Estimation of Antisperm Antibodies in Females with Unexplained Infertility

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To asses antisperm antibodies (ASA) levels in infertile and fertile females, and to look for patterns in the expression of antisperm antibodies in serum and cervical mucus of these subjects. Selective study in females with unexplained infertility (n=30) and fertile females (n=10). Pattern of antisperm antibody positivity in serum and cervical mucus was studied. Samples were collected from gynaecological outdoor, Jinnah Hospital and Hamid Latif Hospital Lahore, from July 1999 to October 2000. Thirty females aged between 19-39 years, with no indication of hormonal or physical causes for their infertility and 10 age matched fertile females as control, were taken as subjects. Serum and cervical mucus samples of all these subjects were tested for ASA by an enzyme linked immunosorbent assay. Antisperm antibodies were positive in 33.3% of infertile females, either in their serum or cervical mucus or in both, while in all the fertile control subjects were negative for these antibodies. ASA were positive in 23.3% of sera and 26.6% of cervical mucus samples obtained from infertile females. Serum and cervical mucus both were positive for ASA in 16.6% of infertile females while 10% exhibited ASA in cervical mucus alone and 6.7% in serum alone. Statistically significant differences in levels of ASAs were obtained between infertile and fertile females. Also in many samples ASA levels in serum did not correlate with ASA levels in cervical mucus. This data emphasizes the importance of detecting ASA, in sera as well as in genital tract secretions, for correct evaluation of sperm immunity.
Key words: antisperm antibodies, unexplained infertility

Infertility among couples of reproductive age is a perplexing condition when the cause cannot be determined. These cases are classified as unexplained infertility[1] In a subset of these subjects antisperm antibodies (ASA) with sperm agglutinating or immobilizing activities have been detected in blood or fluids of reproductive tract[2]. Research studies in animals and human systems have demonstrated conclusively that ASAs can interfere with fertilization[3]. Antisperm antibodies affect fertility at different steps including sperm motility, preventing migration through cervical canal and inhibiting different aspects of the sperm interaction with mature ovum[4] The possibility exists that ASA impair the functional integrity of sperm membrane[5]. Immuno-suppressive treatment of infertile couple, exhibiting antisperm antibodies, has been claimed to improve their reproductive performance[6]. Whereas follow up of untreated couples revealed that fertility was inversely related to the presence of high titres of antibodies[7]. In view of this association between reproductive failure and antisperm antibodies, determination of these antibodies in sera and genital tract secretions is considered an essential step during clinical evaluation of infertile couple[8].

Hence, this study was carried out to asses antisperm antibody levels in infertile and fertile females and to look for patterns in the expression of antisperm antibodies in serum and cervical mucus of these subjects.

Materials and methods

Selection of subjects

This study was carried out on forty subjects selected from Jinnah Hospital and Hamid Latif Hospital, Lahore. These included 30 females aged between 19-39 years, seeking advice for unexplained infertility, of at least 2 years duration, associated with regular ovulation, patent fallopian tubes, no laproscopic evidence of peritubular adhesions, fibroids or endometriosis and normal semen analysis of the husband. Ten age matched fertile apparently healthy females, with one or more children, selected from patients attending gynaecology outdoor for postnatal checkup or for complaints other than infertility were included as controls.

Sample Collection

Serum and cervical mucus samples were obtained from all the subjects. The serum samples were stored frozen at -20^0 C until the assay could be performed. Samples of cervical mucus were collected 48 to 72 hours before ovulation after at least 48 hours of sexual abstinence. Collection of samples was performed using a 3ml graduated syringe to which an extension of sterile plastic tubing was attached. After collection mucus samples were mixed with an equal volume of bromelain (Sigma B4882, 2 mg/ml of PBS) and allowed to liquefy at room temperature for 10 minutes. The mucus/bromelain mixture was spun at 1000 xg for 5 minutes and the supernatant fluid was separated and stored frozen.

Assay of Antisperm Antibodies

All samples collected were tested for antisperm antibodies by an enzyme linked immunosorbent assay (ELISA) using a commercially available kit (Bio Serve, Germany).

Interpretation of Results

The cut-off value was calculated by taking the mean of the optical density of three positive controls divided by the mean of the optical density of three negative controls. The value of samples was taken as: optical density of the sample divided by the mean of three negative controls. Any sample giving a value greater than the cut-off value was considered positive

for ASA. Statistical analysis of the data was performed using student's t-test and Chi square test.

Results

In this study, 10 (33.3%) females with unexplained infertility had antisperm antibodies either in their serum or cervical mucus or in both, while all the normal control subjects were negative for these antibodies. Among 22 females with primary infertility, 8 (36.4%) were positive for ASA. Of 8 females with secondary infertility 2 (25%) were positive for ASA. There was statistically significant difference ($p<0.01$) in ASA positivity when infertile subjects were compared with fertile group (Table I). Antisperm antibodies were detected in sera of 7 (23.3%) and cervical mucus of 8 (26.6%) infertile females. The pattern of distribution of ASA in infertile patients is shown in Table II.

Table 1 Results of antisperm antibody (ASA) analysis in infertile and fertile control subjects.

Subjects	No. of ASA +ve	No. of ASA –Ve
Total infertile (n=30)	10(33.3%)	20(77.7%)
Primary infertile (n=22	8(36.4%)	14(63.6%)
Secondary infertile (n=8)	2(25%)	6(75%)
Fertile (control group) (n=1		10 (100%)

Table II. Pattern of distribution of antisperm antibodies (ASA) in primary and secondary infertile groups.

Subjects	Serum ASA +ve	CM ASA +ve	Both CM & Serum ASA +ve	Total AS +ve
Total infertil (n=30)	2(6.7%)	3(10%)	5(16.6%)	10(33.3%
Primary infertile (n=22)	2(9.1%)	2(9.1%)	4(18.2%)	8(36.3%)
Secondary infertile (n=8)	0	1(12.5%)	1(12.5%)	2(25%)

Key CM: Cervical mucus

Distribution of age and duration of infertility in the infertile patients is shown in Tables III and IV. The mean±SD age of patients positive for ASA was 30±3 years and that of ASA negative females was 28.2±4.8 years with range of 19-39 years. The mean±SD duration of infertility was 8.3±4.3 years and 5.3±3.2 years in ASA positive and negative subjects respectively ($p<0.05$), with range of 3-15 years.

Table III. Age distribution of antisperm antibodies (ASA) positive and negative infertile groups.

Age group (Years)	ASA +ve	ASA –Ve
<20	1(10%)	1(5%)
20-29	3(30%)	9(45%)
30-39	6(60%)	10(50%)

Table IV. Duration of infertility in antisperm antibodies (ASA) positive and negative infertile groups.

Duration of infertility (Years	ASA +ve	ASA –Ve
2-3	2(20%	7(35%)
4-6	2(20%)	&(35%)
7 or more	6(60%)	6(30%)

Discussion

This study showed statistically significant differences between infertile females and fertile controls for sperm antibody positivity in serum and cervical mucus both. The percentage of our patients found positive for these antibodies corresponds well with the one reported in some of the current studies[9] Mathur et al[6] have observed that 44% of women with primary infertility had antisperm antibodies either in serum or cervical mucus while 48% of secondary infertile women were positive for antisperm antibodies. There is sufficient evidence to support the hypothesis that ASA play a role in selected couples with unexplained infertility. Both the prevalence and magnitude of this role remains controversial. Witkin and David[10] observed that incidence of ASA in women who were infertile was 38% and in those who were fertile was 12%. Low levels of circulating ASA are commonly detected in sera of fertile women[11] Critser et al[12] have shown that frequency of circulating ASA is similar in fertile and infertile populations. Recent reviews and literature surveys indicate the incidence of antisperm antibodies varying from 5-40%[13] The difference in the incidence of these antibodies may be due to different type of population studied considering their general health, economic problems, cultural factors and the availability and utilization of different diagnostic tests and procedures[14]

In this study the frequency of antisperm antibodies was higher in cervical mucus than in the sera of infertile females. The occurrence of sperm antibody in cervical mucus was not significantly related to sperm antibody in serum from the same person. Antisperm antibodies were present in both serum and cervical mucus in 16.6% of infertile patients while 10% patients exhibited ASA in cervical mucus alone and 6.7% in serum alone. These individuals may have been misevaluated and considered negative for sperm immunity if only either serum or cervical mucus had been tested. Similar observations regarding the higher incidence and high titer of local antibody activity in female patients have been reported by others[15-16]. Mathur et al[6] demonstrated that 35% of patients with primary infertility had ASA in both their sera and CM, 4% had ASA in serum only and 6% had ASA in CM only. In patients with secondary infertility 12% had ASA in serum only, 4% had ASA in CM only and 32% had ASA in both serum and cervical mucus. The occurrence of sperm antibodies in cervical mucus cannot be explained

merely on the basis that it is transudated from the blood. The source of these antibodies may be local synthesis. Negative results from serum detection cannot exclude the presence of antibodies in the cervical mucus[17].

The majority of the patients in this study were in age group 30-39 years. The age related decline in a woman's infertility is widely acknowledged[18]. The decline in spontaneous pregnancy rate with advancing age may be related to progressive ovulatory dysfunction and exhaustion of normal oocytes, but unexplained infertility is most common factor in older women[19]. In this study ASA positivity was more frequent in older females and with longer duration of infertility. This finding is supported by Daitoh et al[20].

We conclude that ASA play a major role in causing infertility in a subset of females with unexplained infertility. We recommend antisperm antibody testing in serum, as well as cervical mucus for correct evaluation of sperm immunity.

References

1. Koide SS, Wang L, Kamada M. Antisperm antibodies associated with infertility: properties and encoding genes of target antigens. Proc Soc Exp Biol Med 2000; 224(3): 123-32.
2. Bronson R, Cooper G, Rosenfeld D. Sperm antibodies: their role infertility. Fertil Steril 1984; 42: 171-83.
3. Gubin DA, Dmochowski R, Kutteh WH. Multivariant analysis of men from infertile couples with and without antisperm antibodies. Am J Reprod Immunol 1998; 39(2): 157-60.
4. Menge AC, Christman GM, Ohl DA, Naz RK. Fertilization antigen-1 removes antisperm autoantibodies from spermatozoa of infertile men and results in increased rates of Acrosome reaction. Fertil Steril 1999; 71: 251-60.
5. Jairaj S, Check JH, Bollendorf A. Do antisperm antibodies cause functional impairment of the sperm membrane as manifested by a low hypoosmotic swelling test score? Arch Androl 2000; 44(3): 231-35.
6. Mathur S, Baker ER, Williamson HO, Derrick FC, Teague KJ, Fundenberg HH. Clinical significance of sperm Antibodies in infertility. Fertil Steril 1981; 36: 486-95.
7. Marshburn PB, Kutteh WH. The role of antisperm antibodies in infertility. Fertil Steril 1994; 61: 799-811.
8. Shai S, Bar-Yoseph N, Peer E, Naot Y. A reverse (antibody capture) enzyme-linked immunosorbent assay for detection of antisperm antibodies in sera and genital tract secretions. Fertil Steril 1990; 54: 894-901.
9. Moghissi KS, Sacco AG, Borin K. Immunological infertility. 1. Cervical mucus antibodies and post coital test. Am J Obstet Gynecol. 1980; 136: 941-48.
10. Witkin SS, David SS. Effect of sperm antibodies on pregnancy outcome in a subfertile population. Am J Obstet Gynecol 1988; 158: 59-62.
11. Bronson RA. Antisperm antibodies diagnosis and treatment. In: Hammed MG, Talbert LM (eds). Infertility. A Practical Guide for the Physician. 3rd Ed. Boston: Blackwell Scientific Publication 1992: 84-101.
12. Critser JK, Villines PM, Coulam CB, Crister ES. Evaluation of circulating antisperm antibodies in fertile and patient populations. Am J Repord Immunol 1989; 21: 137-42.
13. Mazumdar S, Levine AS. Antisperm antibodies: Etiology, pathogenesis, diagnosis and treatment. Fertil Steril 1998; 70: 799-810.
14. Lunenfeld B, Insler V.Infertility: Insler V, Lunenfeld B (eds). Infertility: Male and Female. 2nd Ed. Edinburgh: Churchill Livingstove, 1993: 3-7.
15. Moghissi KS, Sacco AG, Borin K. Immunological infertility. 1. Cervical mucus antibodies and post coital test. Am J Obstet Gynecol. 1980; 136: 941-48.
16. Menge AC, Medky NE, Mangione CM, Dietrich JW. The incidence and influence of antisperm antibodies in infertile couples on sperm cervical mucus interation and subsequent fertility. Fertil Steril 1982; 38: 439-46.
17. Shulman S, Hu C. A study of the detection of sperm antibody in cervical mucus with a modified immumobead method. Fertil Steril 1992; 58: 387-91.
18. Lim AST, Tsakok MFH. Age related decline in fertility: a link to degenerative oocytes? Fertil Steril 1997; 68: 265-75.
19. Tulandi T, Arronet GH, McInnes RA. Infertility in women over the age of 36. Fertil Steril 1981; 35: 611-14.
20. Daitoh T, Kamada M, Yamano S et al. High implantation rate and consequently high pregnancy rate in vitro fertilization embryo transfer treatment in infertile women with anti -sperm antibody. Fertil steril 1995;63:87-91.