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Effect of antispermatozoal antibodies in seminal plasma upon spermatozoal function

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Summary

The indirect immunobead test for antispermatozoal antibodies of the class IgA, IgG and IgM was applied to the seminal plasma of male partners of infertile couples. The presence of both IgA and IgG was associated with a decreased incidence of good post-coital test results and a reduced rate of fertilization of human oocytes. No significant differences were found for men with IgA or IgG alone when compared to men with no detectable antispermatozoal antibodies.

Keywords: antisperm antibodies, immunobead test, post-coital test, in-vitro fertilization

Introduction

Antispermatozoal antibodies can be identified in approximately 5–10% of male partners of infertile couples (Ansbacher, Mararang-Panga & Serivannaboon, 1971; Hanafiah, Epstein & Sobrero, 1972; Clarke *et al.*, 1985a; Junk *et al.*, 1986). Despite considerable evidence to support a significant role (Rumke *et al.*, 1974; Menge *et al.*, 1982), the importance of antispermatozoal antibodies as a cause of male infertility has remained controversial. Doubt has centred mainly upon the incidence of spontaneous conception in couples in which the male partner has antispermatozoal antibodies. For example, various studies have shown that 31% (Ansbacher, Keung-Yeung & Behrman, 1973) and 46% (Hanafiah *et al.*, 1972) of wives may become pregnant without treatment when sperm agglutinating activity is present in the male partner's serum.

The effect of antispermatozoal antibodies on sperm function has remained equally unclear. Whilst several studies have shown a clear association between the presence of antispermatozoal antibodies in men and the impairment of sperm–mucus interaction (Menge *et al.*, 1982; Parslow *et al.*, 1985; Fjallbrant, 1986), earlier reports failed to show such a relationship (Schwimmer, Ustay & Behrman, 1967; Ansbacher *et al.*, 1971; Hanafiah *et al.*, 1972). Similarly, the ability of antibodies to interfere with the interaction of the sperm with oocytes requires further clarification. It has been demonstrated, using zona-free hamster ova (Dor, Rudak & Aitken, 1981), that antibodies detected using the gelatin and tray agglutination tests do not impede penetration of the ova by sperm, but that there is

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a good correlation between antifertility effects of the sera and antibody titres obtained with the Franklin-Dukes tube slide test. Nevertheless, there is now evidence to suggest that certain antibodies in semen, detected using the direct immunobead test (Clarke *et al.*, 1985b) are responsible for reducing the fertilization rate of human oocytes *in vitro*.

The principal aims of the present study were to investigate the role of anti-spermatozoal antibodies in seminal plasma, detected using the indirect immuno-bead test (IBT) upon the results of the post-coital test (PCT) and the fertilization of human oocytes *in vitro*.

Patients and methods

Patients

A total of 105 couples was included in the study with 45 couples being tested with the IBT and the PCT, and 60 couples undergoing in-vitro fertilization (IVF) and embryo transfer after the IBT. The IBT was performed a maximum of 2 months before the PCT or IVF. Couples in which the female partner had endometriosis or antispermatozoal antibodies in the serum were excluded from the study. All male partners of the couples included were normospermic ($\geq 12 \times 10^6$ motile sperm/ml semen). This definition was derived from the recommendations of the World Health Organization (WHO) Task Force on the Diagnosis and Treatment of Infertility (Yovich & Stanger, 1984), and is based on a correlation with infertility when the total spermatozoal concentration falls below 20×10^6/ml with 60% motility.

Indirect immunobead test (IBT)

The IBT was performed on seminal fluid during initial investigation of the couple. The classes of antibody tested were IgA, IgG and IgM, and the techniques have been reported elsewhere (Junk *et al.*, 1986).

Briefly, 4×10^6 motile donor sperm were incubated at 37°C for 1 h with 0.2 ml heat-inactivated seminal fluid for testing. Meanwhile, suspensions of the immuno-beads (BioRad Laboratories, Richmond, CA, U.S.A.) at 10–15 mg/ml were pre-pared. After washing the incubated sperm, a drop of the bead suspension was mixed with a drop of the sperm suspension on a microscope slide, incubated for 10 min at room temperature and scored under phrase-contrast optics at × 320. A motile sperm was scored as positive if one or more beads were bound to its surface, and a specimen classified as positive if ≥20% motile sperm showed positive binding. The proportion of sperm with beads bound was recorded to the nearest 5%.

Post-coital test (PCT)

The PCT was performed as described previously (Matson *et al.*, 1986). Briefly, the cervix and mucus of the female partners were examined daily from day 8 of the menstrual cycle, and a subjective scoring system applied. Cervical mucus was obtained for scoring, using a blue inoculation loop (Nunc; Intermed, Roskilde, Denmark) taking care not to contaminate the sample with vaginal secretions. Four

characteristics were assessed, namely the quantity and clarity of the mucus, the length of the spinnbarkeit, the complexity of the ferning pattern after the mucus was allowed to dry on a slide, and the degree of dilatation of the cervical canal. Each characteristic was scored out of 3, giving a possible total of 12. Sexual intercourse occurred when the female partner was preovulatory and the cervical score was $\geq$ 6/12. Mucus from the endocervical canal was taken 8–12 h later using long forceps with slim blades to traverse 1.5–3.0 cm into the cervical canal for examination. The result was classified as either poor (no progressively motile sperm per high-power field) or good (1 or more motile sperm per high-power field).

In-vitro fertilization (IVF)

The technical details of the IVF programme have been documented elsewhere (Yovich & Stanger, 1984). Follicle growth was stimulated by the administration of clomiphene citrate (Clomid; Merrell-Dow Pharmaceuticals, Inc., Cincinnati, U.S.A.) and human menopausal gonadotrophin (hMG; Pergonal, Serono, Rome, Italy), or hMG alone. The response to treatment was monitored by daily measurement of serum oestradiol, progesterone and LH by radioimmunoassay from day 8. Ovulation was triggered by the occurrence of an endogenous LH surge or the administration of 10 000 IU human chorionic gonadotrophin (hCG; Primogonyl, Schering, Berlin, FRG) at an appropriate time and oocytes collected 32–36 h later.

The method of semen collection, preparation of sperm and culture conditions have been described previously (Yovich & Stanger, 1984). Oocytes were inseminated with 100 000 washed motile sperm 4–6 h after collection, and pronuclei identified 16–20 h later. The presence of two pronuclei confirmed that fertilization was under way.

Statistical analysis

Data were analysed in 2 × 2 contingency tables using the χ^2 test.

Results

The incidence of good PCT test results is shown in Fig. 1. In those 20 couples in which the male partner had no antisperm antibodies in seminal plasma, 17 (85%) gave good PCT results. A slight reduction in the incidence of good PCT results was seen when IgA (54.6%) or IgG (50%) antibodies were present alone in seminal fluid, but this reduction was significant ($P < 0.05$) when IgA and IgG were both present (14.3%).

The fertilization rate of human oocytes is given in Fig. 2. Compared with couples in which no antibodies were detected, a significantly reduced rate (38.5%) of fertilization was observed when IgA and IgG antibodies were both present in seminal fluid. However, all couples had at least one oocyte fertilized. No significant difference was observed when IgA or IgG antibodies alone were present.

Discussion

The indirect IBT was used in this study in preference to the direct method for two main logistic reasons. Firstly, the samples can be collected, frozen and then

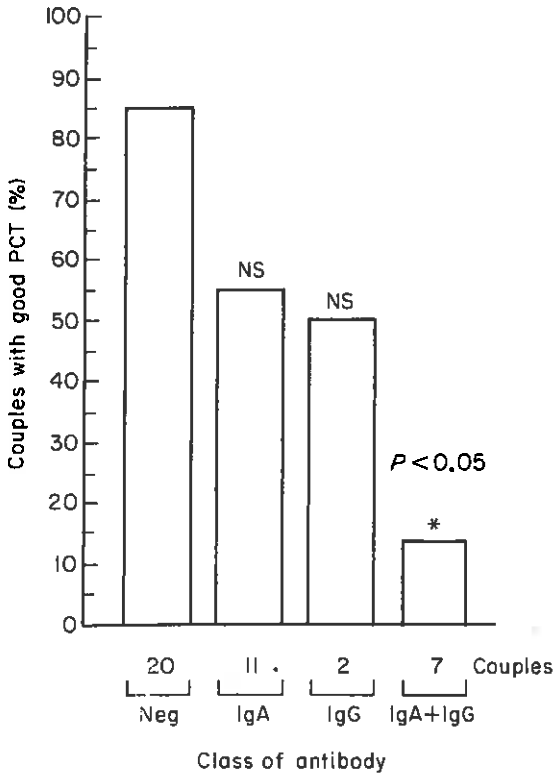

Fig. 1. The incidence of couples with a good post-coital test (PCT) in which the male partner either had antisperm antibodies present or absent (Neg) in seminal plasma.

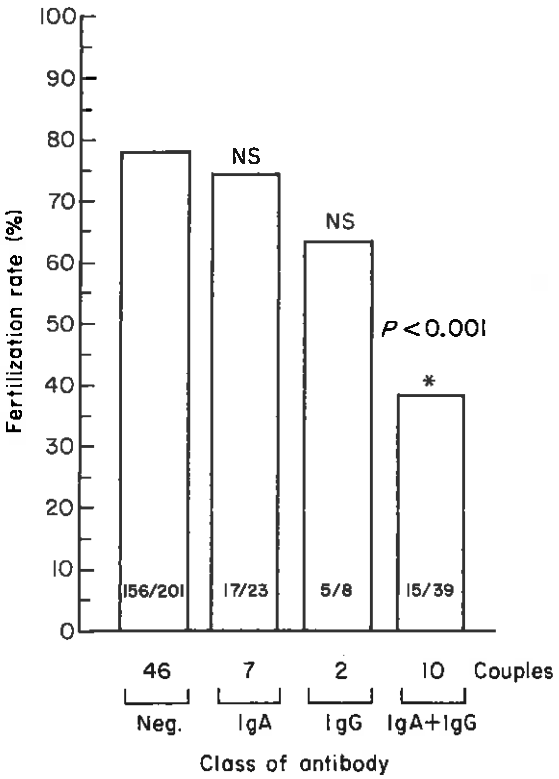

Fig. 2. The fertilization rates of human oocytes by sperm from men with antisperm antibodies present or absent (Neg) in seminal plasma.

analysed on a weekly basis by one technician, using the sperm from one donor. Secondly, the indirect IBT can be applied to blood (Clarke *et al.*, 1985a; Junk *et al.*, 1986) and cervical mucus (Clarke, 1984) samples in the same assay.

The present study has demonstrated that the detection of IgA and IgG antibodies together in seminal plasma using the indirect immunobead test are of particular clinical importance, as they were associated with an increased incidence of poor post-coital tests and a decrease in the fertilization rate of oocytes. The fact that both aspects of sperm function were affected gives credence to the role of this combination of IgA and IgG being physiologically active. However, when either IgA or IgG antibodies were present alone there was no statistically significant effect upon sperm–mucus interaction or fertilization. These results demonstrate primarily that the presence of antisperm antibodies *per se* does not impair sperm function, but rather that identification of the specific class of immunoglobulin is necessary. The data also show that the presence of IgA and IgG antibodies together is not an absolute cause of infertility, since one of the seven couples with IgA and IgG antibodies in the male partner's semen had a positive PCT result, and none of these couples had fertilization failure. It, therefore, appears, as Bronson, Cooper & Rosenfield (1984) have already stated, that antisperm antibodies will reduce, but not always totally prevent, the likely occurrence of a pregnancy, and that affected couples should be considered subfertile rather than sterile.

The ability of antisperm antibodies to affect the interaction of sperm with cervical mucus has been suggested by other workers. In particular, impairment of the penetration of mucus has been shown when sperm are coated with IgA (Parslow *et al.*, 1985) or IgG (Jager *et al.*, 1981). Nevertheless, a significant reduction in the proportion of good PCT results in the present study was only seen in the presence of IgA and IgG antibodies together. It will be interesting to see whether an increased incidence of poor PCT results, and depressed fertilization rates, will occur with greater numbers in the groups with only IgA or IgG antibodies.

The results of the present study have shown that there is not a simple relationship between the presence of antisperm antibodies and the fertilization of oocytes, but that it is the presence of both IgA and IgG antibodies that is important. Interestingly, Clarke *et al.* (1985b) have suggested a synergistic action of the two classes of antibody in affecting fertilization. Using the direct immunobead test to identify the class of immunoglobulin, a fertilization rate of 72% was found for those men with high levels of IgG and low levels of IgA antibodies upon the sperm, whereas men with high levels of both IgG and IgA antibodies had a reduced fertilization rate of 27%. The present study used the indirect immunobead test and it is accepted that some antibody will have been removed from the seminal fluid by the patients' own sperm, although the diagnostic picture remains similar. Other workers (Haas *et al.*, 1985) have shown that IgG and IgA/IgG in combination reduce the penetration of zona-free hamster ova by human sperm.

In summary, the determination of antisperm antibodies in seminal plasma using the indirect immunobead test has proved useful clinically. The identity of the immunoglobulin class is important in affecting sperm function in regard to both the PCT and fertilization. The identification of IgA and IgG antibodies together in

seminal fluid is associated with a reduced incidence of good PCT results and a reduced fertilization rate of human oocytes.

References

Ansbacher, R., Keung-Yeung, K. & Behrman, S. J. (1973) Clinical significance of sperm antibodies in infertile couples. *Fertility and Sterility*, **24**, 305–308.

Ansbacher, R., Mararang-Panga, S. & Srivannaboon, S. (1971) Sperm antibodies in infertile couples. *Fertility and Sterility*, **22**, 298–302.

Bronson, R., Cooper, G. & Rosenfield, D. (1984) Sperm antibodies: their role in infertility. *Fertility and Sterility*, **42**, 171–183.

Clarke, G. N. (1984) Detection of antispermatozoal antibodies of IgA, IgG and IgM immunoglobulin classes in cervical mucus. *American Journal of Reproductive Immunology*, **6**, 195–197.

Clarke, G. N., Lopata, A., McBain, J. C., Baker, H. W. G. & Johnston, W. I. H. (1985b) Effect of sperm antibodies in males on human in vitro fertilisation (IVF). *American Journal of Reproductive Immunology*, **8**, 62–66.

Clarke, G. N., Stojanoff, A., Cauch, M. N. & Johnston, W. I. H. (1985a) The immunoglobulin class of antispermatozoal antibodies in serum. *American Journal of Reproductive Immunology and Microbiology*, **7**, 143–147.

Dor, J., Rudak, E. & Aitken, R. J. (1981) Antisperm antibodies; their effect on the process of fertilization studied in vitro. *Fertility and Sterility*, **35**, 535–541.

Fjallbrant, B. (1986) Interrelation between high levels of sperm antibodies, reduced penetration of cervical mucus by spermatozoa, and sterility of men. *Acta Obstetrica et Gynaecologica Scandinavica*, **47**, 102–118.

Haas, G. G., Ausmanas, M., Culp, L., Tureck, R. W., Blasco, L. K. (1985) The effect of immunoglobulin occurring on the human sperm/hamster ova penetration assay. *American Journal of Reproductive Immunology and Microbiology*, **7**, 109–112.

Hanafiah, M. J., Epstein, J. A. & Sobrero, A. J. (1972) Sperm-agglutinating antibodies in 236 infertile couples. *Fertility and Sterility*, **23**, 493–497.

Jager, S., Kremer, J., Kuiken, J., Van Stochteren-Draasima, T., Mulder, I. & Wilde-Janssen, W. (1981) Induction of the shaking phenomenon by pretreatment of spermatozoa with sera containing antispermatozoal antibodies. *Fertility and Sterility*, **36**, 784–791.

Junk, S. M., Matson, P. L., O'Halloran, F. & Yovich, J. L. (1986) Use of immunobeads to detect human antispermatozoal antibodies. *Clinical Reproduction and Fertility*, **4**, 199–206.

Matson, P. L., Ruvik, A. I., O'Halloran, F. & Yovich, J. L. (1986) The value of the postcoital test in predicting the fertilisation of human oocytes. *Journal of In vitro Fertilization and Embryo Transfer*, **3**, 110–113.

Menge, A. C., Medley, N. E., Mangione, C. M. & Dietrich, J. W. (1982) The incidence and influence of antisperm antibodies in infertile human couples on sperm–cervical mucus interactions and subsequent fertility. *Fertility and Sterility*, **38**, 439–446.

Parslow, J. M., Poulton, T. A., Besser, G. M. & Hendry, W. F. (1985) The clinical relevance of classes of immunoglobulins on spermatozoa from infertile and vasovasostomised males. *Fertility and Sterility*, **43**, 621–627.

Rumke, P., Amstel, N. V., Metser, E. N. & Bezemer, P. D. (1974) Prognosis of fertility of men with sperm agglutinins in the serum. *Fertility and Sterility*, **25**, 393–398.

Schwimmer, W. B., Ustay, K. A. & Behrman, S. J. (1967) An evaluation of immunologic factors of infertility. *Fertility and Sterility*, **18**, 167–180.

Yovich, J. L. & Stanger, J. D. (1984) The limitations of in vitro fertilization from males with severe oligospermia and abnormal sperm morphology. *Journal of In vitro Fertilization and Embryo Transfer*, **1**, 172–179.

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