

Antisperm antibody treatment mode: levels of antisperm antibodies after incubation with TEST-yolk buffer and filtration using the SpermPrep™II method

Panayiotis M. Zavos, Ed.S., Ph.D.,*† Juan R. Correa, Ph.D.,* and Panayota N. Zarmakoupis-Zavos, M.D.*†

Andrology Institute of Lexington and Kentucky Center for Reproductive Medicine, Lexington, Kentucky

Objective: To assess whether incubation in TEST-yolk buffer (TYB) or human tubal fluid (HTF) could alter the sperm membrane characteristics and its relationship to antisperm antibodies (ASA) and/or antigen detachment from the sperm membrane and to evaluate the filtration of those specimens and possible recovery of ASA-free spermatozoa.

Design: A prospective clinical study.

Setting: Andrology Institute of Lexington, Lexington, Kentucky.

Patient(s): Twenty patients undergoing infertility treatment.

Main Outcome Measure(s): Recovery of spermatozoa with reduced levels or antisperm antibody-free sperm after treatment with TYB or HTF, followed by filtration using the SpermPrep™II method (Sephadex based).

Result(s): Assessment of ASA using the direct immunobead test showed no significant differences between specimens incubated for 2 hours in seminal plasma (fresh) or HTF with regard to levels of IgA and IgG. The percentage binding of anti-IgA and anti-IgG immunobeads was significantly reduced in specimens incubated for 2 hours in TYB compared with specimens incubated in seminal plasma or HTF. Furthermore, selection of spermatozoa using the SpermPrep™II filtration method significantly reduced the percentage binding of anti-IgA and anti-IgG immunobeads compared with specimens incubated in HTF.

Conclusion(s): The results suggest that TYB either altered the sperm membrane properties so that there was a decreased affinity at the antibody and/or antigen sites or that the egg yolk proteins were absorbing the antibodies and/or antigens complexes from the sperm membrane surface. Incubation of spermatozoa in TYB followed by filtration with the SpermPrep™II method improved the recovery of ASA-free spermatozoa by selectively entrapping spermatozoa with ASA bound to its surface. (Fertil Steril® 1998;69:517-21. ©1998 by American Society for Reproductive Medicine.)

Key Words: Antisperm antibodies, immunobead test, TEST-yolk buffer, filtration

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Reprint requests:
Panayiotis M. Zavos, Ed.S.,
Ph.D., Andrology Institute
of Lexington, P.O. Box
23777, Lexington,
Kentucky, 40523
(FAX: 606-278-6906).

* Andrology Institute of
Lexington, Lexington,
Kentucky, 40523.

† Kentucky Center for
Reproductive Medicine,
Lexington, Kentucky,
40508.

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It is well established that antisperm antibodies (ASA) are etiologically implicated in female infertility. Sperm antibodies are a relative rather than an absolute cause of infertility. In general, the presence of ASA will reduce the likely occurrence of a pregnancy (1-3). The presence of ASA in the female reproductive tract may impair sperm-egg interaction by interfering with the dispersion of cumulus mass and sperm binding, penetration of the sperm into the zona pellucida, and sperm-egg fusion (4, 5).

The presence of ASA in the male repro-

ductive tract affects sperm function by possibly causing premature acrosome reaction and sperm immobilization (agglutination) as well as by decreasing membrane integrity and opsonizing sperm for phagocytosis (6-10). Antisperm antibodies may bind to the sperm surface within the testis or epididymis (before ejaculation) or during the mixing of the sperm and seminal plasma at ejaculation.

Bronson et al. (10) and Shulman et al. (11) suggested the use of semen manipulation techniques such as competitive in vitro immunosorption and rapid semen dilution to reduce the

concentration of ASA in the seminal plasma of men with low binding of immunoglobulins to the sperm surface. However, in most cases the affinity of ASA for their corresponding antigens are high and the ASA-antigen complex binding occurs rapidly after ejaculation (2, 12).

ASA have a high affinity for the sperm membrane, and consequently it is extremely difficult to either prevent the formation of ASA-antigen complexes or to remove these complexes (by traditional sperm processing methods) after binding of the ASA and antigens (2, 12). Sperm washing alone or high speed centrifugation methods do not seem to remove the ASA once the high affinity binding occurs (10, 12). It is difficult to prepare sperm specimens for IUI or assisted reproductive technologies (ART) when ASA are present because of the agglutination, immobilization, and reduction in membrane integrity. Attempts at dissociating the ASA-antigen complexes by reducing the pH or increasing the ionic concentration of the sperm's immediate environment result in irreversible loss of motility (10).

Incubation of spermatozoa in TEST-yolk buffer (TYB) has been evaluated as a cryoprotective medium; in addition, TYB has the ability to enhance capacitation and synchronization of the acrosome reaction through modification or stabilization of the sperm membrane characteristics or to mask the sperm membrane surface by the egg yolk-coating properties (13–19). Incubation of sperm specimens with associated ASA in TYB could reduce the binding of ASA to antigen sites or remove the antigen sites by a similar mechanism of action. This study was designed to assess whether TYB could mask or alter the membrane characteristics so that the ASA and/or the antigens would become detached from the sperm membrane and to evaluate whether subsequent sperm selection using Sephadex filtration could enable the recovery of high-quality ASA-free spermatozoa.

MATERIALS AND METHODS

Semen Collection and Preparation

Twenty patients undergoing infertility treatment participated in this study. Subjects were selected from a population of patients over a period of 2 years. Semen specimens were collected by each patient via masturbation. The methodology and protocols used in this study were approved by the Institutional Review Board of the Andrology Institute of Lexington, Lexington, Kentucky. Semen specimens were allowed to liquefy for 30 minutes, and each specimen was split into three aliquots (aliquot 1–3). Aliquot 1 was used as control without further manipulation and was incubated for 2 hours at 32°C. Aliquots 2 and 3 were diluted 1:1 (vol/vol) using TYB or human tubal fluid (HTF) medium (ZDL, Inc., Lexington, KY), were washed three times by centrifugation (400 *g* for 10 minutes), and were reconstituted with the corresponding medium.

Assessment of Antisperm Antibody Levels

Fresh sperm specimens (aliquot 1) were washed and resuspended in HTF before the immunobead test was performed. Sperm specimens (aliquots 2 and 3) washed with TYB or HTF were incubated for 2 hours (32°C); the incubation was followed by a second sperm wash, and the sperm were resuspended with the corresponding (fresh) media before the immunobead test was performed. All sperm specimens were adjusted to a concentration of 20×10^6 spermatozoa per mL after the final wash, followed by ASA testing for human IgA and IgG levels by direct immunobead testing.

Immunobeads (Polyacrylamide; BioRad Laboratories, Richmond, CA) prepared with covalently bound rabbit antibody to human IgG and IgA were used for the performance of the immunobead test. After the sperm washing procedures and ASA assessment, sperm specimens were filtered using the SpermPrepTMII method and assessed for ASA.

The immunobeads were reconstituted in TYB and HTF. A 10- μ L immunobead sample was mixed with 10 μ L of the sperm specimen on a microscope slide, a coverslip was applied, and the mixture was incubated for 10 minutes (21°C) in a moist Petri dish. The samples were examined at 200–400 \times magnification using a phase-contrast microscope, which was used to determine the percentage of motile spermatozoa (200 sperm counted per specimen) with attached beads. Immunobead binding to $\geq 10\%$ motile sperm (2 or more beads bound to sperm surface) was considered as a positive ASA binding (20, 21).

SpermPrepTMII Filtration

The SpermPrepTMII filtration method (ZDL, Inc.) was used as previously described (22–25). The Sephadex beads used in the SpermPrepTMII filters are made of a physiologically inert polysaccharide derivative; at hydration and subsequent swelling and enlargement (3 times the original size), these beads develop rough ridges on their surface that further aid in the entrapment and removal of dead or immotile spermatozoa during the filtration process. Before use, the SpermPrepTMII was hydrated by placing 6.0 mL of the corresponding media in the barrel of the column. The filter matrix was gently mixed with the media to form a suspension, ensuring that air bubbles were removed from the bottom of the filter. The beads were allowed to settle for 10 minutes to the bottom (sedimentation) and to undergo complete hydration.

When the sedimentation was completed, the bottom closure was removed and 2–3 mL of media was allowed to run through. This step normally enables the removal of any small bubbles and debris from the filter line. The filter was capped again with the bottom closure, and the filtrate was discarded. The capped filter was filled with 2–3 mL of media and placed into a 15-mL conical-centrifuge tube and held until the sperm specimen was ready for filtration. The sperm specimen was placed in the filter and was gently mixed with the

TABLE 1

Antisperm antibody levels after incubation for 2 hours at 32°C in TEST-yolk buffer or human tubal fluid.

Treatment	Immunoglobulin class assessed	
	IgA (%)	IgG (%)
Fresh*	95.5 ± 3.6	96.1 ± 4.1
TYB incubation†	58.2 ± 4.7§	45.3 ± 5.3§
HTF incubation‡	89.3 ± 3.8	91.4 ± 4.0

Note: All values are means ± SD.

* Fresh semen specimens were incubated without dilution for 2 hours at 37°C.

† Semen specimens were washed and incubated in TEST-yolk buffer (TYB) for 2 hours at 37°C.

‡ Semen specimens were washed and incubated in human tubal fluid (HTF) for 2 hours at 37°C.

§ $P < 0.05$ versus fresh specimens and specimens treated with HTF.

corresponding medium to form a uniform suspension and to prevent all the sperm from settling directly onto the filter and possibly clotting the filter. As the sperm specimen was added and mixed, the bottom closure was removed and filtration began.

Filtration was continued for 10 minutes, and more medium was periodically added to the filter to maintain the level of medium at its original level. This step maintained a uniform hydrostatic pressure on the filter during the total filtration time. At the end of filtration, the filter was closed with the bottom closure and removed from the centrifuge tube. The filtrate was collected in the centrifuge tube and centrifuged, and the recovered sperm pellet was resuspended and evaluated as previously described.

The total functional sperm fraction (TFSF; $\times 10^6$ spermatozoa), an inclusive term that incorporates the quantitative and qualitative sperm characteristics (26), was calculated for fresh specimens and for specimens recovered after SpermPrep™II filtration. The TFSF is the product of sperm count by percent motility by percent normal morphology by percent swollen spermatozoa (HOS test).

RESULTS

The results obtained in this study are summarized in Tables 1 to 3. Assessment of ASA using the direct immunobead test showed no statistically significant differences ($P > 0.05$) between specimens incubated for 2 hours in seminal plasma (fresh) or HTF with regard to levels of IgA and IgG (Table 1). The percentage binding of anti-IgA and anti-IgG immunobeads was significantly reduced in specimens incubated for 2 hours in TYB compared with specimens incubated in seminal plasma or HTF (Table 1). Furthermore, the percentage binding of anti-IgA and anti-IgG immunobeads was significantly reduced ($P < 0.05$) for sper-

TABLE 2

Antisperm antibody levels after filtration using the SpermPrep™II filtration method.

Treatment	Immunoglobulin class assessed	
	IgA (%)	IgG (%)
TYB incubation*	21.3 ± 5.7‡	27.1 ± 6.0‡
HTF incubation†	78.9 ± 4.5	73.9 ± 5.1

Note: All values are means ± SD.

* Semen specimens were washed and incubated in TEST-yolk buffer (TYB) for 2 hours at 37°C, followed by filtration using the SpermPrep™II filtration method.

† Semen specimens were washed and incubated in human tubal fluid (HTF) for 2 hours at 37°C, followed by filtration using the SpermPrep™II filtration method.

‡ Significant differences in the level of IgA and IgG were noticed between specimens incubated in TYB and HTF ($P < 0.05$).

matozoa previously incubated in TYB using the Sephadex (SpermPrep™) filtration compared with specimens incubated in HTF (Table 2).

The reduction in the percentage binding of anti-IgA and anti-IgG immunobeads in specimens filtered using TYB was 77.6% and 71.6%, whereas the reduction for fresh specimens was 95.5% and 96.1%. The reduction in the percentage binding of anti-IgA and anti-IgG immunobeads in specimens filtered using HTF was 17% and 23% when compared to fresh specimens, respectively. Sperm qualitative characteristics were significantly higher ($P < 0.05$) in specimens filtered using the SpermPrep™II filtration method, regardless of the incubation media used (Table 3).

The total number of spermatozoa recovered was significantly higher ($P < 0.05$) in specimens incubated and filtered with TYB than in those prepared with HTF. The TFSF for fresh specimens and for those incubated and filtered using TYB and HTF was 18.2×10^6 , 38.8×10^6 , and 22.4×10^6 spermatozoa, respectively. The TFSF was significantly higher in specimens incubated and filtered using TYB than in fresh or HTF-prepared specimens ($P < 0.05$).

DISCUSSION

TEST (TES and Tris) is a zwitterion buffer system that is generally supplemented with 20% (vol/vol) chicken egg yolk and is known as TYB (13–18, 27). The properties of TYB medium include pH and CO₂ level stabilization and binding of heavy metals; TYB is also a protein–lipid-rich medium. TEST-yolk buffer maintains sperm viability, especially during cryostorage (5°C) or transport conditions (15, 16, 18, 27, 28). It is believed that egg yolk components stabilize the sperm membrane surface by coating it with cholesterol, phospholipids, and lipoproteins (15, 16, 18). This type of interaction may also result in the removal of sperm surface

TABLE 3

Sperm characteristics of specimens after filtration using the SpermPrep™II filtration method.

Treatment	Sperm characteristics assessed				
	Count ($\times 10^6$)	Motility (%)	Grade (0–4)	Morphology (percent normal)	Hypoosmotic swelling test (%)*
Fresh†	138.7 \pm 10.2	48.3 \pm 5.1	2.8 \pm 0.2	49.2 \pm 5.6	55.3 \pm 7.8
TYB incubation‡	77.8 \pm 5.1	78.9 \pm 3.5	3.4 \pm 0.1	76.0 \pm 5.1	83.2 \pm 5.1
HTF incubation§	60.3 \pm 4.2 [#]	70.1 \pm 3.2	3.1 \pm 0.1 [#]	73.2 \pm 4.7	72.6 \pm 4.2 [#]

Note: All values are means \pm SD. HTF = human tubal fluid; TYB = TEST-yolk buffer.

* Percentage of swollen spermatozoa.

† Fresh semen specimens were incubated without dilution at 37°C.

‡ Semen specimens were washed and incubated in TEST-yolk buffer for 2 hours at 37°C, followed by filtration using the SpermPrep™II filtration method.

§ Semen specimens were washed and incubated in human tubal fluid (HTF) for 2 hours at 37°C, followed by filtration using the SpermPrep™II filtration method.

||, ||, # Denote significant differences ($P < 0.05$) among fresh sperm specimens and specimens treated with TYB or HTF.

components such as antigen sites or ASA–antigen complexes.

Hensleigh and co-workers (19) recently evaluated this type of interaction after incubating spermatozoa in TYB for 24 hours at 4°C, followed by assessment of anti-IgA and anti-IgG immunobead binding using the immunobead test. The researchers reported that no significant changes were observed in the levels of anti-immunoglobulins measured before and after TYB incubation under the conditions of the study. The levels of ASA tended to be less but not significantly different than before TYB incubation. Furthermore, the results obtained after performance of the indirect immunobead test, which involves de novo binding of ASA to spermatozoa, indicated the possibility that changes could have taken place in the sperm surface antigens when incubated in TYB.

In this study, spermatozoa were incubated in TYB for 2 hours at 32°C. Differences in time and temperature of incubation could have accounted for the discrepancies noted between the two studies. It is known that refrigeration of spermatozoa, usually at 5°C, alters the sperm membrane arrangement and possibly stabilizes its components, which resume their normal function or structure after controlled refrigeration, storage, and rewarming (15, 16, 18). It is also possible that extended cryostorage may affect antigen sites or antigen–ASA complexes attached to the sperm surface in a different way than short-term incubation at higher temperatures.

Improvements in sperm qualitative characteristics have occurred when spermatozoa with a variety of abnormalities or deficiencies have been filtered (29–32). Such deficiencies include oligoteratoasthenozoospermia, spermatozoa with single-stranded DNA and abnormal acrosome membranes, and spermatozoa with a high incidence of coiled tails (24, 29–32). The SpermPrep™II (Sephadex) filtration method entraps spermatozoa with morphologic abnormalities or other qualitative deficiencies by isolating spermatozoa

in the filter matrix that consists of a physiologically inert polysaccharide derivative (beads); at hydration and subsequent bead swelling and enlargement (3 times the original size), these beads develop rough ridges on their surface that further aid in the entrapment and removal of undesirable spermatozoa (selective filtration).

In this study, the filtration process using the SpermPrep™II method enhanced the sperm qualitative characteristics and significantly improved the number of ASA-free spermatozoa for clinical use after TYB incubation. The results suggest that TYB either altered the sperm membrane properties so that there was a decreased affinity at the antibody and/or antigen sites or the egg yolk proteins were absorbing or neutralizing the antibodies and/or antigens complexes from the sperm membrane surface.

Incubation of spermatozoa in TYB followed by filtration using the SpermPrep™II method improved the recovery of ASA-free spermatozoa by selectively entrapping spermatozoa with ASA bound to its surface. This method could prove to be of significant clinical importance as it could yield ASA-free spermatozoa for further use in the various ART procedures including IUI. Further clinical studies are currently underway at our facilities to confirm the findings of this study, to assess egg and sperm interactions in vitro, and to assess establishment of clinical pregnancies.

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