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Stacey Lee Mandelbaum
Yale University

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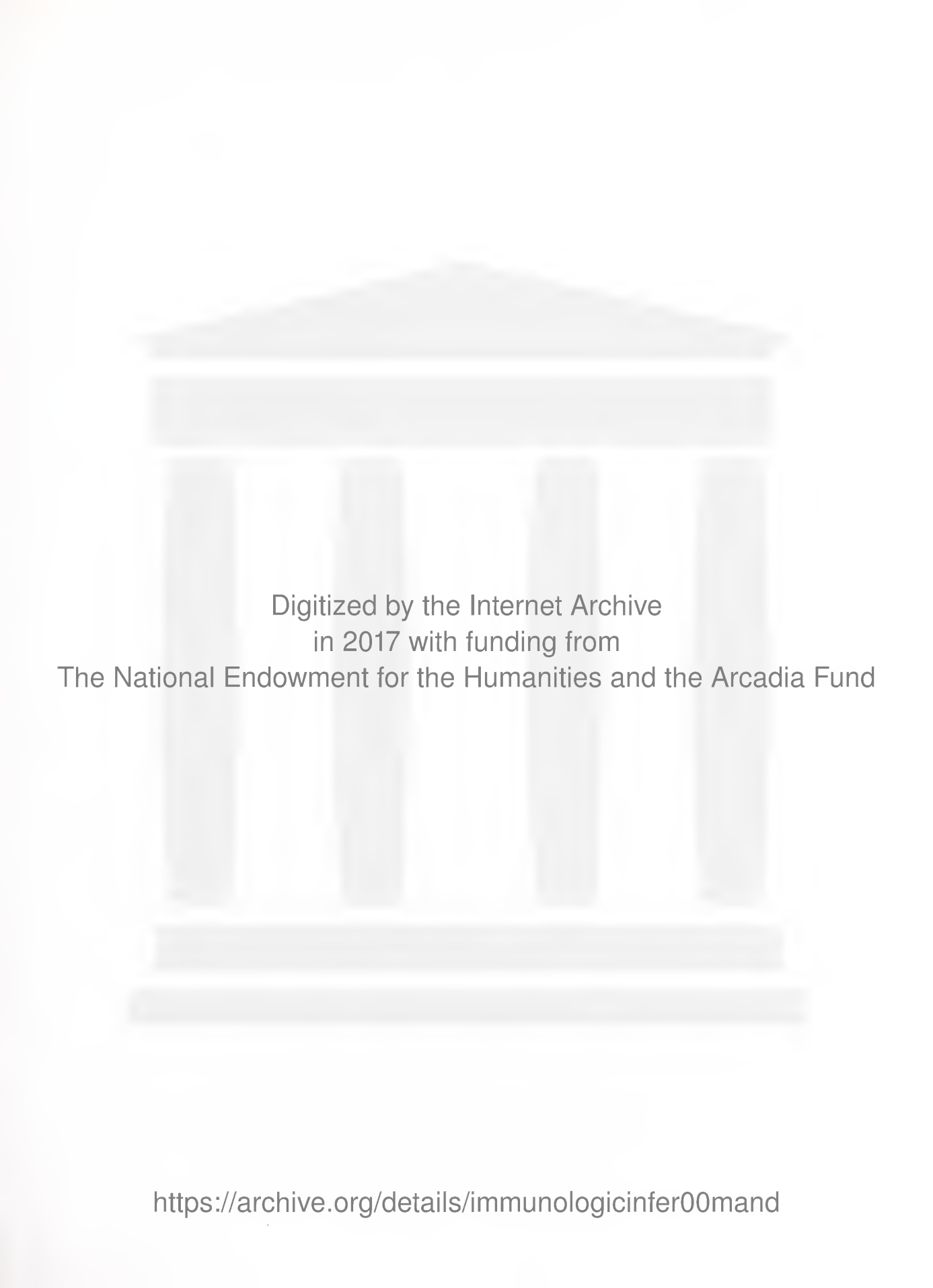

Immunologic Infertility and In Vitro Fertilization/Embryo Transfer (IVF/ET): The Clinical Significance of Antisperm Antibodies

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

by

Stacey Lee Mandelbaum

1986

To Alex, Mom and Dad, Jay and Sherry

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ABBREVIATIONS USED

BSA	bovine serum albumin
ELISA	enzyme-linked immunosorbent assay
FSH	follicle stimulating hormone
GAT	gel agglutination test
hCG	human chorionic gonadotropin
hMG	human menopausal gonadotropin
HLA	human leukocyte antigen
IBA	immunobead binding assay
IVF/ET	in vitro fertilization/embryo transfer
MAR	mixed agglutination reaction
NS	not statistically significant
PBS	phosphate buffered saline
PCT	postcoital test
SCMCT	sperm-cervical mucus contact test
SEM	standard error of the mean
SIT	sperm immobilization test
TAT	tray agglutination test
TSAT	tray-slide agglutination test

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ABSTRACT

IMMUNOLOGIC INFERTILITY
AND IN VITRO FERTILIZATION/EMBRYO TRANSFER (IVF/ET):
THE CLINICAL SIGNIFICANCE OF ANTISPERM ANTIBODIES

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Antisperm antibodies are believed to impair reproduction and have been detected in infertile couples, particularly those whose infertility is unexplained. An unselected infertile population undergoing IVF/ET was studied. The semen, serum and follicular fluid of 40 couples who underwent IVF/ET between August and October 1985 at Yale-New Haven Hospital were tested with the immunobead binding assay to identify antisperm antibodies of IgA, IgG and IgM isotypes, directed to sperm head, tail and tail tip. Antibody binding to sperm tail tip did not affect fertilization. Antibodies to sperm head in female serum reduced the oocyte fertilization rate significantly from 71% to 31% ($p<0.001$). Antibodies to sperm head in female serum also reduced the zygote cleavage rate from 91% to 67% ($p=0.051$). Serum antibodies to sperm head were present in 60% of women in whom no ova fertilized ($n=5$), compared to 6% of women whose ova did fertilize ($n=35$; $p<0.05$). The oocyte fertilization rate was also lower in women with antibodies to sperm head in follicular fluid (29%) than in those without these antibodies (66%; N.S.). Analysis of single antisperm antibody isotypes was not as useful as the use of

all isotypes combined in predicting fertilization. Fifty-five percent (n=11) of the couples with unexplained infertility had antibodies to sperm head in one or more fluid tested, compared to an 8% incidence in couples with tubal infertility (n=25; p=0.005). In summary, 1) clinically significant antibodies to sperm were present in an unselected IVF/ET population, particularly in those patients whose infertility was unexplained; 2) the presence of antibodies to sperm head in female serum significantly reduced fertilization rate.

INTRODUCTION

Approximately 10% of couples who desire pregnancy are infertile.[1] For most infertile couples, medical investigation detects an abnormality in one or both partners which accounts for infertility. However, in 10-20% of infertile couples, the investigation reveals a normal semen analysis in the man and regular ovulation, a normal uterine cavity and patent fallopian tubes without peri-tubal adhesions or endometriosis in the woman.[2,3] If these couples have been having regular unprotected sexual intercourse for at least two years and no cause for infertility is found, their infertility is described as "unexplained."

Immunologic factors are probably responsible for a number of cases of unexplained infertility. More than 20 years ago, Franklin and Dukes[4] reported finding antisperm antibodies in 79% of women with unexplained infertility. Recent studies with newer methods have shown a somewhat lower incidence of antisperm antibodies in unexplained infertility. Antisperm antibodies were detected in the semen of 21% of men with otherwise unexplained infertility.[5] Circulating antisperm antibodies were found in 13% of women and 7% of men with otherwise unexplained infertility.[6] In general, immunologic factors play a significant role in the pathogenesis of approximately 10-20% of unexplained infertility.[7,8,9,10]

The chance of conception has been shown to be significantly decreased in couples with antisperm antibodies. In couples where antibody binding of >50% of the sperm in the man's ejaculate was the only known cause of infertility, the pregnancy rate without treatment was 15%, as compared with a 67% conception rate of infertile men with <50% of their sperm bound.[11] Among women with unexplained or minor organic infertility of three or more years duration, the pregnancy rate without treatment was 13% in those with circulating antisperm antibodies and 45% in those without. Clearly, current investigations must identify clinically significant antibodies to sperm and develop effective therapies against them.

Spontaneously-occurring antibodies to sperm were first detected by Rumke and Hellinga (1959)[12] in infertile men. Since then, a number of methods to detect antisperm antibodies have been developed. However, most of these have gained only qualified acceptance because they have failed to reliably measure clinically significant antibodies to sperm.[7,8,13,14,15,16] Bronson et al[17] have recently described an immunobead binding assay to detect antibodies to sperm in serum, semen and other reproductive fluids. The assay detects immunoglobulin bound to the surface of living sperm and identifies the region of antibody binding, the isotype of immunoglobulin bound, and the extent of binding. Use of the assay has been reported by only two groups[17,18]

and is in its infancy. In early studies by Bronson's group,[11,19,20] the assay has been shown to identify clinically significant antisperm antibodies.

Why test for antibodies to sperm in an In Vitro Fertilization/Embryo Transfer (IVF/ET) population? IVF/ET, which is most often used to treat patients with tubal infertility, is also being used empirically to treat patients with immunologic infertility[21,22,23,24,25,26] and patients with unexplained infertility,[27] who are at risk for immunologic infertility but may not have been tested for antibodies to sperm. There are no published data on the incidence of antibodies to sperm in an unselected infertile population undergoing IVF/ET. In addition to incidence, studying an unselected IVF/ET population would define the clinical and prognostic significance of these antibodies. It would indicate the therapeutic value of the IVF/ET process for these patients and might provide the opportunity to tailor IVF/ET methods to maximize the chance of conception for these patients in the future. Moreover, results of IVF outcome in these patients provide a direct opportunity to evaluate the effects of antibodies to sperm on human fertilization, cleavage and conception in IVF/ET.

In this study, infertile couples undergoing IVF/ET were studied, using the immunobead binding assay, to determine the incidences of antibodies to sperm in male and female serum, semen and follicular fluid. The effects of various

types of antibodies to sperm in men and women on fertilization, cleavage and subsequent development of pregnancy in an IVF/ET program were analyzed.

MATERIALS AND METHODS

Patients

All consenting couples who underwent IVF/ET with retrieval of at least one oocyte between August and October 1985 at Yale-New Haven Hospital were studied prospectively. This represented an unselected IVF/ET population of 40 couples with infertility secondary to one of three etiologies: tubal disease (n=25), male factor (n=4) or unexplained (n=11). The etiology and type (primary vs. secondary) of infertility, previous postcoital test (PCT) results, and patient age were obtained by chart review. In the classification of infertility,[27] tubal infertility was defined by either absent tubes, bilaterally occluded tubes after tubal surgery or failure to conceive within 18 months after tubal surgery for tubal disease even with apparently patent tubes. Infertility due to male factor was defined as infertility associated with abnormal semen analyses ($<20 \times 10^6$ cells/ml, <60% normal morphology or <50% good progressive motility) on at least two occasions separated by more than three months. Unexplained infertility was defined as infertility for which no cause was found despite a complete investigation, including semen analysis, PCT, endometrial biopsy for histologic dating, hysterosalpingogram and laparoscopy.

Informed consent was obtained from all couples prior to

collection of samples, and each participating couple was given an information sheet describing the study. There were three couples with tubal infertility who underwent IVF/ET during the interval who declined to participate. The protocol used was reviewed and approved by the Yale University Human Investigations Committee (Protocol #3638).

Collection of Samples

Male and female serum, follicular fluid and semen were obtained from each couple for antisperm antibody testing. All samples were collected on the day of laparoscopic aspiration of oocytes for all but two couples studied. In one case, a semen sample was obtained two weeks after laparoscopy because all semen provided on the day of laparoscopy had been used for oocyte insemination. In the other case, the couple provided serum and semen several months after laparoscopy because samples were again not available from the time of laparoscopy.

Blood was obtained from male and female patients by venipuncture and was left to clot at room temperature for 15-30 minutes. Clotted blood was centrifuged at 800g for 8 minutes and serum was removed for testing. Similarly collected female serum, which was heat-inactivated, was used in various media for IVF/ET as described below.

Follicular fluid was obtained during laparoscopic

aspiration of oocytes for IVF/ET at Yale-New Haven Hospital according to the procedures previously described by Laufer et al.[28] Follicular fluid from a single aspirating suction trap, which in the vast majority of cases contained a single oocyte, was tested for each patient. When blood-tinged follicular fluid was obtained, warm heparinized medium devoid of serum was added to the trap and the dilutional factor was calculated. The particular oocyte(s) retrieved from the trap were noted for future evaluation of fertilization and cleavage parameters. After oocyte removal, the follicular fluid was centrifuged at 800g for 8 minutes and the supernatant was removed for testing.

Semen samples tested were those used to inseminate oocytes for IVF/ET except in the two cases described above. Semen was collected by patients at the hospital after several days of abstinence, as per IVF/ET protocol,[27] and was brought to the laboratory immediately after collection. Semen was allowed to liquify at room temperature, and an aliquot was removed for antibody testing after semen analyses were performed using a Makler counting chamber as previously described.[29]

Testing on all fluids was begun 1-2 hours after collection of semen. Testing of serum and follicular fluid for each couple was performed using sperm obtained from that couple except in cases where a man's semen was positive for antibodies. In those cases, donor sperm was used. Any

serum and follicular fluid not used immediately for testing was stored at -70° C.

Sera known to be positive for antisperm antibodies of all three isotypes by immunobead binding were kindly provided by Richard Bronson, M.D., Division of Human Reproduction, North Shore University Hospital, Manhasset, New York, and were stored in aliquots at -70° C for use as positive controls. Sera obtained from fertile volunteers, which were negative for antisperm antibodies by immunobead binding, were stored in aliquots at -70° C for use as negative controls.

Donor semen was obtained from fertile donors participating in the donor artificial insemination program. This semen and its sperm were found to be negative for antibodies to sperm by immunobead binding. Subsequently, these sperm were used in testing positive and negative control sera. In addition, in couples with semen that was positive for antibodies to sperm, these donor sperm were used to test for antisperm antibodies in the sera and follicular fluid.

Immunobead Binding Assays

Testing for antisperm antibodies in male and female serum, semen and follicular fluid was performed by immunobead binding assay according to the methods described by Bronson et al.[30,31] This assay is done using immunobeads,

polyacrylamide spheres of 5-10 μm diameter with covalently bound rabbit antibodies to the constant domain of human IgA (α-chain specific), IgG (γ-chain specific) or IgM (μ-chain specific; Bio-Rad Laboratories, Richmond, CA; Anti-IgA, cat. no 170-5114; Anti-IgG, cat. no 170-5100; Anti-IgM, cat. no. 170-5120). Lyophilized anti-IgA, anti-IgG, or anti-IgM immunobeads were washed once by centrifugation (800g, 8 minutes) with Dulbecco's phosphate buffered saline (PBS; Gibco Laboratories, Grand Island, NY) and were resuspended at 5mg/ml in PBS containing bovine serum albumin (BSA, fraction V; Calbiochem, La Jolla, CA) 10 mg/ml which had been passed through a 0.22 μm sterile filter.

Fresh immunobead suspensions of all three isotypes, enough for several days of testing, were prepared in this manner every 3-4 days, and stored refrigerated at 4° C. Each fresh immunobead suspension was tested with positive and negative controls. Lyophilized beads could be stored as received from Bio-Rad refrigerated at 4° C for several months.

Semen Immunobead Binding Assay

Semen was kept at room temperature for one hour after ejaculation to allow unbound antisperm antibodies in the seminal fluid, if present, to bind to spermatozoa prior to testing. After adequate sperm number and motility ($\geq 1 \times 10^6$

motile sperm) for testing were assured by semen analysis, sperm were washed once by centrifugation (800g, 8 minutes) in PBS containing BSA 5mg/ml (BSA/PBS), resuspended, and washed in BSA/PBS three times using a Beckman Microfuge B (Beckman Instruments, Palo Alto, CA; 6 seconds per spin). Following the third wash, the sperm pellet was resuspended in BSA/PBS to 10-20 x 10^6 sperm/ml. BSA/PBS was remade every 2 days and was stored refrigerated at 4° C.

Five μl of this sperm suspension were added to 50 μl of well-mixed immunobead suspension, and a drop of the mixture was placed on a glass slide and covered with a coverslip. The preparation was kept at room temperature for 10 minutes, allowing immunobead binding to occur. The reaction was observed and counted using phase-contrast microscopy at x200.

Two counts of 100 motile sperm for immunobead binding were made on each preparation. Sperm cells without bound antisperm antibodies progressed through the immunobead suspension without binding any of the beads. Sperm cells with bound antisperm antibodies became bound to one or more beads, upon sperm-bead contact, at the region of the sperm surface where antibody was bound. Immunobead binding was designated as directed to the head, tail or tail tip of the sperm, indicating the presence of bound antisperm antibodies to that region of the spermatozoan.

Figure 1 represents the positive immunobead binding

reaction diagramatically. Photographs of sperm cells with positive and negative immunobead binding are shown in figures 2-4.

Nonmotile sperm were not counted, both because they lacked ample opportunity to contact and bind beads, and because their tendency to be "stuck" nonspecifically to beads was difficult to distinguish from specific binding in the absence of motility. Sperm-bead contact was maximized by gentle tapping on the coverslip to prevent sticking and to redistribute beads.

The region of sperm surface to which antibody was directed and the percent of motile sperm cells bound was determined for each of three isotypes of immunoglobulin (IgA,IgG,IgM). A specimen was deemed positive if $\geq$20% of the motile sperm were bound to one or more bead for one or more of the immunoglobulin isotypes tested, as previously described by Clarke.[18]

All specimens found to be positive were tested for the specificity of bead binding for each positive isotype, using an inhibition method described by Clarke.[18] Immunobead binding sites were blocked by adding a drop of human IgA (colostral) to the anti-IgA bead suspension, IgG (polyclonal) to anti-IgG beads, or IgM (polyclonal) to anti-IgM beads and leaving it at room temperature for at least 1 minute prior to mixing with the antibody-bound sperm (Calbiochem, La Jolla, CA; IgA, cat no.401698, IgG, cat no

401105, IgM, cat no 401107, respectively). The positive sperm were then added to these beads and counted for binding as described above.

Serum and Follicular Fluid Immunobead Binding Assay

Each couple's male and female serum and follicular fluid were tested for antibody binding using the husband's sperm, if his sperm were negative for immunobead binding. In cases where a husband's sperm were positive for immunobead binding, antibody-negative donor sperm were used.

Spermatozoa were selected for motility using a swim-up method[32] in which antibody-negative semen (containing $\geq$ 2 x 10^6 motile sperm) was overlaid with BSA/PBS in a test tube and incubated for one hour at 37° C. Motile spermatozoa, which migrate to the top of the test tube, were collected, and 0.5-1.0 x 10^6 of these were added to 0.4 ml of serum or follicular fluid, or the equivalent volume of follicular fluid if diluted with heparin. PBS was added for a total volume of 1.6 ml, making the serum or follicular fluid dilution 1:4, and the preparation was incubated for one hour at 37° C. The spermatozoa were than washed and tested for surface-bound antibody by immunobead binding as described above for semen.

In Vitro Fertilization/Embryo Transfer (IVF/ET)

All patients underwent IVF/ET procedures at Yale-New Haven Hospital as previously described. Induction of follicular growth was achieved using either human menopausal gonadotrophins (hMG, Pergonal; Serono Laboratories Inc., Randolph, MA), hMG + clomiphene citrate (Clomid, Merrell-Dow; Cincinatti, OH), or purified follicular stimulating hormone (FSH; Metrodin; Serono Laboratories Inc.). Follicular development was monitored by ovarian ultrasonography and daily serum estradiol measurements beginning on day 8 of the cycle. Human chorionic gonadotrophin (hCG, Pregnyl; Organon, West Orange, NJ) was administered to stimulate ovum maturation once two follicles $\geq$1.6 cm in diameter were seen on ultrasound and serum estradiol was >400 pg/ml.[32]

Oocytes were aspirated by laparoscopy and graded for maturity, based on the degree of cumulus mucification and corona cell dispersion, as immature, intermediate and mature as previously described.[28] Intermediate and mature oocytes were uniformly inseminated, 6-8 hours after retrieval, with 0.5×10^6 spermatozoa washed twice with insemination medium and obtained by swim-up. Modified Ham`s F-10 medium enriched with 10% of the individual woman`s heat-inactivated serum was used as insemination medium. Ova were moved from insemination medium to growth medium, consisting of modified Ham`s F-10 with 20% of the woman`s serum, 16 to 18 hours

after insemination. The oocytes were examined for the formation of pronuclei at this time. Embryos of two or more cells were transferred to the uterus 38-40 hours after insemination, in transfer medium containing modified Ham's F-10 with 90% of the woman's serum. The luteal phase was supported with daily intramuscular progesterone. Women were followed for pregnancy with radioimmunoassays for β- hCG and serum estradiol as previously described.[28]

Fertilization rate was calculated including only oocytes graded as intermediate and mature which were inseminated uniformly as described above. Fertilization was defined by the presence of two or more pronuclei. Cleavage rate was calculated as the proportion of fertilized oocytes which cleaved. Cleavage was defined as zygote division.

Statistical Analysis

Appropriate statistical analysis was performed using Student T-tests, Chi-square analysis, and Fischer's exact test.[33] Data are expressed as mean±SEM.

RESULTS

Negative controls tested were found to have occasional binding (0-10%) of the motile spermatozoa, particularly to tail tip. Consequently, a cutoff of $\geq$20% immunobead binding was used to define positives in table 1 and all subsequent data reported.

The specificity of antibody binding to sperm in all positive specimens was affirmed by an inhibition test. In this test, a specific positive reaction to a particular isotype of immunoglobulin was inhibited when an adequate concentration of that immunoglobulin to block the immunobead binding sites was first mixed with beads. Thus, in this study, immunobead binding was inhibited for all samples which were positive for IgA class antibodies to sperm after free IgA had been mixed with the anti-IgA immunobeads. Similarly, positive reactions were inhibited for samples positive for IgG class antibodies to sperm after addition of free IgG to anti-IgG immunobead beads and for samples positive for IgM class antibodies to sperm after addition of free IgM to anti-IgM immunobeads.

Incidence of Antibodies to Sperm

A complete listing by couple of the results of immunobead binding assays and of IVF/ET outcome for the 40

couples studied is shown in table 1. For each couple studied, immunobead binding results are given for three regions of antibody binding to sperm--head, tail tip and tail, in each of the 4 fluids tested. IVF/ET results shown include fertilization rates (listed in increasing order), cleavage rates and pregnancy outcome. The etiology of infertility for each couple is also shown.

The immunobead binding data shown in table 1 is summarized in table 2. The frequency of immunobead binding by fluid and region of antibody binding on sperm may be seen. Thus, in an unselected population of IVF/ET patients, antibody binding to sperm head was present in 15% of female sera and 13% of follicular fluid samples. Antibodies to sperm head were present in the follicular fluid of 5 of the 6 women with these antibodies in their serum (83%, table 1, couples# 1,3,11,13,33). All women with antibodies to sperm head in follicular fluid also had antibodies to sperm head in serum. In each of these 5 cases, the follicular fluid was positive only for immunoglobulin isotypes which were also positive in female serum (table 3).

Antibody binding to sperm head was present in 8% of semen samples and 8% of male serum tested (table 2). As seen in table 1, there was overlap between these two groups. Two men (table 1, couples# 33,34) had antibodies to sperm head in both semen and serum. There was one man with

antibodies to sperm head in semen alone (couple# 2) and one man with antibodies to sperm head in his serum alone (couple# 14). The mean sperm concentration and percent motility by semen analysis in men with antibodies to sperm head in either semen, serum or both (n=4) did not differ significantly from those in men without antibodies to sperm head (n=36; 105$\pm$23 x 10^6 sperm/ml, 60$\pm$8% and 113$\pm$13 x 10^6 sperm/ml, 64$\pm$2%, respectively).

Antibody binding to sperm tail tip, the region of sperm most frequently bound, was present in 30% of the female sera, 25% of the follicular fluid samples, 10% of semen samples, and 30% of male sera tested.

Antibody binding to sperm tailpiece (tail), the region of sperm least frequently bound, was present in 5% of female sera, 3% of follicular fluid samples, 3% of semen samples and 5% of male sera tested. All specimens with binding to sperm tailpiece also had binding to sperm head (table 1, couples# 1,11, 33,34).

The distribution of binding by immunoglobulin isotype (IgA,IgG,IgM) for each region of antibody binding on sperm in each fluid tested is shown in table 3. Analysis of individual antibody isotypes was not as useful as the use of all three isotypes combined in predicting fertilization in any of the fluids tested. Thus, the isotypes have been grouped together for all analyses described here.

Antibodies to Sperm Head and Subsequent Fertilization

Analysis of the effect of antisperm antibodies on fertilization will be reported for each region of antibody binding to sperm in each fluid tested, following the organization of table 2. As shown in table 2, fifteen percent (6/40) of women tested had antibodies to sperm head in their serum. Among these women, the oocyte ferilization rate of uniformly inseminated mature oocytes was significantly reduced. As shown in figure 5, 9 of the 27 oocytes (33%) inseminated were fertilized, as compared with 118 of 167 oocytes (71%) of women without antibodies to sperm head in their serum ($p<0.001$). Of the women in whom no oocytes fertilized, 60% (3/5; table 1, couples# 1,2,3) had antibodies to sperm head in their serum, while only 9% (3/35; couples# 11,13,33; $p<0.05$) of those who had at least one oocyte which fertilized had antibodies to sperm head. Serum antibodies to sperm head were found in 5 of the 13 women whose fertilization rate was <60% (table 1, couples# 1,2,3,11,13), and in 1 of the 27 women whose fertilization rate was >60% (couple# 33; $p<0.05$). For all couples with antibodies to sperm head in female serum (n=6), the mean fertilization rate was 34 ± 17% compared to 74 ± 6% in those without antibodies to sperm head (n=34; p=0.01).

As shown in table 2, 13% of women tested had antibodies to sperm head in follicular fluid. Fertilization rates of

uniformly inseminated mature oocytes were calculated for the particular oocyte(s) retrieved from follicular fluid tested. The presence of antibodies to sperm head in follicular fluid was associated with decreased fertilization. As seen in figure 5, among women with antibodies to sperm head in follicular fluid, 29% (2/7) of oocytes were fertilized as compared with 66% (27/41) of oocytes in women without. Although this is not a statistically significant difference because of the small numbers involved, it is highly suggestive of a trend.

As summarized in table 2, the incidence of antibodies to sperm head was 8% in semen and 8% in male serum. Neither semen nor serum antibodies to sperm head significantly affected the oocyte fertilization rate (figure 5).

Antibodies to Sperm Tail Tip and Tail and Subsequent Fertilization

Antibody binding to sperm tail tip occurred more frequently than binding to either sperm head or tail in each fluid tested (table 2). It occurred in 30% of female sera, 25% of follicular fluid samples, 10% of semen and 30% of male sera tested. Despite its frequent occurrence, antibody binding to sperm tail tip did not reduce the oocyte fertilization rate significantly in any of the fluids tested (figure 6).

Antibody binding to sperm tailpiece was rare in all fluids (table 2). The number of patients with antibodies to tailpiece was too few to examine the effect of tailpiece antibodies on fertilization.

Subsequent analyses examine the clinical significance of antibodies to sperm head, particularly in female serum, for two reasons. First, antibodies to sperm head in female serum and follicular fluid have apparent importance to fertilization. Second, antibodies to sperm tail and tail tip lacked clinical relevance to fertilization in this study.

Antibodies to Sperm Head in Female Serum and Cleavage

Among women with serum antibodies to sperm head, oocytes that did fertilize were capable of cleavage. However, the zygote cleavage rate was reduced in the presence of serum antibodies to sperm head (figure 7). Among women with serum antibodies to sperm head, 67% of zygotes (6/9) were cleaved, as compared with 91% of zygotes in women without serum antibodies to sperm head (107/117; $p=0.051$). Of the 6 cleaved embryos in the former group, 4 were 3-4 cell embryos and 2 were >4 cell embryos at the time of transfer to the uterus.

Antibodies to Sperm Head in Female Serum and Pregnancy by IVF/ET

Among women with serum antibodies to sperm head, oocytes that fertilized and cleaved were capable of implantation leading to a normal pregnancy. There was no significant difference in the pregnancy rate of women with and without serum antibodies to sperm head in this sampling (figure 7). One of the 6 women with serum antibodies to sperm head (17%) conceived and has continued with a normal pregnancy confirmed by ultrasound. Seven of the 34 women without serum antibodies to sperm head (21%) became pregnant; 3 have ongoing pregnancies documented by ultrasound; 2 had spontaneous abortions after their pregnancies were documented by ultrasound, and 2 had chemical pregnancies.

Antibodies to Sperm Head and Etiology of Infertility

Sixty-three percent of couples tested had tubal infertility, 10% were infertile secondary to a male factor, and 28% had unexplained infertility (table 1). The incidence of antibodies to sperm head in unexplained infertility and tubal infertility are shown in figure 8. Comparing couples with unexplained (n=11) vs. tubal (n=25) infertility, 46% and 4% respectively had antibodies to sperm head in female serum (p=0.006), 36% and 4% had antibodies to sperm

head in follicular fluid (p=0.023), 27% and 0% had antibodies to sperm head in semen (p=0.023), and 18% and 4% had antibodies to sperm head in male serum (p=0.022). Antibodies to sperm head were present in one or more fluids tested in 55% of the patients with unexplained infertility compared to 8% of patients with tubal infertility (p=0.005). Of the 6 women with antibodies to sperm head in their serum, 83% (5/6) were undergoing IVF for unexplained infertility compared to 17% (1/6; p=0.08; figure 9) who had tubal infertility.

Serum Antibodies to Sperm Head and Primary vs. Secondary Infertility

Of the 6 couples with antibodies to sperm head in female serum, infertility was primary for 4 and secondary for 2. In these couples, the mean sperm concentration was almost twice as high for those couples with secondary infertility as for couples with primary infertility. Specifically, the mean sperm concentration and percent motility of couples with secondary infertility and antibodies to sperm head in female serum were $102 \pm 11 \times 10^6$ sperm/ml with $66\% \pm 9$ motility, compared to $65 \pm 30 \times 10^6$ sperm/ml with $57\% \pm 6$ motility for couples with with primary infertility antibodies to sperm head in female serum. This difference is not statistically significant because of the small

numbers involved, but suggests a trend. Among couples with antibodies to sperm head in female serum, a higher number of motile sperm was associated with previous fertility.

Women with serum antibodies to sperm head were similar in age to women without (mean ages 35 ± 2 and 34 ± 1, respectively).

Antibodies to Sperm Head in Female Serum and Semen and PCTs

Results of previous PCTs were available in the medical charts of 23 of 40 of the couples tested. Because of inconsistencies in the methods of documenting PCT results, a controlled comparison cannot be made. However, available results for couples with antibodies to sperm head in female serum or semen are reported here. Of the 6 women with antibodies to sperm head in serum, 4 (67%) had had only abnormal PCTs in the past, 1 had had a good PCT (17%), and 1 had no PCT documented in the chart. Two of the 3 men with antibodies to sperm head in semen had PCTs documented in the chart: one had had abnormal PCTs in the past; the other had had a PCT considered to be within normal limits.

DISCUSSION

The Immunology of Reproduction

The growth of the immunology of conception as a field of scientific and clinical interest should not be surprising in view of our knowledge of the importance of the immunology of pregnancy. In fact, more than 40 years before Landsteiner and Weiner (1940)[34] and Levine et al (1941)[35] delineated isoimmunization as the cause of hemolytic disease in the fetus and newborn, Landsteiner (1899)[36] had demonstrated the antigenicity of sperm. In 1932, Baskin[37] reported the induction of transient sterility in 20 women as a result of immunization with human spermatozoa for contraception. Subsequent studies[38,39,40,41,42,43] have reported the antifertility effects of experimentally-induced antibodies to sperm in many animal species. In recent years, in vitro studies using animal models[44,45,46,47,48,49,50,51] which demonstrate impairment of the egg-sperm interaction in the presence of antisperm antibodies have provided further evidence that antibodies against sperm may compromise human reproduction.

Pathogenesis of Immunity to Sperm

The pathogenesis of immunity to sperm still remains largely a mystery. Mature sperm cells first appear in the male at puberty, long after the development of immunocompetence and tolerance to "self" antigens. Since spermatozoa express antigens which are foreign to the immune system, their immunogenicity is not surprising.

Sperm are normally sequestered from the immune system by a blood-testis barrier formed by the tight junctions of Sertoli cells in the seminiferous tubules[52] and epithelial cell barriers elsewhere in the male reproductive tract.[53] Disruption of the blood-testis barrier by vasectomy has been associated in many men with the formation of antibodies to sperm, presumably due to sperm antigen leakage.[53,54] Other processes,[43] like trauma, inflammation or irradiation of the testes, or obstruction of the tubules or ducts of the male reproductive tract, might similarly stimulate antisperm antibody formation. This might occur either by increasing the permeability of the male reproductive tract to immunocompetent cells or by allowing the passage of sperm antigens through the blood-testis barrier. In fact, these associations have led to the speculation that the formation of autoantibodies to sperm could be a part of a reproductive strategy in the male which prevents genetically abnormal spermatozoa from participation in fertilization.[55]

However, large gaps exist in our understanding of the pathogenesis of immunologic infertility in the male. Some men who have undergone vasectomy do not develop autoantibodies to sperm,[54] while many infertile men are found to have antisperm antibodies, but have no known history to explain sperm extravasation across the blood-testis barrier.[43] The relevance of the quantity, rate and type of antigen exposure, and variation in the immune response among individuals is undetermined at present.

The mechanisms of isoimmunization to sperm in the female are even less clear than in the male. Through sexual activity, the female reproductive tract is repeatedly innoculated with immunologically-foreign sperm cells, and yet only a minority of women develop immunity to sperm. Several factors are felt to inhibit an immune response to sperm in most women. First, most sperm cells remaining in the female reproductive tract after coitus are normally phagocytosed by macrophages and neutrophils before an immune response develops.[8] Second, one or more immunosuppressive factors in seminal fluid are believed to prevent sensitization to sperm.[56]

Despite these factors, an immune response to sperm develops in some women. After sexual intercourse, antibodies to sperm may develop locally or sperm may reach the peritoneal cavity where immunologic stimulation may occur.[8] Factors, such as infection, in the female reproductive tract

may either act as adjuvants which stimulate local immune responsiveness, alter the effectiveness of immunosuppressive factors in seminal plasma or lead to the formation of antibodies that cross-react with sperm.[8,57] Alternately, sperm may innoculate the gastrointestinal tract. Gastrointestinal exposure to sperm after oral or anal intercourse has been associated with the formation of antisperm antibodies in studies of homosexual men.[58,59]

The need to identify specific antigens on sperm involved in immunologic infertility is apparent. Several labs [60,61,62] have produced monoclonal antibodies to human sperm components in efforts to delineate specific sperm antigens. One monoclonal antibody, which cross reacts with human sperm and testes, monkey sperm and mouse sperm, has been shown to block penetration by human sperm of zona-free hamster ova.[60] Characterization of the antigens involved in immunologic infertility would permit highly specific identification of affected couples and definition of the sites of reproductive impairment and leading to more effective therapy. In addition, identification of a specific antigen which impairs fertilization in a well-defined and circumscribed manner would make the development of immunocontraception possible.[7,63]

Assays of Antisperm Antibodies

A number of assays have been used in attempts to detect antibodies to sperm (table 4).[13,64] The methods which rely on sperm cell agglutination [tray-slide agglutination test (TSAT),[4] gel agglutination test (GAT),[65] tray agglutination test (TAT)[66]] lack specificity and reliability because sperm cells agglutinate in the absence of specific antibody under a variety of conditions.[7,13,64] These include the presence of bacteria, fungi or amorphous materials in semen, and sex steroids or nonimmunoglobulin proteins in serum. Menge[8] compared the mean incidences of agglutination determined by investigators using various agglutination methods in more than ten reports and found that the mean incidence of sperm agglutination in the sera of pregnant controls was 35-38%, higher than the 15-23% incidence found in the women with unexplained infertility. Perplexing variation was found, particularly with the TSAT, where mean incidences of serum antisperm antibodies in seven studies ranged from 14-67% of women with unexplained infertility. Agglutination methods, in addition to their nonspecificity and unreliability, do not define the isotype(s) of immunoglobulin involved, and none but the TAT indicate the region of sperm to which antibody is bound.[67]

The sperm immobilization test (SIT),[64,68] which detects complement-dependent immobilization and cytotoxicity, is

highly specific and reproducible. However, Bronson[31] has demonstrated its insensitivity to IgA isotypes which do not fix complement, and to antibodies bound to sperm head which do not cause sperm immobilization. Some of these undetected antibodies have been shown to interfere with the sperm-egg interaction.[19] The SIT is a useful, reliable clinical test for antisperm antibody activity, but its utility is limited by its insensitivity to certain antisperm antibodies and its inability to identify the region of antibody binding to sperm.

Immunofluoresence techniques[69,70] have been associated with a high incidence of false positives and poor correlation with fertility status because methanol fixation exposes internal sperm antigens. Antibodies to these antigens are felt to play no role in infertility.[13] This test is not suitable for routine clinical screening, but may be a valuable research tool because it identifies the region of antibody binding to sperm.[43]

Enzyme-linked Immunosorbent Assays (ELISA),[71,72,73,74] use an enzyme-labeled antiglobulin which binds to human immunoglobulins on the sperm surface. The presence of the enzyme may be detected and measured colorimetrically upon addition of the enzyme substrate. Fixation of either whole sperm[71,73,74] or sperm membrane extracts[72,73] are required as antigenic targets. However, sperm antigens appear to be altered by the process of sperm fixation involved, contri-

buting to a high false positive and negative rate.[74,75] Assays using whole sperm have not shown clinical correlation with infertility.[75] Until clinically significant antigens are identified and isolated for use as antigen targets, this test has limited clinical utility.

In the recently described "panning" assay,[76] antibody bound sperm sticks to wells coated with antiglobulin molecules. The assay, which is qualitative and does not identify the region of antibody binding to sperm, has been shown to detect those antibodies detected by agglutination but not immunofluoresence assays.[75] Its sensitivity, specificity and clinical value are unknown.

Although cervical mucus appears to be an important site of immunologic infertility, it is often not tested for antibodies to sperm because of the difficulties in collecting and processing it.[7] Antibodies to sperm in cervical mucus and semen may be detected by simple visual observation of the "shaking phenomenon" using the Sperm-Cervical Mucus Contact Test (SCMCT).[77] When antibody-bound sperm contact cervical mucus on a slide, the characteristic shaking reaction seen is felt to result from cross-linking of motile antibody-coated sperm to the cervical mucus gel via the Fc portion of the antibody molecule.[78] "Crossover" testing using donor sperm and cervical mucus discriminates between antibodies in the male and female. Although it is a qualitative test with unknown specificity and sensitivity,

it is a simple, valuable method to detect antisperm antibodies in cervical mucus.[79]

In radiolabeled antiglobulin assays,[6] radiolabeled antibodies detect human immunoglobulins bound to sperm. These techniques are highly sensitive and specific.[13] The technique, best used on living sperm, can determine isotype of immunoglobulin in semen or serum and supply quantitative results. However, since the regional specificity of binding to sperm cannot be determined, the assay provides no information about the antigens involved in the immune response detected.

The mixed agglutination reaction (MAR)[80] is based on the Coombs mixed agglutination reaction.[81] Rh-positive human red blood cells sensitized with a human IgG anti-Rh antibody is mixed with semen and rabbit anti-human IgG antiserum. A mixed agglutinate of sperm and red blood cells occurs in the presence of antibody-bound sperm. The test is highly specific and useful to detect antibodies bound to sperm in semen,[82] however, it has only been used reliably to detect IgG isotype.[18] In addition, the region of antibody binding to sperm and extent of binding are difficult to determine.[13]

The immunobead binding assay provides a highly specific and reproducible method of detecting antibody bound to the sperm surface.[17] It determines the isotype of immunoglobulin bound, the percent of sperm which are antibody bound and most importantly, the region of sperm to which specific

antibody is bound. It can measure antibodies in males and females, in both sera and local reproductive secretions and fluids. The sensitivity of the assay has not been determined,[13] because there is no assay accepted for its clinically-relevant sensitivity for use as a standard for comparision. However, its findings are proving to correlate highly with clinical infertility status. In various studies using immunobead binding, Bronson has found significant correlation between antibody binding and decreased conception rates,[11] decreased in vitro binding to the zona pellucida by human spermatozoa,[19] and abnormal PCTs.[20]

Sites of Immunologic Infertility

The various sites where antibodies to sperm may interfere with reproductive success are shown in table 5. Antisperm antibodies may impair reproduction at the level of spermatogenesis, sperm transport, sperm survival, gamete interaction, and embryo survival.[8,13,14,43,83] In addition, several immunologic responses other than humoral immunity to sperm have been suspected of immunologic impairment of fertility.[7,43] Cellular immunity to sperm[84,85] and humoral immunity to the zona pellucida of the ova[86,87,88,89,90] have recently received attention for their potential role in infertility. Studies of these immunologic responses in infertility have taken a back seat to the study of antisperm

antibodies, because of some of the technical difficulties of conducting studies in these areas, and because of the weight of evidence implicating antisperm antibodies in infertility.[7] The current study examines the effect of antisperm antibodies on gamete interaction.

A number of studies[23,30,91,92] have used zona-free hamster ova[93] to assess fertilization by human spermatozoa with bound antisperm antibodies. All but one of these studies[30] indicates that antisperm antibodies inhibit sperm binding to the zona-free hamster ova. Bronson[19] showed that normal human sperm with antibodies bound to the sperm head failed to bind to salt-stored human zona-pellucidae. Kamada[23] demonstrated that zona penetration, in addition to zona binding, is inhibited by antibodies bound to sperm. The implication of these studies is that antisperm antibodies interfere with the sperm-egg interaction, either the acrosome reaction, zona binding and/or penetration, or sperm-egg fusion.

None of these studies provides a look at both sperm-zona interaction and sperm-egg fusion in the same system. Clarke[26] studied men with sperm antibodies undergoing IVF/ET and found that in the presence of high levels of IgA and IgG class isoantibodies to sperm, fertilization was reduced. However, he did not make distinctions on the basis of region of antibody binding to sperm. In this study, Yale-New Haven Hospital IVF/ET data from both women and men with antibodies

to sperm was analysed to further examine the effect of antibodies to sperm in female and male sera and local reproductive fluids on the total sperm-egg interaction.

Immunobead binding to at least 20% of sperm was used in the current study as the cutoff for positive antibody binding, based on the low levels of nonspecific binding (<10%) found in fertile negative controls. The cutoff correlated well with clinical findings by identifying a group of positives with a significantly decreased fertilization rate in IVF and is consistent with the 20% cutoff used by Clarke.[18] Bronson's group[11,20] has used a cutoff of 50%, based on his findings of a significantly reduced spontaneous conception rate and impaired cervical mucus penetration in those with >50% binding. The 50% cutoff was not used in the current study because it would exclude a substantial number of couples with antibodies to sperm who had no fertilization of oocytes.

Clarke recently reported the finding that IgG and IgA are the two major immunoglobulin classes of antibodies to sperm in female and male serum,[94] and in follicular fluid.[95] This was not confirmed in the current study. No statistically significant relationship was found between isotype of immunoglobulin and either location in particular biologic fluids or clinical significance in this study. Further investigation is necessary to define the role, if any, of immunoglobulin class in infertility.

Antibody-negative donor sperm were used as an antigen source while testing for antisperm antibodies in control and other sera where necessary. This widespread practice has been adopted based on reports that antibodies to sperm are tissue-specific rather than human leukocyte antigen (HLA)-specific.[7] Because HLA or ABO group antigens are not expressed on human sperm,[96] the antibody findings for a given fluid (serum, follicular fluid, seminal plasma) are the same when sperm from different individuals is used as the antigenic target. As expected, this was confirmed in the current study by the uniform antibody binding pattern detected in positive control serum when sperm from several donors were used as the antigen source. In addition, analysis of HLA antigens in couples with unexplained infertility in two studies[97,98] has revealed no association between infertility and intracouple HLA compatability.

Antibodies to Sperm Head and Subsequent Fertilization

In women with serum antibodies to sperm head, the ova and/or sperm were directly exposed to these antibodies from several sources in vitro, in addition to any possible in vivo exposures. These include exposure to antisperm antibodies from female serum in insemination, growth and transfer media, from follicular fluid in the aspirating trap and from any follicular fluid antibodies remaining in the

cumulus mass at the time of insemination. Thus, the finding in this study that IVF/ET fertilization rates are reduced in women with antibody to sperm head in serum and follicular fluid indicates that fertilization is impaired in the presence of antibodies to sperm head. This is consistent with previous findings which suggested that antisperm antibodies interfere with the sperm-egg interaction[23,91,92] and that women with immune infertility have impaired fertilization.[99]

The presence of antibodies to sperm in the follicular fluid showed a clear relationship to antibody findings in serum. All women with antibodies to sperm head in follicular fluid also had antibodies in serum, and each isotype that was positive in follicular fluid was also positive in serum. This confirms the findings of Ackerman, et al.[21] In the one case for which the serum was positive and the follicular fluid was not, and the few cases in which one isotype in serum was not found in follicular fluid, a dilutional effect appears to be the cause; these women had lower concentrations of antibody in serum and the concentrations of antibody in their follicular fluid just missed the 20% cutoff involved.

The antibodies in follicular fluid may be due to local production,[21] transudation from serum[99] or from vascular injury by the aspirating needle during aspiration of follicles[21] resulting in collection of blood with the

follicular fluid. In any case, follicular fluid antibodies are clinically relevant in IVF/ET because the ova are exposed to the antibody-containing follicular fluid and antibodies may remain on the cumulus at the time of insemination. In vivo, the ovum is similarly exposed to follicular fluid antibodies within the follicle, and may retain those antibodies in the cumulus mass. In addition, follicular fluid from the ruptured follicle may enter the fallopian tube and impair the egg-sperm interaction.[99]

Antibody binding to sperm head in semen and male serum did not impair fertilization in the current study. This lack of correlation with fertilization cannot be regarded as conclusive because the number of positives in this group were so few. Clarke[26] has reported a reduced fertilization rate in IVF in men with $\geq$80% isoantibodies bound to sperm and a normal fertilization rate in men with <80% of sperm bound. Further studies must be conducted to investigate the clinical significance of antibody binding to sperm head in the male.

Antibodies to Sperm Tail Tip and Subsequent Fertilization

Particularly significant is the finding that antibodies to sperm tail tip had no effect on fertilization. Thus, the region of antibody binding to sperm appears clinically relevant. This is consistent with previous studies by

Bronson which indicate that antibodies to sperm head inhibit sperm binding to the zona pellucida[19] and antibodies to sperm tail tip probably play no role in infertility.[13] These findings indicate that organization of antibody findings by region of binding is a critical step in distinguishing between clinically significant and insignificant antibodies.

Antibodies to Sperm Head and Subsequent Cleavage and Pregnancy

Once fertilized, oocytes from women with serum antibodies to sperm head were capable of cleavage and pregnancy. However, since sperm-specific surface isoantigens have been detected on the egg after fertilization, antisperm antibodies may act directly to impair cleavage, implantation or embryo survival.[100] In fact, in the current study, antibodies to sperm head in female serum were associated with a reduced rate of zygote cleavage. This is in contrast to a previous study involving 3 women with immunologic infertility undergoing IVF/ET in which cleavage was not impaired.[24] Since the total number of fertilized oocytes from women with antibodies to sperm head in the current study was nine, future studies of cleavage in women with head-directed antisperm antibodies must be conducted on a larger scale.

The pregnancy rates using IVF/ET in women with and

without serum antibodies to sperm head were not significantly different in the current study. Since the spontaneous pregnancy rate is reduced in couples with antisperm antibodies,[7,11] this finding suggests a therapeutic advantage for IVF/ET for couples with antisperm antibodies. Further studies must assess the pregnancy rate in IVF/ET in women with serum antibodies to sperm head on a larger scale.

Antibodies to Sperm Head and Unexplained Infertility

Populations of infertile couples undergoing IVF/ET include a large percentage of patients whose infertility is unexplained. An immune etiology has been suspected for a subset of these patients.[2] The immunobead testing in this study indicates that antibodies to sperm are present in members of an IVF population, particularly in couples whose infertility is considered unexplained.

The strong correlation between antibodies to sperm head and unexplained etiology is not surprising. Previous studies which did not identify the region of antibody binding to sperm have reported a high incidence of antisperm antibody findings in populations with unexplained infertility. The added definition of region of sperm binding in the current study permits greater specificity in identifying patients affected with infertility on an immune basis. That

is, we can now distinguish in a clinically relevant way between types of positives by region of binding, in view of the apparent clinical insignificance of binding at some sperm locations and possibly in some fluids.

Antisperm Antibodies and Primary vs. Secondary Infertility

Among couples with antibodies to sperm head in female serum, the history of prior conceptions in those with higher numbers of motile sperm suggests several important concepts. First, immunologic infertility may be relative rather than absolute,[13] since conceptions in vivo occurred in this group. Second, factors in infertility are additive, and it appears to be advantageous to maximize all factors contributing to fertility. A higher number of motile sperm in a couple with antibodies to sperm head in female serum appears to be prognostically better. Third, some or all of the couples who had conceived in the past may not have had antibodies to sperm when they previously conceived. Study of couples with immunologic infertility who have previously conceived might help us identify those couples at risk for developing immune infertility.

Antisperm Antibodies and PCTs

A PCT[1] is an examination of the cervical mucus after sexual intercourse around the time of ovulation to assess the quality and number of motile sperm in the cervical mucus, as well as the quality of the cervical mucus itself. PCT results were available in only some of the patients studied. The criteria used in these tests and the types of documentation in the medical charts were nonuniform. However, four out of five of those women with antibodies to sperm head who had PCT results documented in their charts had abnormal PCTs. This confirms a number of previous studies which have correlated antisperm antibodies with abnormal PCTs.[13,20,77,101]

The PCT is a good nonspecific way of screening for antisperm antibodies because immunoglobulin-bound sperm have impaired ability to penetrate cervical mucus.[77] In one study,[13] intermediate to high levels of circulating antisperm antibodies were present in 23% of men and 35% of women in an infertile population with abnormal PCTs, despite normal semen analyses.

However, an abnormal PCT may be caused by many factors, including occult infections like chlamydia or mycoplasma, poor mucus quality due to hormonal factors, poor semen quality or improper timing of PCT.[1,2,101] In cases where an abnormal PCT is unexplained, in vitro sperm-mucus testing

should be performed.[2] The SCMCT[77] provides a controlled method of examining the semen and cervical mucus for antisperm antibodies and can distinguish between antibodies to sperm in the semen and cervical mucus using a crossover technique.

IVF/ET as Therapy for Immunologic Infertility

IVF/ET compares well with other currently employed methods of treatment for immunologic infertility, including condom or occlusive therapy,[102] immunosuppression with corticosteroids[104] and intrauterine insemination.[103] Varying success rates have been reported for these methods but none have been shown to be very effective therapy for immunologic infertility.[13] Isojima et al[104] reported that titers of antibodies to sperm remained unchanged in a woman whose husband used condoms to decrease his wife's sensitization to sperm by removing exposure to sperm antigens. Studies using corticosteroid immunosuppression have yielded contradictory results.[105,106] A recent double-blind, randomized, placebo-controlled clinical trial[106] demonstrated no significant effect of intermittent high doses of prednisolone on antisperm antibody levels and semen characteristics.

Various methods[13] of in vitro manipulation of semen, like sperm washing,[102] prior to intrauterine insemination

have been used for men with autoimmune infertility. Sperm washing techniques have failed to reduce the proportion of sperm which are antibody bound and may lead to loss of sperm function.[13] Techniques which use rapid high volume dilution of freshly-ejaculated unbound sperm[13] and freeze-thawed sperm membrane fragments as immunosorbent[107] have been reported to reduce the proportion of antibody-bound sperm and must be tested further. Intrauterine insemination to bypass antisperm antibodies in the cervical mucus of isoimmune women does not eliminate the problem of impaired fertilization due to antisperm antibodies in serum and within the reproductive tract in these women.[108]

The promise of IVF/ET for immunologic infertility lies in the opportunity to employ appropriate methods to minimize antibody impairment of fertilization. In autoimmune men, in-vitro manipulations of sperm, such as rapid semen dilution, might be used to reduce the proportion of antibody-bound sperm. In women with antisperm antibodies in serum and follicular fluid, elimination of the antibody-containing female serum from all IVF/ET media would reduce direct exposure of the ova and sperm to antibodies in female serum. Washing follicular fluid from the cumulus mass might remove residual antibodies from the fertilization environment. These and other modifications of IVF/ET methodology should improve fertilizing ability for couples with antibodies to sperm head.

The few reports of IVF/ET in couples with immunologic infertility indicate that IVF/ET may offer a chance for conception to couples with antibodies to sperm who cannot conceive otherwise. This may be accomplished using IVF/ET with or without the use of methodological modifications. In the current study, pregnancies were achieved in couples with male and female immunologic infertility without any modification of IVF/ET methods. Ackerman et al[21] and Yovich et al[22,102] each reported pregnancies in women with serum antisperm antibodies (table 6). Both of these groups used IVF/ET media which contained no patient serum, substituting either human fetal cord or donor serum, and employed a procedure to wash the cumulus mass free of follicular fluid prior to insemination. Kamada et al[23] and Clarke et al[24] eliminated patient serum from IVF/ET media but did not employ additional washing procedures of the cumulus mass. These groups achieved improved fertilization rates but neither achieved any pregnancies. All but one fertilized ovum in these studies underwent cleavage.

The relative value and effects on fertilization rate of fetal cord and donor serum are somewhat difficult to assess from these cases. However, replacement of antibody-containing serum in IVF/ET media with antibody-negative serum appears to improve fertilization. The combination of washing the ova and eliminating patient serum from IVF/ET media would be expected to improve the chances of concep-

tion. On the basis of this limited data, IVF/ET seems to be a suitable treatment for women with antisperm antibodies. However, since IVF/ET is a major undertaking for an infertile couple, the therapeutic value of IVF/ET, particularly with these two modifications, must be tested further.

For couples with autoimmunity to sperm in the male undergoing IVF/ET, pregnancies were achieved in the current study without any apparent modification of methodology, and have similarly been reported by Naaktgeboren et al[25] and Clarke et al[26] (table 7). However, Clarke does not indicate whether cryopreserved semen collected and frozen during previous prednisone therapy was used for the patients who achieved pregnancies. Clarke did report poor fertilization rates in those men with $\geq$80% of their sperm bound as compared with a normal fertilization and conception rate in IVF/ET for those men with <80% of their sperm bound. Further studies must evaluate possible in vitro manipulations of sperm in IVF/ET to reduce antisperm antibody binding, particularly in men with high levels of antisperm antibodies. Clearly, conception is possible in at least a subset of men with immunologic infertility by IVF/ET alone.

The findings of the current study further define the relevance of antisperm antibodies in IVF/ET. Clinically significant antibodies to sperm were present in an unselected population undergoing IVF/ET, particularly in those patients whose infertility was unexplained. Antibodies to

sperm head in female serum and follicular fluid reduced fertilization in IVF/ET. Cleavage was also reduced in the presence of these antibodies in female serum. The immunobead binding assay proved to be an excellent detector of clinically significant antibodies, particularly because it determines the region of antibody binding to sperm. Using the immunobead binding assay, we were able to identify those patients with immunologic infertility which was clinically significant.

Based on these findings, we will initiate clinical screening for antisperm antibodies at Yale among couples at high risk for immunologic infertility. Couples with either unexplained infertility or a history of impaired fertilization in a previous IVF/ET cycle will be tested with the immunobead binding assay for antisperm antibodies. Subsequently, couples whose infertility is immunologic will have the opportunity to undergo IVF/ET with appropriate modifications to maximize their chances of conception.

Figure 1

Immunobead Binding to Sperm Head and Tail Tip

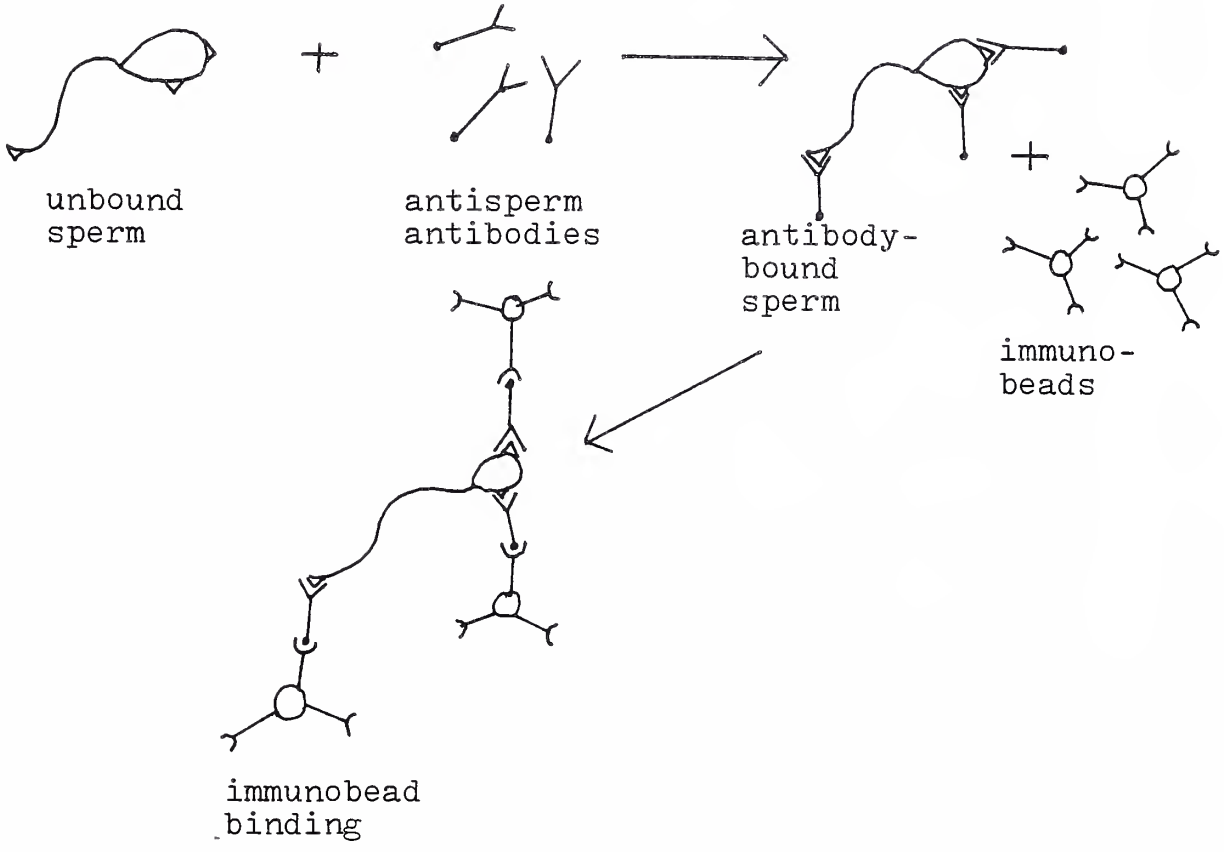


Figure 1. Immunobeads (polyacrylamide beads with covalently bound rabbit antibodies to human IgA, IgG or IgM) bind immunoglobulin bound to the sperm plasma membrane. In the presence of antibody-bound sperm (as illustrated), immunobeads are seen bound to sperm surface by phase contrast microscopy. In the absence of antibody-bound sperm, no immunobead binding occurs.

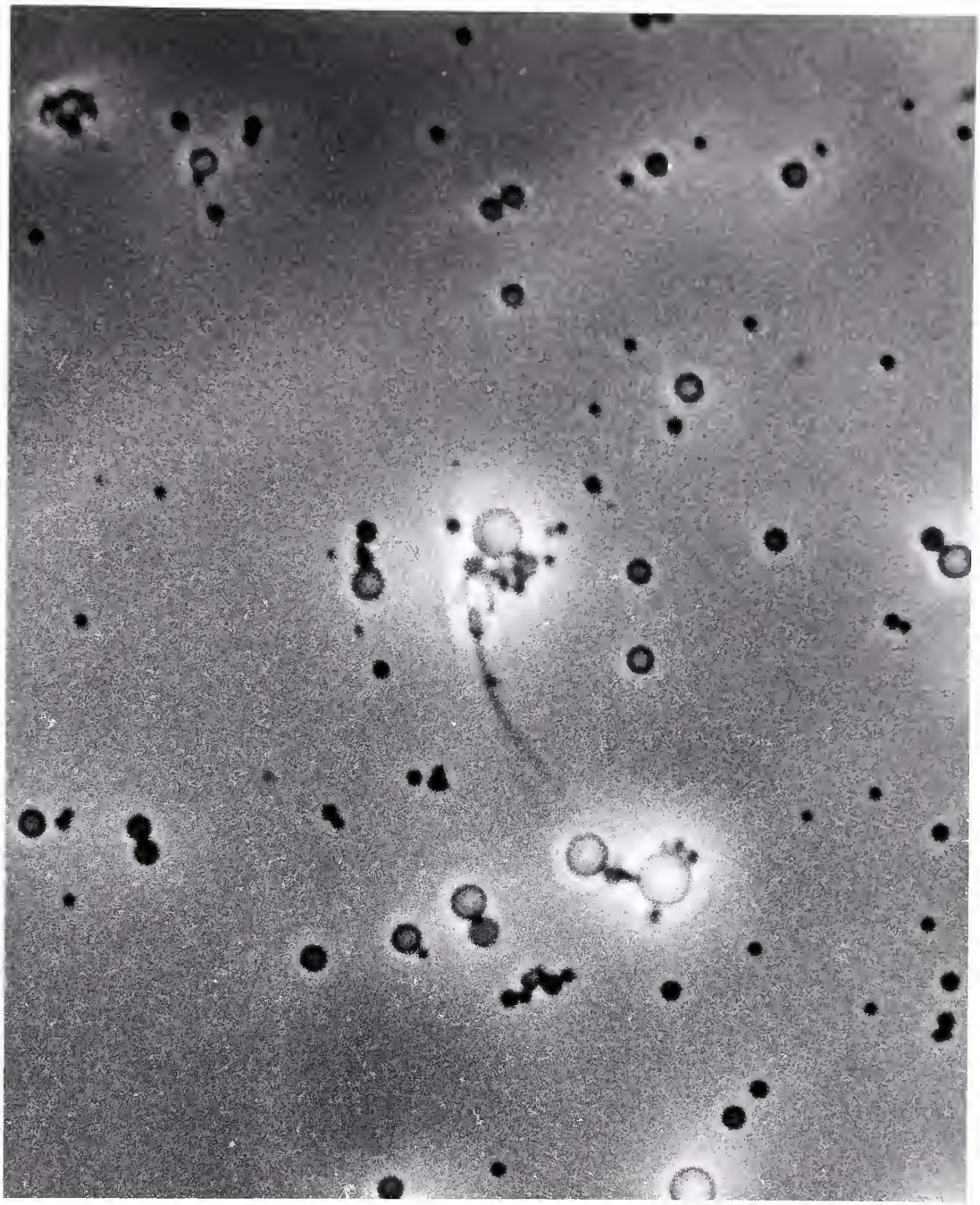


Figure 2. Immunobead Binding to Sperm Head and Tail Tip
(original magnification x200)

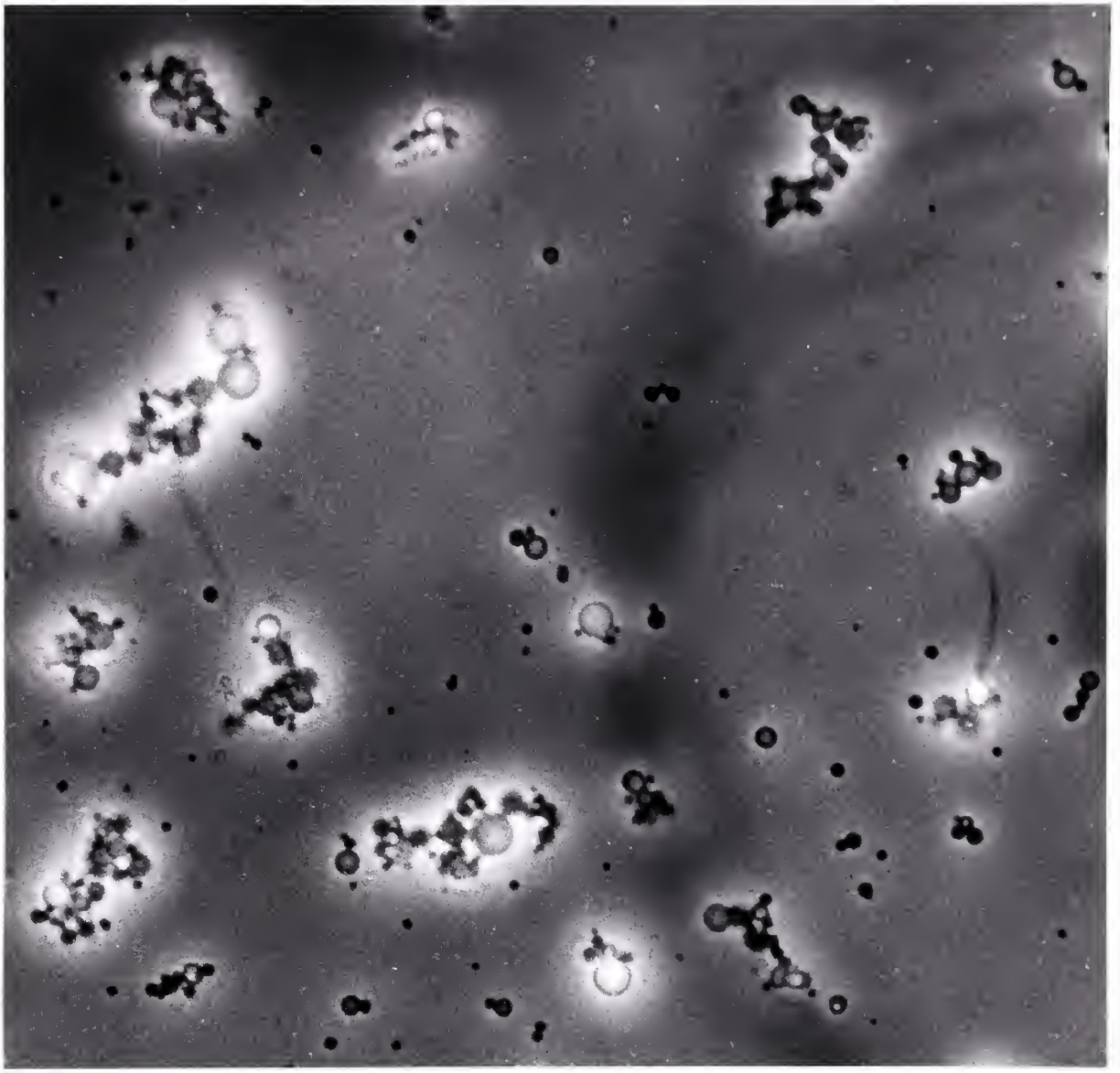


Figure 3. Immunobead Binding to Sperm Head and Tail Tip
(original magnification x200)

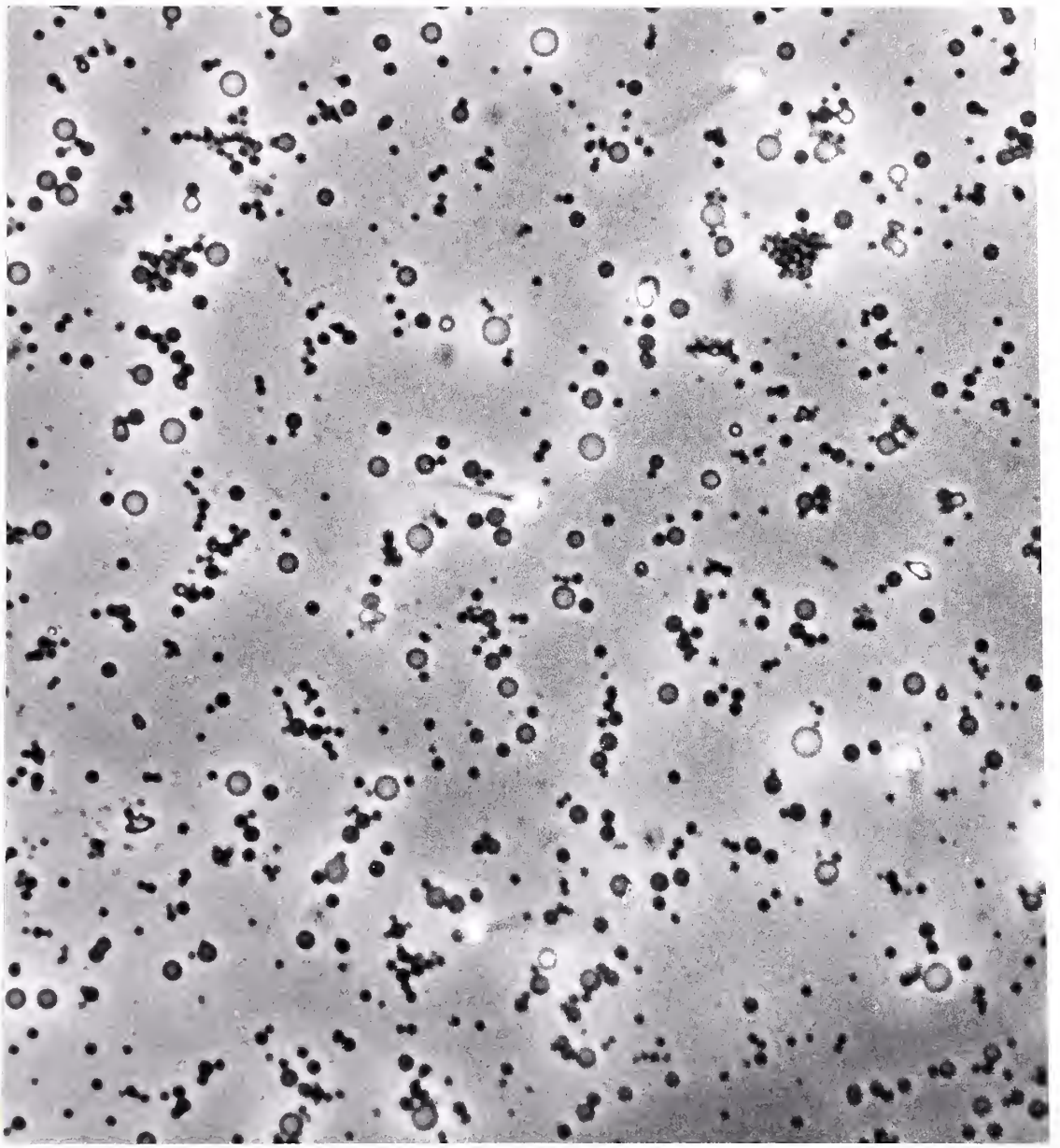


Figure 4. No Immunobead Binding to Sperm
(original magnification x200)

Figure 5
Oocyte Fertilization Rates in the Presence vs. Absence of Antibodies to Sperm Head in Female Serum, Follicular Fluid, Semen, and Male Serum

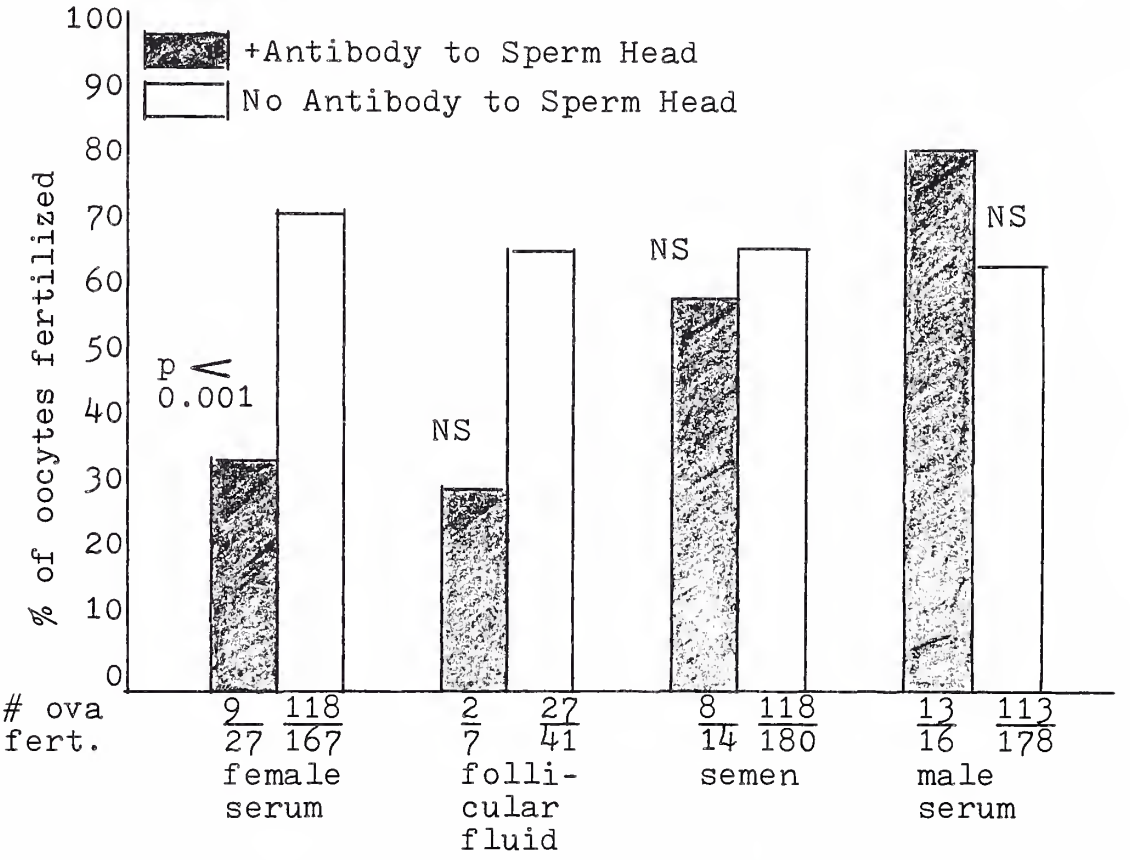


Figure 5. Fertilization was significantly impaired in the presence of antibodies to sperm head in female serum. Fertilization was also impaired in the presence of antibodies to sperm head in follicular fluid. However, due to the small number of oocytes involved (only oocytes from traps of follicular fluid tested are reported), this difference is not statistically significant. There was no significant impairment of fertilization in the presence of these antibodies in semen or male serum.

Figure 6
Oocyte Fertilization Rates in the Presence vs. Absence of Antibodies to Sperm Tail Tip in Female Serum, Follicular Fluid, Semen, and Male Serum

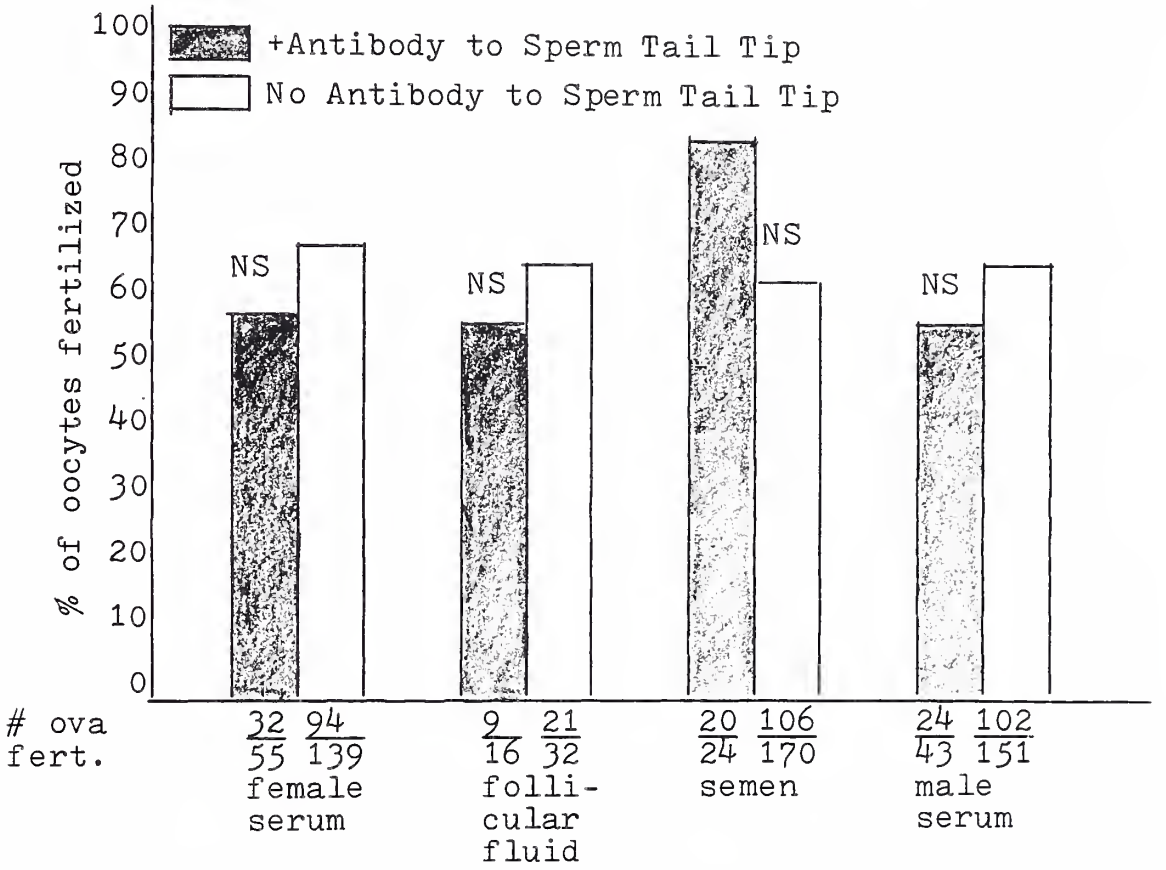


Figure 6.Oocyte fertilization was not significantly impaired in the presence of antibodies to sperm tail tip in any of the fluids tested.

Figure 7
Zygote Cleavage Rate and Pregnancy Rate by IVF/ET in the Presence vs. Absence of Antibodies to Sperm Head in Female Serum

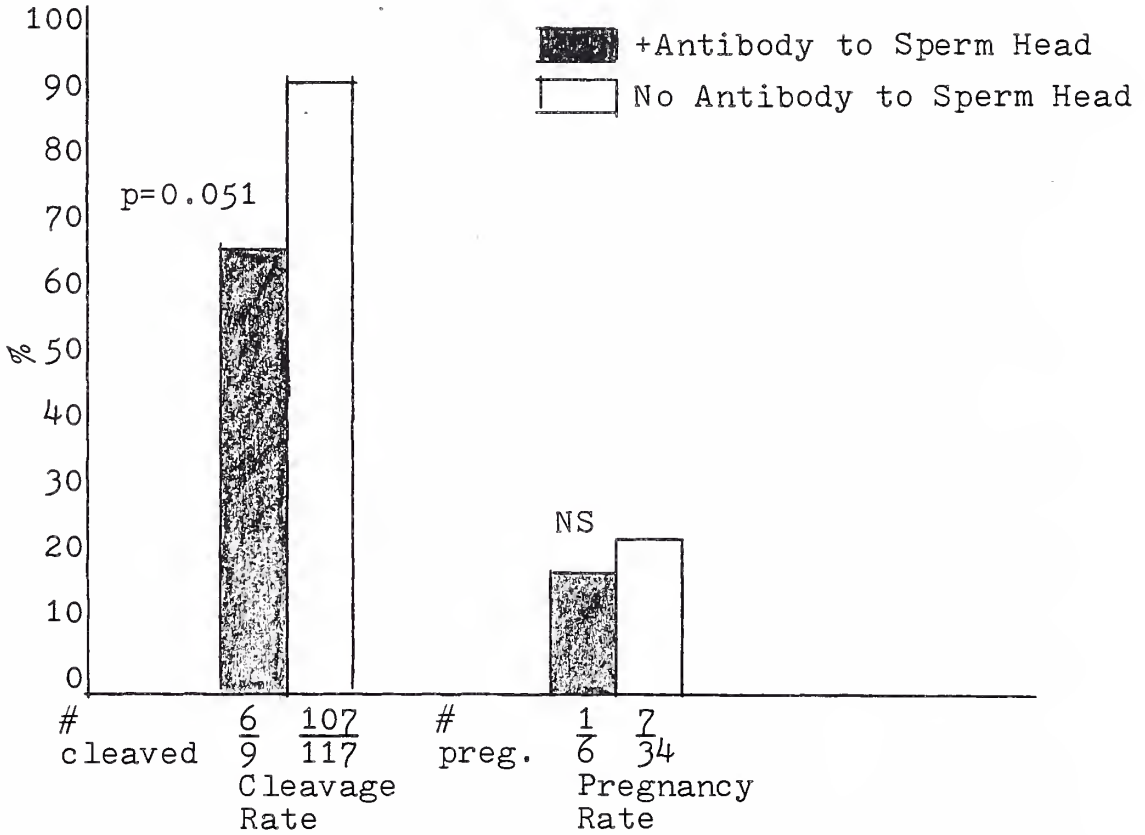


Figure 7.Zygote cleavage was impaired in the presence of antibodies to sperm head in female serum. Pregnancy was not significantly impaired in the presence of these antibodies in female serum.

Figure 8
Incidence of Antibodies to Sperm Head in Couples with Unexplained Infertility vs. Tubal Infertility by Fluid Tested

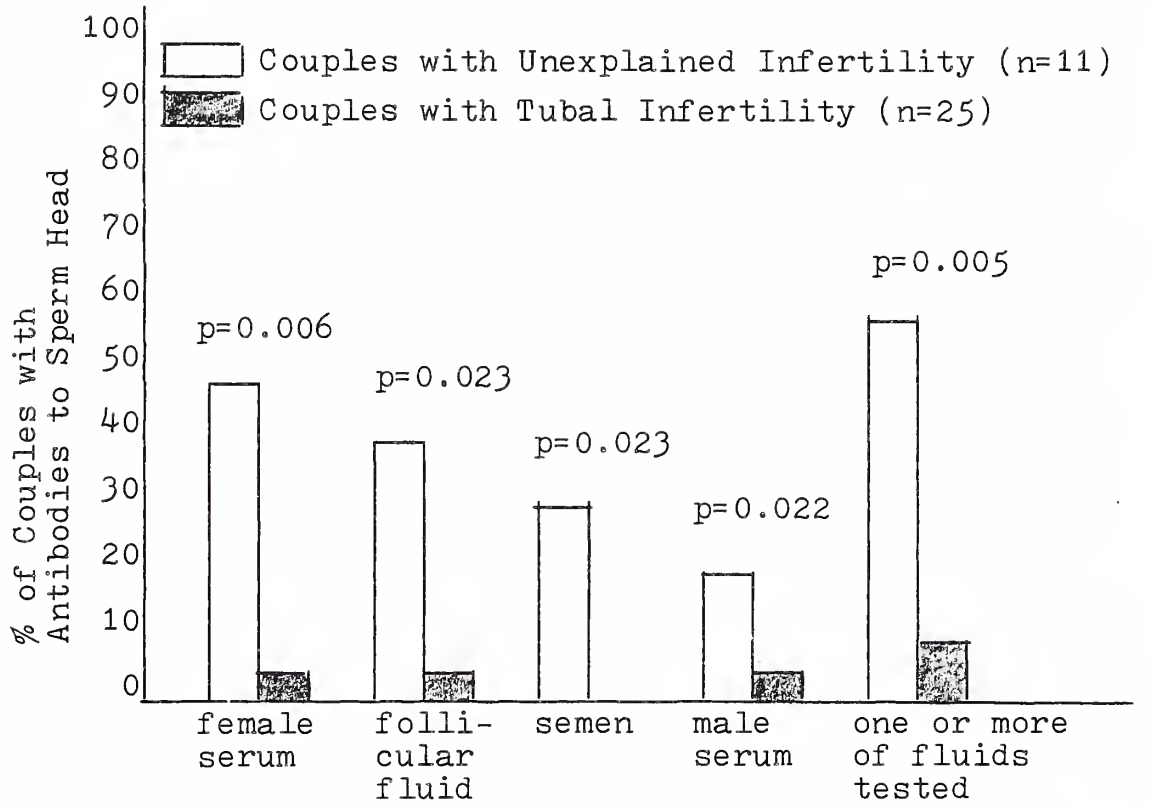
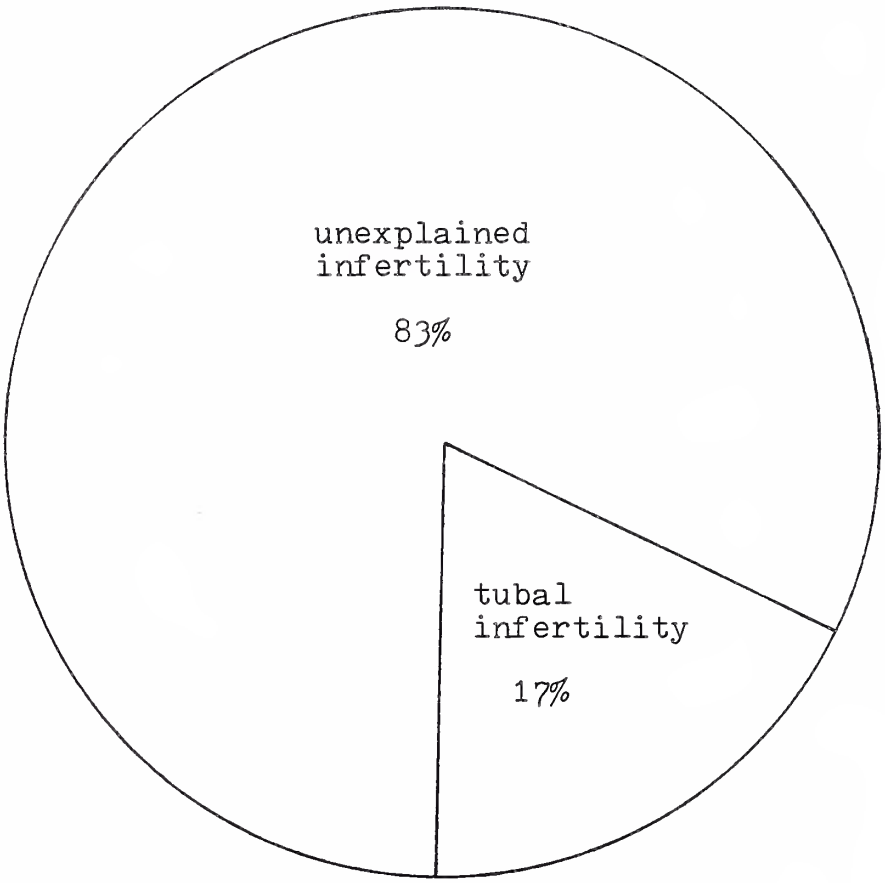


Figure 8. Antibodies to sperm head were detected in a significantly higher proportion of patients with unexplained infertility than tubal infertility in each of the 4 fluids tested and in all 4 fluids combined.

Figure 9
Distribution of Patients with Antibodies to Sperm Head in Female Serum in Unexplained vs. Tubal Infertility*



*p=0.08 (NS)

Figure 9. The vast majority of patients with antibodies to sperm head in female serum had otherwise unexplained infertility.

Results of Immunobead Binding and IVF/ET By Couple Table 1

Cp #	IMMUNOBEAD BINDING RESULTS												IVF/ET RESULTS				
	Head				Tail tip				Tail				Fert.		Cl. #	Pr.	IVF ind
	FS	FF	S	MS	FS	FF	S	MS	FS	FF	S	MS	#	%			
1	+	+	−	−	+	+	−	+	+	+	−	−	0/4	0	0/0	NP	U
2	+	−	+	−	+	−	−	−	−	−	−	−	0/6	0	0/0	NP	U
3	+	+	−	−	+	+	−	+	−	−	−	−	0/2	0	0/0	NP	U
4	−	−	−	−	−	−	−	−	−	−	−	−	0/6	0	0/0	NP	U
5	−	−	−	−	−	−	−	−	−	−	−	−	0/1	0	0/0	NP	T
6	−	−	−	−	−	−	−	−	−	−	−	−	1/8	14	1/1	NP	T
7	−	−	−	−	−	−	−	−	−	−	−	−	1/5	20	1/1	P	M
8	−	−	−	−	−	−	−	+	−	−	−	−	2/9	22	2/2	P	U
9	−	−	−	−	+	−	−	+	−	−	−	−	1/4	25	0/1	NP	T
10	−	−	−	−	−	−	−	−	−	−	−	−	2/5	40	1/2	NP	M
11	+	+	−	−	+	+	−	−	+	−	−	−	3/7	43	0/3	NP	U
12	−	−	−	−	+	−	−	−	−	−	−	−	1/2	50	1/1	NP	T
13	+	+	−	−	−	+	−	−	−	−	−	−	3/5	60	3/3	NP	T
14	−	−	−	+	−	+	−	−	−	−	−	−	5/8	63	4/5	NP	T
15	−	−	−	−	−	−	−	+	−	−	−	−	2/3	67	2/2	NP	T
16	−	−	−	−	−	−	−	+	−	−	−	−	2/3	67	2/2	P	T
17	−	−	−	−	−	+	−	+	−	−	−	−	3/4	75	2/3	NP	T
18	−	−	−	−	−	−	−	−	−	−	−	−	3/4	75	3/3	NP	U
19	−	−	−	−	+	+	−	−	−	−	−	−	6/8	75	6/6	NP	T
20	−	−	−	−	−	−	+	−	−	−	−	−	3/4	75	3/3	NP	T
21	−	−	−	−	−	−	+	−	−	−	−	−	10/13	77	9/10	NP	T
22	−	−	−	−	−	−	−	−	−	−	−	−	7/9	78	7/7	P	T
23	−	−	−	−	−	−	−	−	−	−	−	−	6/7	86	6/6	NP	T
24	−	−	−	−	+	−	−	−	−	−	−	−	6/7	86	5/6	P	T
25	−	−	−	−	−	−	−	−	−	−	−	−	7/8	88	6/7	NP	T
26	−	−	−	−	−	−	−	−	−	−	−	−	1/1	100	0/1	NP	T
27	−	−	−	−	−	−	−	−	−	−	−	−	7/7	100	6/7	P	T
28	−	−	−	−	−	−	−	−	−	−	−	−	1/1	100	1/1	NP	U
29	−	−	−	−	−	−	−	−	−	−	−	−	4/4	100	4/4	NP	T
30	−	−	−	−	−	−	−	−	−	−	−	−	2/2	100	2/2	P	T
31	−	−	−	−	−	−	−	−	−	−	−	−	8/8	100	8/8	NP	M
32	−	−	−	−	+	−	−	−	−	−	−	−	5/5	100	5/5	NP	M
33	+	+	+	+	−	+	−	+	−	−	−	+	3/3	100	3/3	P	U
34	−	−	+	+	−	−	+	+	−	−	+	+	5/5	100	5/5	NP	T
35	−	−	−	−	−	−	−	+	−	−	−	−	3/3	100	3/3	NP	T
36	−	−	−	−	−	−	+	+	−	−	−	−	2/2	100	1/2	NP	T
37	−	−	−	−	−	−	−	+	−	−	−	−	1/1	100	1/1	NP	T
38	−	−	−	−	+	+	−	−	−	−	−	−	5/5	100	5/5	NP	U
39	−	−	−	−	+	+	−	−	−	−	−	−	2/2	100	2/2	NP	T
40	−	−	−	−	+	−	−	−	−	−	−	−	3/3	100	3/3	NP	T

Cp #, couple number; FS, female serum; FF, follicular fluid; S, serum; MS, male serum; Fert., oocyte fertilization; Cl.#, # zygotes cleaved; Pr., pregnancy outcome; P, pregnant; NP, nonpregnant; IVF Ind., Indication for IVF/ET; T, tubal factor; U, unexplained infertility; M, male factor.

Table 2
Incidence of Antisperm Antibodies in 40 IVF/ET Couples by Fluid and Region of Antibody Binding to Sperm

	Head No. (%)	Tail tip No. (%)	Tail No. (%)
Female serum	6 (15)	12 (30)	2 (5)
Follicular fluid	5 (13)	10 (25)	1 (3)
Semen	3 (8)	4 (10)	1 (3)
Male serum	3 (8)	12 (30)	2 (5)

Table 3
Distribution of Antisperm Antibodies by Isotype of Immunoglobulin for Each Fluid and Region of Binding to Sperm

	Head (No.)	Tail tip (No.)	Tail (No.)
Female serum	*A=1 G=1 M=1 A,G=2 G,M=1	A=2 M=5 A,G=2 A,M=2 A,G,M=1	G=2
Follicular fluid	A=1 G=2 A,G=2	A=3 G=1 M=3 A,G=2 A,M=1	G=1
Semen	G=2 A,G=1	A=1 G=1 A,G=2	A,G=1
Male serum	A=1 G=2	A=3 G=1 M=3 A,G=2 A,M=1 A,G,M=2	G=2

*A=IgA; G=IgG; M=IgM.

Tests for Detection of Antisperm Antibodies Table 4

Test	Methodology	Advantages	Disadvantages
Sperm Agglutination Tests (TSAT,[4] GAT,[65] TAT[66])	Sperm agglutinate in presence of bivalent antibody bound to sperm surface (serum test)	Widely available	1.Not specific, i.e, clumping on nonimmune basis 2.Poor correlation with fertility status 3.None except TAT localize
Sperm Immobilizing Test[68] (SIT)	Antibody binding to sperm activates complement system to cause sperm immobilization or cytotoxicity (serum test)	1.Specific 2.Reproducible 3.Widely available	1.False negatives, i.e., won't detect antibodies that don't fix complement or immobilize 2.Doesn't localize sperm binding
Indirect Immunofluorescence Tests[69,70]	Fluorescence-labeled antiglobulins bind to antibodies bound to sperm prepared by fixation with methanol (serum test)	1.Highly sensitive 2.Determines region of sperm bound	1.False positives due to sperm internal antigens exposed by fixation 2.Poor correlation with fertility status
Enzyme-Linked Immunosorbent Assay[71-4] (ELISA)	Enzyme-linked antiglobulins bind antibody on sperm surface of whole sperm or sperm membrane extracts and substrate is added (semen, serum, biologic fluid test)	Quantitative	1.False positives and negatives due to denaturation of sperm membrane with fixation 2.Doesn't localize sperm binding

(continued)

Detection of Antisperm Antibodies (continued) Table 4

Tests	Methodology	Advantages	Disadvantages
Panning Assay[76]	Antibody-bound sperm stick to antiglobulin-coated wells (semen, serum biologic fluid test)	Can be used on sperm with low motility	1. Specificity, sensitivity unknown 2. Qualitative 3. Doesn't localize sperm binding
Sperm-Cervical Mucus Contact Test[77] (SCMCT)	Antisperm antibodies in semen or cervical mucus (CM) cause "shaking" reaction when sperm contact CM (CM, (semen test)	Can detect antibodies in cervical mucus or semen	1. Specificity, sensitivity unknown 2. Qualitative 3. Doesn't localize sperm binding
Radiolabeled Antiglobulin Assay[6]	Radiolabeled antiglobulin bind to antibodies bound to sperm (semen, serum, biologic fluid test)	1. Highly sensitive 2. Specific 3. Quantitative 4. Determines Ig isotype	1. Doesn't determine region of sperm bound
Mixed Agglutination Reaction (MAR)[80]	Mixed agglutination of sperm & sensitized Rh+ RBCs occurs when RBCs are mixed with semen and anti-IgG anti-serum (semen test)	1. Specific 2. Can detect antibodies bound to sperm in semen	1. Sensitivity unknown 2. Qualitative 3. Can only detect IgG 4. Region of sperm bound difficult to determine
Immunobead Binding Tests[17,31] (IBA)	Immunobeads with bound antiglobulin molecules bind antibodies bound to sperm surface (semen, serum, biologic fluid test)	1. Specific 2. Localizes region of sperm bound 3. Determines proportion of ejaculate that's antibody-bound 4. Determines Ig isotype	1. Sensitivity unknown

Table 5
Sites Where Antisperm Antibodies Might Impair Reproduction

Spermatogenesis	Autoimmunity may lead to aspermatogenesis, oligospermatogenesis or abnormal sperm morphology.
Sperm Transport	Immunoglobulins in semen, cervical mucus or female reproductive tract fluids may bind sperm surface and decrease sperm motility or impair sperm penetration of cervical mucus.
Sperm Survival	Antibody binding of sperm may lead to lysis of antibody-coated sperm by complement components or enhance macrophage phagocytosis of sperm in the reproductive tract through binding of the Fc portion of antisperm antibody to the macrophage Fc receptor.
Gamete Interaction	Antisperm antibodies may impair sperm capacitation, the acrosome reaction, sperm binding or penetration of the zona pellucida, sperm-ova fusion or zygote cleavage.
Embryo Survival	Antisperm antibodies may react with antigenic determinants on the embryo and block implantation or lead to spontaneous abortion after implantation.

Reports of IVF/ET Outcomes in Females with Immunologic Infertility — Table 6

Authors	Immunol. Findings	# Cps	Total #IVF Cyc.	Total Fert. (no.)	Total Preg. (no.)	Special IVF Methods Used
Ackerman et al[21]	+GAT & +SIT in female serum	1	2	2/7	1 TM	1) Cumulus washed free of foll. fluid x 4 2) Human fetal cord serum used in media
Yovich et al[22]	≥1: +GAT, +TSAT, +SIT in female serum	5	7	26/32	2 TM	1) Cumulus washed free of foll. fluid x 3 2) Donor serum used in media
Clarke	+TAT & +SIT; +IBA in female serum & foll. fluid	3	4	6/16	0	Type of serum in media: Patient serum
			2	4/8	0	Donor serum
			3	11/14	0	Human fetal cord serum
Kamada et al[23]	+SIT in female serum	1	1	0/2	0	Type of serum in media: Patient serum
			1	3/3	1	Human fetal cord serum
Mandel-baum et al	+IBA in female serum and foll. fluid: to sperm head	6	6	9/27	1	None
	to sperm tail tip	12	12	32/55	1	

Immunol., immunologic; Cps, couples; Cyc., cycles; Fert., fertilization; Preg., pregnancies; foll. fluid, follicular fluid; TM, term.

Reports of IVF/ET Outcomes in Males with Immunolgic Infertility — Table 7

Authors	Immunol. Findings	# Cps	Total #IVF Cyc.	Total Fert. (no.)	Total Preg. (no.)	Special IVF Methods Used
Clarke et al[26]	+SIT in male serum & +IBA in semen a) ≥80% sperm IgA-bound	17	12	27/100	0	Cryopreserved semen from previous prednisolone Rx used in 7/17 patients
	b) <80% sperm IgA-bound		18	47/65	2 SAB 2 TM	
Naaktgeboren et al[25]	+TAT in male serum & +MAR in semen	1	2	5/6	1 SAB	None
Mandelbaum et al	+IBA in semen: to sperm head	3	3	8/14	1	None
	to sperm tail tip	4	4	20/24	0	
	+IBA in male serum: to sperm head	3	3	13/16	1	
	to sperm tail tip	12	12	24/43	3	

Immunol., immunologic; Cps, couples; Fert., fertilization; Preg., pregnancies; foll. fluid; follicular fluid; TM=term; SAB=spontaneous abortion

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