

IMMUNOGLOBULIN PROFILE OF INFERTILE COUPLES

By

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SUMMARY

This study was conducted to find out nature of Immunoglobulin present in the sera, cervical mucus and semen in 30 infertile couples, suffering from infertility of unexplained origin. The antisperm antibodies were detected by Indirect Immunofluorescence test (IFT). IFT positive cases were evaluated for the Immunofluorescence pattern on the spermatozoa and the class of Immunoglobulin present. In 60% of cases systemic antisperm antibodies in sera of couples were found and in 46.6% local antisperm antibodies were demonstrated in the cervical mucus and semen. The commonest morphology of spermatozoa showing fluorescence on the donor's spermatozoa in all the samples was acrosome. The Immunoglobulin present in serum was mainly IgM whereas locally in the cervical mucus and semen was IgA. The Immunoglobulin staining acrosomal part of the spermatozoa was found to be IgM and postnuclear cap IgG. The tail piece stained exclusively by IgG immunoglobulin. Ten of the fertile couples serving as control were negative for antisperm antibodies.

Introduction

Autoantigenicity of Mammalian spermatozoa and that of seminal plasma was first demonstrated by Landsteiner in the year 1899. In women with unexplained infertility of several years duration antisperm antibodies appear to be involved in a significant percentage (Isojima *et al* 1972). Over all results suggest that 7 to 17% of women with unexplained infertility exhibit antisperm antibodies. Antibodies have been demonstrated in both serum and seminal plasma and may be in the IgG, IgA or IgM antibody classes (Sullivan and Quinlivan 1974, Friberg 1974) Immunoglobulins are also found in

the cervical mucus and may be IgA or IgG classes.

Material and Methods

This study was carried out in 30 couples with unexplained infertility of more than two years duration attending sterility clinic of LNJP Hospital, New Delhi during the period April, 1983 to April, 1984. Basic investigations for infertility including Hysterosalpingography and diagnostic Laparoscopy with chromotubation were within normal limits and post coital test showed a hostile cervical factor according to criteria laid down by Marcus and Marcus (1963). The couple was investigated for the presence of systemic and/or local antisperm antibodies and IFT positive, cases

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were further evaluated for the Immunoglobulin classes of antibodies on the spermatozoa. The patterns of the fluorescence on the spermatozoa were also studied and documented. 3 c.c. of serum from each partner was collected. Cervical mucus was aspirated by a syringe at the ovulatory phase and a semen sample of husband was also collected after 5 days of abstinence by masturbation for examination. Indirect Immunofluorescence technique—method of Hjort and Hansen (1971) was followed using fluorescein Isothiocyanate conjugated sera. The samples showing fluorescence at a dilution of 1:4 and above were taken as positive.

Observations

In the present study 60% of cases had systemic antisperm antibodies and 46.6% showed local antibodies.

The numerator indicates number of positives. The denominator indicates the number of samples stained.

The commonest area showing fluorescence was acrosome, followed by the post nuclear cap. Isolated equator staining was not observed.

Numerator indicates the number of positive cases. Denominator indicates the number of total cases.

It was observed that IgM was mainly present in the sera while IgA was secreted locally in the semen and cervical mucus.

Thus, the acrosome staining is mainly IgM while the post nuclear staining is IgG mainly. The tail staining is exclusively IgG.

Discussion

In the present study 40% sera from females and 20% from male partners were positive for antisperm antibodies. Hansen (1972) investigated the immunoglobulin classes that were involved in carrying the sperm antibody activity in the sera of infertile females and found most of the cases

TABLE I

Morphological of the Immunofluorescence Observed in the Donor's Spermatozoa During Indirect Immunofluorescent Test

| Spermatozoal morphology | Total | Wife's serum (1:4) | Husband's serum (1:4) | Cervical mucus | Semen |
|-------------------------|-------|--------------------|-----------------------|----------------|-------|
| Acrosome | 26/32 | 11/12 | 3/6 | 9/10 | 3/4 |
| Equator | 0/32 | 0/12 | 0/6 | 0/10 | 0/4 |
| Acrosome + Equator | 1/32 | 0/12 | 0/6 | 1/10 | 0/4 |
| Post nuclear cap | 7/32 | 2/12 | 0/6 | 3/10 | 2/4 |
| Main tail piece | 3/32 | 2/12 | 0/6 | 1/10 | 0/4 |
| Whole sperm | 4/32 | 1/12 | 3/6 | 0/10 | 0/4 |

TABLE II

Class of Immunoglobulins Present in the Antisperm Antibodies

| Group | Total | IgG | IgM | IgA | IgG + IgM |
|-----------------|-------|------|------|------|-----------|
| Wife's serum | 12/30 | 1/12 | 7/12 | 1/12 | 3/12 |
| Husband's serum | 6/30 | 1/6 | 5/6 | — | — |
| Cervical mucus | 10/30 | 1/10 | — | 9/10 | — |
| Semen | 4/30 | 1/4 | — | 3/4 | — |

TABLE III

Coreation of the Class of Immunoglobulin and the Morphology of Spermatozoa Showing Immunoflourescene

| Spermatozoal morphology | Total | IgG | | IgM | | IgA | | IgG+IgM | |
|-------------------------|-------|-----|------|-----|------|-----|------|---------|------|
| | | No. | % | No. | % | No. | % | No. | % |
| Acrosome | 26 | 3 | 21.7 | 12 | 46.1 | 8 | 30.7 | 3 | 11.1 |
| Equator + acrosome | 1 | — | — | 1 | 100 | — | — | — | — |
| Post nuclear cap | 7 | 4 | 57.1 | 1 | 14.2 | — | — | 2 | 28.5 |
| Main tail piece | 3 | 3 | 100 | — | — | — | — | — | — |
| Whole sperm | 4 | — | — | 1 | 25.0 | 1 | 25.0 | 2 | 50.0 |

had either IgG or IgM or various proportions of the two immunoglobulins on the other hand IgA was detected only in small amounts as sperm antibody. In present study the immunoglobulin present in the serum was mainly IgM (Table II).

It is recognised that immunoglobulins are present and in some cases are synthesised in the human cervix. Localised inflammation appears to contribute to the development of sperm antibody. There is very high incidence of sperm antibodies in prostitutes (Schwimmer *et al* 1967). IgA positive cells have been shown in cervical tissue in 53 out of 54 women with abnormal post coital test (Sinha *et al* 1977). Shulman (1975) measured Ig, IgM and IgA levels in cervical mucus. IgM was essentially absent from cervical mucus and IgG was usually predominant over IgA. In the present study antisperm antibody by IFT was detected in 10 (33.3%) out of 30 Cervical mucus samples from infertile women. In 9 cases (90%) it was IgA and only in one case (10%) IgG class. (Table II). Coelingh and Menge (1974) detected sperm antibody by IFT in 3 out of 13 cervical mucus samples of infertile women. In two cases it concerned IgA and in one case IgG.

In our study the commonest area showing the immunoflourescence was acrosome followed by post nuclear region. Isolated

equator staining was not observed in any cases. The whole sperm showing flourescence was seen in 4 cases (Table I) Fig. 1 showing principal staining patterns of

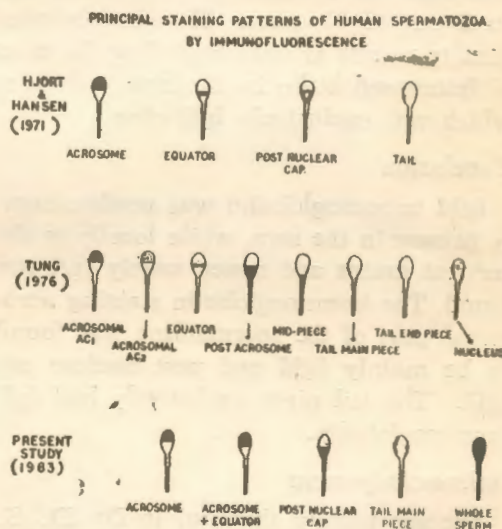


Fig. 1

human spermatozoa by Immunoflourescence as reported by Hjort and Hansen (1971) and Tung (1976).

In the present study 13.3% of cases had antisperm antibodies in the semen and class of immunoglobulin present was mainly IgA followed by IgG. Rumke *et al* (1974) found that in seminal plasma only IgG and IgA immunoglobulins were present, but IgM was absent. In our

series also IgM was not found in the semen. It was observed that immunoglobulin staining the acrosomal part of spermatozoa was mainly IgM (46.1%) whereas IgG and IgA contributed 11.7 and 30.7% respectively (Table III). Isolated equator staining was not observed. Post nuclear cap had mainly IgG (57.1%) followed by IgG + IgM (28.5%) and IgM (14.3%). Main tail piece was stained exclusively by IgG, Immunoglobulin. Whole sperm was fluorescent in IgG + IgM (50%), IgM (25%) and IgA (25%) class of Immunoglobulins. In a series Husted (1975) reported antibody against the front part of acrosome mainly IgM but also reported combination of IgM and IgG in 6 out of 63 cases. He also observed similar results as ours regarding the class of Immunoglobulin in the main tail piece which was exclusively IgG class.

Conclusion

IgM immunoglobulin was predominantly present in the sera, while locally in the cervical mucus and semen mainly IgA was found. The immunoglobulin staining acrosomal part of the spermatozoa was found to be mainly IgM and post nuclear cap IgG. The tail piece exclusively had IgG immunoglobulin.

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