play different roles in immunologic infertility.

Yeh et al. (1995) reported that IgG and IgA antibody levels showed no significant

correlation with total fertilization rate, though when IgA showed very high levels of binding (>68%) and IgM binding was >40%, the rate fell significantly. Their data also revealed that IgA induced a significantly lower fertilization rate. Clarke et al. (1985) showed that ASAs in men, especially class IgA, reduced the fertilization rate if >80% of spermatozoa were affected. Junk et al. (1986) and Matson et al. (1988) concluded that for significantly reduced fertilization, both IgG and IgA were needed; either class alone did not produce any effect. Studies by Clarke et al. (1985) demonstrated that in men whose motile sperm was coated with a high level of IgA antibodies, the fertilization rate was significantly lower. In a subsequent study, Clarke et al. (1986) reported that when IgG and IgA titers were 10 and the IVF medium was supplemented with the wife's serum, the fertilization rate was significantly lower; if only IgG titers were 10, there was, however, no decrease. Later, when spermatozoa were treated with sera from women with ASAs, a significantly detrimental effect on *in-vitro* fertilization was seen, though this could be reduced by immuno-absorption of the IgG fraction of the serum (Clarke et al., 1988).

With regard to the impact of the antibody binding site on fertilizing capacity, Bronson et al. (1988) showed that ASA on the sperm head impaired gamete interaction, which suggested that ASA on the tip of the sperm tail, whether or not clinically significant, probably involved antigens relating to sperm transport rather than fertilization. This was confirmed by Mandelbaum et al. (1987), who demonstrated that ASA on the tail tip had no effect on fertilization, whereas ASA on the head caused significant impairment. Yeh et al. (1995) reported that IgA induced a significantly lower fertilization rate only when present on the head.

This study was designed to investigate whether Ig isotypes and the regional distribution of ASA on the surface of human sperm affected fertilizing capacity and quantitative semen parameters. To detect sperm fertilizing capacity, we performed SPA, though it has been suggested that this procedure provides no information as to whether spermatozoa can penetrate human ova complete with their vestments; a positive result in this assay does not necessarily mean, therefore, that the fertilizing capacity of the sperm is normal. Nevertheless, clinical validation of the results of our assay with human IVF showed significantly high correlation (sensitivity, 100%; specificity, 80%; positive predictive value, 97%; and negative predictive value, 100%) between the two sets of results (Pang et al., 1993). Fertilizing capacity is defined as the average number of penetrations per zona-free hamster ovum, and where this was <3, there was a lower probability of achieving fertilization *in vitro*. The results obtained under our study conditions showed that in those with positive ASAs in semen, fertilizing capacity was poor. With regard to the impact of the antibody binding site on fertilizing capacity, stepwise multiple regression analysis for IgA antibodies by binding sites showed a statistically significant reduction in this capacity only when tail-directed ASAs were present. Moreover, stepwise multiple regression analysis for IgG antibodies by binding sites showed a statistically significant reduction in fertilizing capacity only when head-directed ASAs were present

The association of ASA with infertility has been substantially documented, as mentioned above. The effect of ASA on semen quality is, however, uncertain; little is known about the relation between ASA and quantitative semen parameters. Infection, trauma and blockage of sperm ducts are associated with autoimmunization of sperm (Hendry et al., 1977; Sotolongo, 1982) and the toxic effect of infection also directly affects spermatogenesis. Some investigators have reported that ASA in the semen of infertile men had a negative effect on initial sperm motility characteristics in ejaculate (Mathur et al., 1986; Barratt et al., 1989) whereas others showed no relationship between ASAs and sperm motility (Cerasaro et al., 1985; Adeghe et al., 1989). Using a computer-aided sperm analysis system, Mathur et al. (1986) showed that sperm from men with ASA exhibited poorer motion characteristics than that from fertile donors. They also observed that during postcoital test in infertile couples, the motility of sperm found from cervical mucus showed negative correlation with ASA levels.

Gonzales et al. (1992) reported that the occurrence of sperm-bound ASAs was associated with alterations in some semen parameters, mainly low sperm count, low sperm morphology and low corrected fructose levels. In 50% of men with ASA, asthenozoospermia, teratozoospermia, leukocytospermia or hypofunction of the seminal vesicles was observed. Semen parameters were altered most frequently when IgM was present in association with IgG and/or IgA. They therefore concluded that there was an active inflammatory process in the reproductive tract, as evidenced by leukocytospermia, and this could be responsible for the abnormal semen quality.

In the present study, oligozoospermia and/or asthenozoospermia was found in 60% of men with ASA. Semen parameters, especially percentage motility, were altered most frequently (in 89% of cases) when IgM was present in association with IgG and/or IgA. ASAs were associated with lowered sperm count and motility, and sperm kinetics. It has been suggested that these phenomena are related to the production of antibodies against sperm, and that reduced concentration may be a result of local lesions appearing in the seminiferous tubules, blocking sperm passage, disrupting the blood-testis barrier, and inducing an immune response against sperm antigens (Hendry et al., 1977; Sotolongo et al., 1982; Menge and Beitner, 1989).

The relationship between ASA and sperm morphology reported by Gonzales et al. (1992) is in agreement with other findings (Menge and Beitner, 1989); they (Gonzales et al., 1992) also reported that teratozoospermia was more common in the simultaneous presence of the three isotypes (IgG, IgA and IgM) than in the presence of IgA only or a combination of IgG and IgA, and therefore concluded that the synergistic action of Igs affected sperm morphology.

For many years, efforts have been made to establish an effective therapy against autoimmune infertility in males, and these have included. Ekwere et al. (1995) reported that after corticosteroids therapy only in patients with mild histological change, antibody titers decreased significantly and there was a sixfold improvement in mean sperm concentration

and a threefold improvement in motility and morphological characteristics. Omu et al. (1996) also demonstrated that corticosteroid therapy led to significant improvement in sperm motility and in the results of the hypoosmotic swelling test. This evidence indirectly supports our findings that the appearance of sperm-bound ASAs is associated with alterations in some semen parameters, mainly low sperm count, low motility and sperm kinetics.

Our findings collectively demonstrate a direct relationship between ASA in semen and abnormal semen parameters. ASA type and binding region on the sperm surface correlated with fertilizing capacity. With regard to the impact of antibody binding site on fertilizing capacity, the presence of IgA antibodies led to a significant reduction in fertilizing capacity only when tail- and head-directed ASAs were present.

## SUMMARY

To investigate the influences on semen parameters and fertilizing capacity of immunoglobulin (Ig) isotypes and regional distribution of antisperm antibody (ASA) on the human sperm surface. Sixty-seven ASA-positive patients were compared with 96 ASA-negative donors. ASAs in semen showed significant negative effects on both semen parameters and fertilizing capacity; in those with ASAs in the sperm head and/or tail, the reductions were significant. In the head as well as the tail, there was close correlation between fertilizing capacity and both IgG and IgA. Both semen parameters and fertilizing capacity are significantly affected by the presence of ASA in semen. In particular, antibodies IgG to sperm head and/or tail, and antibodies IgA to sperm tail appeared to have a highly detrimental effect on fertilizing capacity

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**Table 1**. Results of sperm count, percentage motility, sperm kinetics and fertilizing capacity in relation to antisperm antibody negatice donors proven to be fertile and to antisperm antibody positive patients

| | Sperm Count ( $\times 10^6$/㎖) | Percentage Motility | Sperm Kinetics | Fertilizing Capacity |
|---|---|---|---|---|
| ASA negative (n=96) | 95.13 ±57.56 | 65.31 ±13.55 | 2.83 ±0.47 | 7.24 ±?.90 |
| ASA positive (n=67) | 58.36 ±50.52* | 46.72 ±20.11* | 2.44 ±0.73* | 2.19 ±2.29* |
| Head (n=31) | 56.10 ±47.31* | 51.29 ±19.15* | 2.55 ±0.51* | 2.44 ±2.64* |
| Tail (n=24) | 68.67 ±62.21* | 42.50 ±16.48* | 2.29 ±0.86* | 2.02 ±1.95* |
| Head+Tail (n=12) | 43.58 ±25.09* | 46.67 ±23.77* | 2.42 ±0.90* | 1.88 ±2.06* |

* Significantly different from ASA negative group; P<0.01, Duncan multiple range test

**Table 2**. Results of sperm count, percentage motility, sperm kinetics and fertilizing capacity in relation to the distribution of antibodies and antibody isotypes on spermatozoa

| | Sperm Count ( $\times 10^6$/㎖) | Percentage Motility | Sperm Kinetics | Fertilizing Capacity |
|---|---|---|---|---|
| ASA negative (n=96) | 95.13 ±47.56 | 65.31 ±13.55 | 2.83 ±0.47 | 7.24 ±4.90 |
| HG+ (n=35)# | 54.57 ±39.32** | 52.57 ±20.63** | 2.60 ±0.60* | 2.36 ±2.56** |
| HA+ (n=19)# | 41.63 ±43.81** | 47.90 ±18.36** | 2.47 ±0.70* | 1.13 ±0.84** |
| HM+ (n=7)# | 50.43 ±29.77* | 37.14 ±14.68** | 2.14 ±0.69** | 2.81 ±2.77* |
| TG+ (n=30)# | 58.37 ±57.69** | 46.50 ±19.26** | 2.47 ±0.86* | 2.20 ±2.07** |
| TA+ (n=23)# | 65.30 ±62.03* | 45.43 ±18.64** | 2.39 ±0.89* | 1.48 ±1.46** |
| TM+ (n=3)# | 70.00 ±27.84* | 30.00 ±17.32** | 2.00 ±0.00* | 1.17 ±0.75** |

* Significantly different from ASA negative group, P<0.05, Student's t-test

** Significantly different from ASA negative group, P<0.01, Student's t-test

# HG+: ASA IgG positive on sperm head; HA+: ASA IgA positive on sperm head; HM+: ASA IgM positive on sperm head; TG+: ASA IgG positive on sperm tail; TA+: ASA IgA positive on sperm tail; TM+: ASA IgM positive on sperm tail

**Table 3**. Correlation coefficient between selected variables

| | Sperm Count | Percentage Motility | Sperm Kinetics | Fertilizing Capacity | HG+ | HA+ |
|---|---|---|---|---|---|---|
| Sperm Count | 1.0000 | | | | | |
| Percentage Motility | 0.4248** | 1.0000 | | | | |
| Sperm Kinetics | 0.2928** | 0.4948** | 1.0000 | | | |
| Fertilizing Capacity | 0.3234** | 0.3445** | 0.3089** | 1.0000 | | |
| HG+# | 0.2570** | 0.1524 | 0.0582 | 0.3103** | 1.0000 | |
| HA+# | 0.2694** | 0.1986* | 0.1148 | 0.3100** | 0.4153** | 1.0000 |
| HM+# | 0.1211 | 0.2401* | 0.1805 | 0.1053 | 0.2577** | 0.3003** |
| TG+# | 0.1986* | 0.2958** | 0.1555 | 0.2979** | 0.1372 | 0.1235 |
| TA+# | 0.1152 | 0.2760** | 0.1822* | 0.3162** | 0.0455 | 0.0724 |
| TM+# | 0.0265 | 0.2086** | 0.1483 | 0.1159 | 0.0396 | 0.0925 |

* Significantly different from ASA negative group, P<0.05, Student's t-test

** Significantly different from ASA negative group, P<0.01, Student's t-test

# HG+: ASA IgG positive on sperm head; HA+: ASA IgA positive on sperm head; HM+: ASA IgM positive on sperm head; TG+: ASA IgG positive on sperm tail; TA+: ASA IgA positive on sperm tail; TM+: ASA IgM positive on sperm tail

**Table 4**. Slective variables affecting fertilizing capacity (multiple regression analysis: stepwise)*

| Variables | β | B | P value |
|---|---|---|---|
| Percentage Motility | 0.134253 | 0.020846 | 0.0990 |
| HG+** | -0.201613 | 0.856855 | 0.0076 |
| TA+** | -0.227031 | 0.954751 | 0.0015 |
| Sperm Kinetics | 0.170581 | 0.595816 | 0.0300 |
| HA+** | 0.163615 | 1.105802 | 0.0309 |

* $R^2$=0.028402, P,0.0001

** HG+: ASA IgG positive on sperm head; HA+: ASA IgA positive on sperm head; TG+: ASA IgG positive on sperm tail