

TREATMENT OF IMMUNOLOGICAL INFERTILITY BY SPERM WASHING AND INTRAUTERINE INSEMINATION

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The purpose of this study is to review our experience with sperm washing and intrauterine insemination as a method to reduce the presence of sperm antibodies in serum of couples with immunological infertility. Our results support the premise that sperm washing and intrauterine insemination can diminish the level of sperm-bound immunoglobulins and can improve the chance of conception in a selected group of couples with a diagnosis of immunological infertility. Patients with >50% attachment of IgG, IgA, or both antisperm antibodies in this study had a greater chance of achieving a pregnancy. The high level of antibody attachment seen in our study appears to represent true immunological infertility and therefore is more likely to respond to therapy that reduces antibody attachment and bypasses cervical mucus.

Key Words: Sperm washing; Motility; Immunological infertility; IUI.

INTRODUCTION

Opinions diverge as to the benefits of homologous male sperm in artificial insemination in an infertile couple. Potential indications for such efforts include oligospermia, asthenospermia, poor postcoital tests, the presence of sperm antibodies, low semen volume, and anatomic abnormalities [24]. Sperm-reactive antibodies are known to affect sperm penetration into, and motility within, cervical mucus [4, 8, 17]. Since the development of methods to identify the attachment of antisperm antibodies to the sperm, there has been an increased interest in the treatment of patients whose infertility is due to the presence of such antibodies [5, 9, 21]. The only therapy recommended for treatment of antisperm antibodies in men has been steroids [12, 14, 28]. Unfortunately, some adverse effects from high-dose steroids have limited their usefulness in treating this condition.

To examine the use of a less risky procedure in the treatment of patients with antibody-induced infertility, we retrospectively analyzed data from patients treated for various causes of infertility by sperm washing and intrauterine insemination (IUI) [1, 7, 15, 25, 29]. Diagnoses for these patients included cervical factor infertility, oligoasthenospermia, unexplained infertility, and infertility secondary to antisperm antibody. Forty-five couples out of a total of 159 in this study underwent therapy because of the presence of antisperm antibodies. The purpose of

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this article is to review our experience with sperm washing and IUI as a method to reduce the presence of sperm antibodies in serum of couples with immunological infertility.

MATERIALS AND METHODS

Selection of Couples. One hundred fifty-nine couples treated for infertility by sperm washing and IUI over a 2-year period were included in this study. The women were considered to be potentially fertile because of a negative history and physical examination, evidence of normal endocrine parameters and patent tubes, or earlier treatment for a known cause of infertility other than cervical factor infertility. The men undergoing this treatment who were diagnosed infertile with causes other than idiopathic oligoasthenospermia or immunological infertility were treated for those conditions, such as obstruction (vasectomy), infection (prostatitis), and varicocele.

Preparation of Semen. Semen specimen was produced at the laboratory after 48–72 h of sexual abstinence. After liquefaction in a 37°C incubator, the semen was examined for sperm concentration, percentage motility, and sperm velocity on a Cell-Soft semen analyzer (Cryo-Resources, New York, NY). The specimen was then diluted (1 : 3) with Ham's F-10 medium (Gibco, Grand Island, NY), supplemented with penicillin G, streptomycin sulfate (Sigma, St. Louis, MO), lactic acid, sodium bicarbonate (Fisher, Pittsburgh, PA), and 0.75 mL of fetal cord serum. This mixture was centrifuged at 300g for 10 min. The supernatant was then aspirated and discarded, except for the pellet, and 1.2 mL residual fluid was placed in a CO₂ incubator at 37°C for 60 min. After the incubation, 0.3–0.5 mL of the upper swim-up portion was used for insemination, and a small portion of it was used for a postwash count and motility analysis.

Macroagglutination. A Kibrick test [18, 19, 26] or gelatin agglutination test (GAT) was performed by suspending semen from a donor without antisperm antibodies mixed with the complement inactivated serum of the suspected subfertile patient in a gelatin mixture in a small glass tube. After incubation for 60 min at 37°C, the formation of large agglutinated clumps of sperm toward the bottom of the tube and clearing of the homogeneous suspension of sperm in the upper portion of the tube indicated that antisperm antibodies were present in the serum sample. Both positive and negative controls were tested with patient specimens.

Mixed Agglutination Reaction (MAR). A MAR test [13, 16] was used to demonstrate membrane-bound antibodies of IgG and IgA type on the surface of sperm. The test also indicates the regionalization of binding on the sperm surface. In this test, red blood cells are coated with IgA or sensitized with IgA or IgG. These cells are then mixed with a monospecific anti-IgA or an anti-IgG. These treated red cells are incubated with the sperm under test. If the sperm carry antisperm antibodies of the appropriate class, then they adhere to the red blood cells.

Indirect Immunobead Test (IBT). Samples for the indirect IBT were stored frozen at –20°C until analyzed. Before use, the sample was heated to 56°C for 30 min to inactivate the complement. Aliquots of the sample were incubated with donor sperm previously proven negative for membrane-bound antibodies by the direct IBT. These sperm were washed to eliminate unreacted immunoglobulin and mixed with immunobeads. Antisperm antibodies in the test sample bind to the donor sperm, which then interact with the antihuman immunoglobulins on the immunobeads [6].

Timing and Technique of Insemination. Ovulation was detected by a monoclonal antibody assay of human luteinizing hormone (LH) in urine beginning on the tenth day of the menstrual cycle (Ovustick,

Monoclonal Antibodies, Inc., Mountainview, CA). Insemination was performed twice in each cycle, first at 24 h after the LH surge and then again at 36–48 h after the LH surge. Transcervical IUI was performed on an ambulatory basis. Patients were asked to lie down in the lithotomy position; the cervix was exposed with a bivalve speculum and wiped with a gauze sponge. A Makler cannula was attached to a 1-mL plastic tuberculin syringe, and 0.3–0.5 mL of concentrated washed sperm were aspirated into the syringe. The cannula was introduced into the uterine cavity by means of a Makler insemination device (Sefi Medical Instruments, Haifa, Israel), and the concentrated sperm were instilled slowly into the uterine cavity. The cannula was removed, and the patient remained recumbent for 15 min.

RESULTS

Among the couples who underwent sperm washing and IUI in a 2-year period, there were 37 pregnancies, for an overall pregnancy rate of 21%. Forty-five of the couples underwent this therapy as treatment for immunological infertility. Among those 45, there were 15 pregnancies, for a pregnancy rate of 32.6%. Thus, 41% of the total pregnancies for the study group occurred in couples being treated for immunological infertility.

The range of age in those women who did not become pregnant and the range of the length of infertility were higher than for those who became pregnant, which may in part account for their lack of success (Table 1). The high number of men who had other possible causes of infertility in the nonpregnant group, compared with the pregnant group, suggests that other infertility factors played a significant role in their lack of success.

Examination of the antibody data in those patients whose wives achieved pregnancy with this method reveals that more than half of them had >50% attachment of either IgG or IgA antisperm antibody to their sperm (Table 2). Out of 15 patients, 13 had IgA antisperm antibodies and 10 had IgG present on their spermatozoa. In couples that did not achieve a pregnancy only 23 of them had attachment of antisperm antibody demonstrated by MAR or IBT. Seven other couples had significant titers of IgG antisperm antibody in their serum. These data suggest that pregnancy is more likely to occur when significant levels of anti-sperm antibody are attached to the sperm. Perhaps the presence of serum antisperm antibody is not really of significance in immunological infertility.

Comparison of the pregnant and nonpregnant patients showed no significant difference in the number of cycles in which they underwent IUI, although the nonpregnant group underwent more cycles of IUI than the pregnant group (Table 3). The mean number of cycles of insemination in couples who achieved pregnancy suggests that if pregnancy was going to occur, it was likely to occur within three cycles. There were actually slightly fewer motile sperm inseminated in those who achieved pregnancy than in those who did not. Comparison of the quality of prewash semen with that of postwash semen showed no difference with regard to sperm count and percentage motility. Results of sperm washing in both groups showed a significant reduction in count but a significant increase in the percentage of motile sperm.

Analysis of the presence of antisperm antibodies before and after sperm washing in 30 randomly selected patients revealed a marked reduction after washing (Table 4). In 17 of these patients, no attachment of antisperm antibodies was found after washing, and in the remaining 13, attachment was reduced significantly and was perhaps clinically insignificant.

TABLE 1 Characteristics of 45 Couples Undergoing Treatment of Immunological Infertility with Intrauterine Insemination

Category	Mean Age in Years (Range)		Mean Length of Infertility in Years (Range)	Number of Patients with Other Associated Infertility Factors (Total %)	
	Men	Women		Men	Women
Pregnant (<i>n</i> = 15)	35 (26–50)	32 (30–37)	3.3 (1–7)	7/15 (47%)	4/15 (27%)
Not pregnant (<i>n</i> = 30)	34 (25–52)	34 (25–42)	3.2 (1–12)	24/30 (80%)	16/30 (53%)

TABLE 2 Results of Antisperm Antibody Tests in 45 Couples Treated for Immunological Infertility with Intrauterine Insemination

Category	Kibrick		MAR-IgG ^a		IBT-IgA ^b	
	Patients with Titer < 1 : 32	Patients with Titer > 1 : 32	Patients with < 50% Attachment	Patients with > 50% Attachment	Patients with < 50% Attachment	Patients with > 50% Attachment
Pregnant (<i>n</i> = 15)	0	3	3	7	5	8
Not pregnant (<i>n</i> = 30)	3	12	8	7	4	4

^aMAR-IgG; Mixed Antibody Reaction Test for IgG.

^bIBT-IgA, Immunobead Test for IgA.

TABLE 3 Treatment Cycle and Semen Quality in 45 Couples Treated for Immunological Infertility with Intrauterine Insemination

Category	IUI Cycles	Prewash Semen Quality		Postwash Semen Quality	
		Count	% Motility	Count	% Motility
Pregnant (<i>n</i> = 15)	Total 107 3 (1–9)	52 (7–173)	47 (25–78)	16 (0.5–60)	85 (67–100)
Not pregnant (<i>n</i> = 30)	Total 136 4 (3–11)	41 (6.5–147)	54 (15–88)	16 (1.4–42)	87 (21–99)

TABLE 4 Results of Sperm Washing for Removal of Antibodies in Randomly Selected Patients Undergoing Intrauterine Insemination

Patients	Number of Patients with Indicated Antibodies			
	Prewash		Postwash	
	IgG (+)	IgA (+)	IgG (+)	IgA (+)
Tested for MAR-IgG (<i>n</i> = 18)	18	0	4	0
Tested for IBT-IgA (<i>n</i> = 3)	0	3	0	1
Tested for both MAR-IgG and IBT-IgG (<i>n</i> = 9)	9	9	2 ^a	7 ^b

^a76% reduction in antibody attachment.

^b52% reduction in antibody attachment.

DISCUSSION

The use of sperm preparation and IUI have been recommended for infertility related to sperm surface antibodies. Although the data suggest that these antibodies have high affinity and are not easily removed by repeated sperm washing [10], several reports indicate pregnancy rates of ~25% with these techniques [20, 21]. Sperm washing followed by insemination has been applied with success [3, 11, 25, 27, 32] to couples in which the man is found to have circulating antisperm antibodies. This approach is based on the rationale that the antisperm antibodies presumably were also present on the man's spermatozoa and could be removed by repeated sperm washing. Since cortical steroids can produce adverse effects, it is worthwhile to consider sperm washing and IUI for patients with sperm surface antibodies.

Associated infertility factors were present in the majority of couples who did not achieve pregnancy and in a significant number who did achieve pregnancy. But the common factor in those who achieved pregnancy was >50% attachment of antisperm antibody IgG, IgA, or both. This suggests that in these patients the immunological factor was the major cause of infertility [2]. Two factors leading to success appear to be the significant reduction in the antibody attachment achieved by the washing technique and the bypassing of cervical mucus by IUI. It has been demonstrated that IgA is more likely to prevent sperm from passing through cervical mucus than IgG and therefore the patients with high levels of IgA attachment may represent a variety of cervical factor infertility [28].

The success of the washing technique may in part be due to the use of human fetal cord serum. A significantly higher pregnancy rate was seen in an in vitro fertilization program when the culture medium was supplemented with fetal cord serum than with maternal pre-ovulatory serum [21]. In the present study, 73% of patients who achieved pregnancy had antibody attachment of >50%, in contrast to previous findings [15] in which the pregnancy rate was greatest (67%) in couples with <50% antibody binding. The high level of antibody attachment seen in our study appears to represent true immunological infertility and therefore is more likely to respond to therapy that reduces antibody attachment and bypasses

cervical mucus. The spontaneous abortion rate in these patients of 27% is consistent with reports from other groups using sperm washing and IUI as a treatment for infertility.

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