

Treatment of male sperm autoimmunity by using the gamete intrafallopian transfer procedure with washed spermatozoa

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Sixteen couples were diagnosed as having immunological infertility. To detect sperm-bound immunoglobulin (Ig), i.e., IgA, IgG, and IgM antibodies, the direct immunobead test (IBT) was used. In each individual patient, the direct IBT was $\geq 70\%$ positive for either IgA or IgG or both. The indirect IBT was positive for IgA and IgG antibodies in the serum of all the patients. Semen was collected in 15 mL medium (Ham's F10 [Gibco, Grand Island, NY] + 10% whole blood serum) and prepared with the wash and swim-up method. Patients in the study group were treated for their immunological infertility problem by performing the gamete intrafallopian transfer (GIFT) procedure. An ongoing pregnancy was achieved in 7 of the 16 (43%) couples treated with the GIFT procedure with an ongoing pregnancy rate of 24.1% (7 of 29) per cycle. The GIFT procedure appears to be an effective and safe way of treating male immunological infertility.

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For many married couples, infertility is a cause of much anxiety. Infertility is commonly defined as the inability to conceive after 1 year of unprotected intercourse. It is important to realize that infertility is not an absolute condition, as some couples will eventually succeed in achieving a pregnancy at some future date.¹

The implications of sperm antibodies in infertility remains an area of controversy. Although most reproductive immunologists agree on the negative influence of antisperm antibodies on conception, many practicing gynecologists do not recognize an immunological basis for infertility and do not test for these antibodies.² Infertility because of sperm

antibodies only means a reduction in fertility of the couple.¹

Multiple assays have been devised for measuring antisperm antibodies. These include sperm agglutination and complement-mediated sperm immobilization assays. A more specific method of measuring antibodies on the sperm surface is the direct immunobead test (IBT) assay.³ This technique is used to visualize the attachment of polyacrylamide beads coated with antihuman immunoglobulin (Ig) to spermatozoa. This technique indicates the attachment of antibodies on the sperm surface and is an important advance in the field of male autoimmune infertility.

Treatment of men with antisperm antibodies is problematic and may be associated with major side effects.^{4,5} We have treated couples with male autoimmune infertility by carrying out the gamete intrafallopian transfer (GIFT) procedure with washed spermatozoa.⁶ The aim of this paper is to present the results of the treatment of male autoimmune infertility by the GIFT method.

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MATERIALS AND METHODS

Infertility Work-up

Sixteen couples were diagnosed as having immunological infertility. All the patients were submitted to the following tests.

Semen Evaluation before GIFT Procedure

The semen samples were obtained by masturbation at the laboratory after 3 to 4 days of abstinence. The quantitative motility or percentage of motile spermatozoa and qualitative motility or speed of forward progression was assessed, as described by MacLeod.⁷ At the same time, presence of agglutination and particulate debris was observed and an estimation of the sperm concentration made. An improved, double-ruled Neubauer hemocytometer (Assistant, Sondheim, Federal Republic of Germany) was used for determining the spermatozoa concentration.^{8,9}

In our laboratory, when evaluating sperm morphology, a spermatozoon is considered normal when the head has a smooth, oval configuration with a well-defined acrosome comprising about 40% to 70% of the spermhead.¹⁰ In addition there must be no neck, midpiece, or tail defects and no cytoplasmic droplets of more than one-half the size of the spermhead. In contrast with Eliasson,⁹ we consider borderline forms abnormal.

The provisional diagnosis of immunological infertility was made after a poor postcoital test (PCT) and a positive mixed antiglobulin reaction (MAR) test.¹¹ The diagnosis was subsequently confirmed, by doing the semen cervical mucus (CM) contact test.¹² Finally, the direct IBT test³ was done on all samples.

The MAR test was performed as described by Jager et al.¹¹ The percentage of motile spermatozoa present in mixed agglutinates with red blood cells was determined (MAR%: >10%, positive; >80%, strongly positive).^{11,13}

A semen CM contact test was also performed with normal donor CM when mucus was available. The method described by Kremer and Jager¹² was used. The percentage of motile spermatozoa showing no progressive movement but a definite "shaking" pattern was determined (semen CM contact %: $\geq 30\%$, positive; $> 75\%$, strongly positive).¹³

To detect sperm-bound IgA, IgG, and IgM, the direct IBT, as described by Clarke et al.,³ was used. The percentage of motile spermatozoa with one or

more immunobeads attached was determined microscopically (IBT %: >20%, positive).³ The indirect IBT as described by Clarke et al.³ was used to detect the presence of circulating antibodies in the seminal plasma. Regular ovulation was confirmed ultrasonographically and biochemically. Adequate cervical mucus was also confirmed during ovulation.

A hysterosalpingogram and laparoscopy were also performed on all patients. Ovarian hyperstimulation was achieved by using a combination of clomiphene citrate and human menopausal gonadotropin.¹⁴ Follow-up in these patients was done by doing serum luteinizing hormone (LH) and estradiol determinations as well as serial ultrasonographical measurement of the Graafian follicle. Human chorionic gonadotropin (hCG) was administered as soon as the leading follicle had reached 18 mm in diameter. Follicle aspiration was done 36 hours after hCG administration.

Semen Preparation on the Day of the GIFT Procedure

The spouse was required to produce a semen sample 1½ hours before laparoscopy was performed. The semen sample was collected in 15 mL medium (Ham's F10 [Gibco, Grand Island, NY] + 10% whole blood serum) at 37°C. The sample was divided into equal volumes and centrifuged at 1,500 rpm for 10 minutes. Supernatants were aspirated and pellets combined in 2 mL of new medium. The sample was centrifuged again at 1,500 rpm for 10 minutes. The supernatant was aspirated and the pellet covered with a 1-mL layer of fresh medium. Spermatozoa were allowed to swim up into the layer of medium for 1 hour.¹⁵

Gamete Intrafallopian Transfer Procedure

Laparoscopy and follicle aspiration was done under general anesthesia. One hundred percent CO₂ was used to obtain pneumoperitoneum. The aspirated oocytes were evaluated for maturity according to the criteria laid down by Veeck.¹⁶ Four oocytes were selected on completion of follicular aspiration. Two oocytes were then loaded into the transfer catheter together with 100,000 sperm. The number of sperm was increased to 500,000 in the case of male patients with a morphology of <14% normal forms.¹⁷ The loaded catheter was passed through the canula used for aspiration. Subsequently the catheter was inserted up to a distance of 2 cm through the fimbrial opening of the fallo-

pian tube. After the gametes were deposited, the procedure was repeated on the other tube. All couples were requested to abstain from intercourse during the course of treatment until the results of the pregnancy tests were available.

Pregnancy was diagnosed by the presence of β -hCG in the serum on the 12th day and an increasing value on the 16th day after the GIFT procedure was performed. The pregnancy was confirmed sonographically during the 7th gestational week when a fetal heart beat could be recorded.

Patients receiving artificial insemination before the GIFT procedure were evaluated as follows: spontaneous cycles were used, follicle growth was followed by ultrasound, and the insemination performed 36 hours after the beginning of the LH surge. Two inseminations on consecutive days were performed on each patient. In vitro fertilization (IVF) was performed before the GIFT cycles in some of the patients. The methodology followed was carefully described in previous publications.¹⁰

RESULTS

The present duration of infertility ranged between 2.5 and 12 years with an average of 6.25 years. Seven pregnancies had ensued previously in 7 of the 16 couples, and 5 children were born. Four of these were spontaneous pregnancies. Two term pregnancies and one ectopic pregnancy resulted after artificial insemination with husband's sperm (AIH).

During the infertility work-up no other cause for infertility was found except for the male abnormalities. One patient, however, had moderate active endometriosis.

The outcome of the PCT in all 16 patients was poor. Spermatozoa were found in the endocervical canal in 6 patients. The movement of the spermatozoa was slow and sluggish in 2 of these patients. In 1 of the 2 patients, 50 spermatozoa per high power field were found and the other had 3 spermatozoa per high power field. One patient had an average of 0.5 spermatozoa with nonprogressive motility per high power field. The same patient also had 5 immotile spermatozoa per high power field. Three patients had immotile spermatozoa in the endocervical canal. Adequate numbers of spermatozoa were found in the vaginal pool sample of all the patients.

The results of mixed antiglobulin reaction,

direct, and indirect IBT are summarized in Table 1. Except for one patient who had a 20% positive MAR, all the other patients were >80% positive for this screening test for IgG antibodies.

The semen CM contact test was positive (>30%) in all patients except in three. The reading was 8%, 21%, and 25%, respectively, in these patients. In two patients the test was not performed because of technical reasons. The direct IBT (IgG) in these three patients ranged between 70%, 99%, and 100% with a MAR test positive in all three (>10%). The two patients with no semen CM contact results had positive MAR tests (100%), direct IBT (IgG) of 70% and 90%, and IgA of 70% and 100%, respectively. All the patients in the study group had a IgM value of <10%. The main localization of antibodies in the spermatozoa is shown in Table 1. The numbers are too small for any conclusion to be made regarding the effect of site and type of antibodies.

The outcome of the patients admitted to the Reproductive Biology Unit for their infertility problem is summarized in Table 2. All the patients in the study group were treated with the GIFT procedure for their immunological infertility problem.

Eight of the 16 patients initially had either AIH or IVF in the clinic as treatment, before the GIFT treatment. Six patients had 28 cycles of AIH resulting in one pregnancy—a pregnancy rate of 3.5% per cycle. From the 4 patients who underwent IVF, 74 oocytes were retrieved of which 16 (21.6%) fertilized and five embryo transfers resulted. There were no pregnancies in this group. An ongoing pregnancy was achieved in 7 of the 16 (43%) couples treated with the GIFT procedure. The pregnancy rate per cycle was 41.4% (12 of 29) with an ongoing pregnancy rate of 24.1% (7 of 29) per cycle. Eleven babies including two sets of triplets were born from the 7 pregnancies.

The semen parameters in these patients are of interest. Only two were oligozoospermic with a concentration of 10×10^6 /mL and 15×10^6 /mL on the day of the procedure. The rest all had a semen concentration of $>20 \times 10^6$. Four patients had a normal morphology of 7%, 7%, 9%, and 13%, respectively, with the remainder above 14% normal forms (normal for our laboratory).^{10,18} The motility in the initial sample ranged between 30% and 60% in all cases.

In 10 of the 16 patients excess ova were available after GIFT for fertilization in vitro. All the oocytes were in the metaphase II stage of maturity at the

Table 1 Results of Mixed Antiglobulin Reaction, Direct and Indirect Immunobead Test, and Sperm Cervical Mucus Contact Test

No.	MAR	Direct percent IBT			Indirect percent IBT			Semen CM Contact
		IgA	IgG	IgM	IgA	IgG	IgM	
	%							%
1	95	95 to 100 H ^a	95 to 100 H + T ^{a,b}	<10	100 T ^b	100 E ^c	0	95
2	100	80 to 90 H ^a	10 T ^b	—	50 T ^b	100 E ^c	0	95
3	100	80 to 100 H ^a	100 H ^a	—	100 T ^b	100 E ^c	<10	68
4	80	40 to 50 T ^b	99 T ^b	<10	30 T ^b	100 E ^c	20	21
5	100	100 H + T ^{a,b}	90 H + T ^{a,b}	—	100 H ^a	100 E ^c	60 T	—
6	100	70 H ^a	70 E ^c	—	95 T ^b	100 E ^c	10	—
7	100	20 to 50 T ^b	95 H + T ^{a,b}	<10	100 T ^b	100 E ^c	<10	70 to 90
8	99	80 H + T ^{a,b}	90 to 99 T ^b	<10	60 T ^b	100 E ^c	10	66
9	100	90 H ^a	90 E ^c	0	100 E ^c	100 E ^c	0	60
10	100	20 T ^b	100 E ^c	<10	90 T ^b	100 E ^c	<10	25
11	100	99 H + T ^{a,b}	100 E ^c	0	50 E ^c	100 E ^c	0	91
12	100	100 E ^c	100 E ^c	0	40 T ^b	100 E ^c	0	98
13	100	80 E ^c	90 T ^b	0	80 T ^b	100 E ^c	<10	100
14	20	50 T ^b	70 T ^b	0	70 T ^b	95 E ^c	0	8
15	100	99 E ^c	80 T ^b	<10	40 T ^b	100 E ^c	0	92
16	100	100 H ^a	95 H ^a	0	99 T ^b	100 E ^c	10	94

^a H, head: bead bound >50% to head.

^b T, tail: bead bound >50% to tail.

^c E, equal: equal distribution of bead on head, neck, and tail tip.

time of fertilization 5 hours after retrieval. Of the 58 oocytes retrieved, 14 fertilized (24.13%). In a control group of 20 patients with unexplained infertility done on the same day or the next day the fertilization rate was 82% per oocyte.

DISCUSSION

Seven pregnancies had ensued previously in 7 of the 16 couples, with five children born. The average period of infertility was 6.25 years, ranging between 2.5 and 12 years. These couples should thus be classified as subfertile.^{1,2} Pregnancies due to antispermatozoal antibodies treated by means of the GIFT procedure may be because of a general improvement of semen parameters; motility, forward progression, and morphology in swim-up samples, and not a decrease in antibody binding.¹⁵ Another advantage of the GIFT procedure is that it brings the ova into direct contact with free swimming spermatozoa in the fallopian tube, thereby excluding all the barriers from the cervical mucus upwards.

It is of interest to note the poor fertilization rate in the study group (24.1%) compared with the 82% in the control group. It is our opinion that poor fertilization in vitro does not reflect the outcome of autoimmune male infertility treated by the GIFT method. We also observed that of the four patients in whom failure of fertilization occurred in vitro, two became pregnant during the course of the study. Because of small numbers, we were not able to correlate the data with the chance of pregnancy in the concurrent GIFT procedure. It is possible that the environment in the fallopian tube facilitates the fertilization process and is superior to the environment in our incubators. We do not believe that the quality of the oocytes played a role in the poor fertilization outcome as all the oocytes were metaphase II oocytes at time of fertilization.

The percentage IgG and IgA antispermatozoal antibodies on the sperm surface was determined by means of the direct IBT. Circulating IgG, IgA, and IgM antisperm antibodies were determined by the indirect IBT (Table 1). Although figures are small, no relationship was apparent between the percent-

Table 2 Summary of the Results of the Three Types of Treatment the Patients had since their Admission in our Unit

Treatment	Patients	Cycles	Ongoing pregnancy/cycle ^a	Miscarriages	Ectopic
AIH	6	28	1 (3.5)	0	1
IVF	4	10	0	0	0
GIFT	16	29	7 (24.1)	4	1

^a Values in parentheses are percents.

age IgA and IgG antisperm antibodies in semen and the ability of the sperm to fertilize ova. Both IgA and IgG have been shown to cause sperm agglutination and immobilization, and to impair sperm ovum penetration.^{19,20}

Before admission to our program, many of these couples were treated with various regimes including long term steroid treatment. Before we decided to use the GIFT method of treatment for patients with immunological infertility, these patients had been treated with AIH and IVF. For AIH, sperm was prepared by the same wash and swim-up method as is done for IVF. Although this was not a comparative study, it is interesting when one compares the success rate of the different modalities of treatment. The 6 patients that had AIH in our study had a total number of 28 insemination cycles with an average of 4.6 insemination cycles per patient. This treatment resulted in one ongoing, and one ectopic pregnancy, an ongoing pregnancy rate of 3.5% per cycle. Four patients had 10 IVF cycles with no pregnancies. The pregnancy rate for the GIFT procedure was 41.3% (12 of 29) with an ongoing pregnancy rate of 24.1% (7 of 29). Seven of the 16 patients (43%) eventually had an ongoing pregnancy beyond 12 weeks. The pregnancy rate with the GIFT procedure compares favorably with the figures in a compendium of studies by Haas,⁴ reporting a success rate of 23% for AIH, 37% for IVF, and 31% for corticosteroid treatment. The latter form of treatment, however, is associated with side effects which include dyspepsia, trunkal skin rashes, intense irritability or depression, fluid retention or weight gain, and avascular necrosis of the hip. The GIFT procedure has less side effects than either corticosteroids or cyclosporin A.^{5,21} The pregnancy rate in the above patients with immunological infertility treated by means of the GIFT procedure corresponds to that for patients treated in our unit by GIFT for endometriosis and unexplained infertility.²²

Most of the male patients had completely normal semen parameters. Four of the patients had a sperm morphology of <14%. In previous studies we have clearly shown that the fertilization rate is impaired in this group.¹⁰ It has been reported that if the morphology was between 5% and 14% normal forms the fertilization rate per oocyte was 63%, and if the normal sperm morphology was <5% normal forms the fertilization rate was 7.6%, if only 100,000 spermatozoa/mL were used to fertilize an oocyte.¹⁸ However, if the concentration was raised

to 500,000/mL then the fertilization rate improved significantly.¹⁷ We applied this principle in the GIFT program in patients with teratozoospermia (<14% normal forms). However, more data is needed in this group of patients before any final conclusion can be drawn.

We conclude that the GIFT procedure, after rapid dilution of semen in a large volume of medium, appears to be one of the safest and most effective treatment modalities available for immunological infertility. A randomized controlled trial is now required to critically evaluate this method of treatment.

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