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Production and immunolocalization of monoclonal antibodies against human sperm

Shuo Tang
Lehigh University

Tang, Shuo

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Shuo Tang

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Dr. Barry Bean, Thesis Adviser

Biological Sciences

Dr. Neal Simon, Chairman of Department

Biological Sciences

Dr. Michael Kuchka, Graduate Coordinator

Biological Sciences

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Abstract:

In order to help identify and characterize antigens involved in sperm functions and immune infertility, monoclonal antibodies (mAbs) were raised against human sperm surface antigens. The IgG fraction of serum from a donor with a spontaneous high titer of IgG-positive antisperm reactivity (as determined by immunobead binding) was purified by ammonium sulfate precipitation. This IgG preparation was coupled to Sepharose 4B, and used for immunoaffinity purification of antigens from a detergent-solubilized extract of pooled normal human sperm. The affinity-purified antigens were used to immunize female mice, and resultant spleen cells were fused with SP 2/0 mouse myeloma cells to generate hybridomas. A single-step semi-solid methylcellulose method was used to isolate hybridomas for selection of positive clones, which were determined by ELISA. Thirty-two positive hybridoma lines were selected for immunolocalization and cross-reactivity studies using avidin-biotin complex (ABC) assay. Distinctive staining patterns and distribution of sperm surface antigens were observed for ten monoclonal antibodies. Among them, the cross-reactivity with human lymphocytes was not observed for four monoclonal antibodies.

Introduction:

The interactions between sperm and oocyte during human fertilization are thought to be highly specific. Sperm are antigenic, and antisperm antibodies have been associated with involuntary infertility in both men and women. Monoclonal antibodies (mAbs) could be a powerful tool for identification of specific sperm antigens that have a role in the reproductive processes, most earlier attempts to produce mAbs against human sperm have used as an immunogen either whole cells, or a detergent-extract, or a sonicate of sperm. To date, many antisperm mAbs have been reported, and a few of the targeted sperm antigens have been characterized. For example, antigens SP-10, PH-20, HSA-63, may have roles in the fertilization process. However, none of these may seem to react with sera from immunoinfertile volunteers (Naz et al., 1994). However, these and other antigens remain interesting candidates for immunocontraceptive technologies (Anderson et al., 1987; Archibong et al., 1995). We hope to identify additional, different sperm immunogens that function in fertilization, and demonstrate affinity for natural immunoglobulins involved in immune infertility. Some such antigens and their corresponding mAbs could be valuable tools for understanding human fertilization, or for diagnosis or treatment of immune infertility, and some might join the list of candidates for immunocontraceptive technologies.

Our strategy to enhance selection for mAbs against sperm surface features follows. A semen donor who shows high titer of IgG directed against the sperm surface was

identified, and is called donor BW in this report. Donor BW was shown by direct and indirect Immunobead Binding Test (IBT) and immunolocalization studies to have high reactivity for serum, seminal plasma, and surface-bound antisperm IgG. A serum IgG fraction from donor BW was used to purify human sperm surface antigens by immunoaffinity chromatography. The eluted antigens were used to immunize mice, and eventually generate hybridoma lines that were selected by a single-step semi-solid methylcellulose method. Thirty-two mAbs were selected for immunolocalization and cross-reactivity studies using avidin-biotin-peroxidase complex [ABC] assay. Distinctive staining patterns on sperm and noncross-reactivity with human lymphocytes was observed for some mAbs.

Materials and Methods

Detection of Antisperm antibodies by Immunobead Binding Test (IBT)

The direct IBT is for the detection of the bound human immunoglobulins (Igs) on the sperm surface. The indirect IBT looks for the presence of antisperm antibodies in serum or in reproductive tract secretions.

Immunobeads

Beads were obtained form Bio-Rad as separate conjugates of polyclonal rabbit-antihuman immunoglobulins conjugated to polyacrylamide beads of diameter 3-10 μm. Separate preparations provide specificity for antihuman IgG, IgA, or IgM. For each, 6.25 mg of lyophilized beads was washed twice in 5 ml PBS (800 g, 10 min) and then was resuspended in 1 ml of buffer B (10 mg/ml BSA in PBS) to a final concentration of 6.25 mg beads /ml. The reconstituted beads can be stored at 4°C for 1 month.

Direct IBT

Direct IBT was performed on donor sperm following IBT protocol for antisperm antibody detection (WHO manual). Briefly, sperm was washed twice in buffer A (5 mg/ml BSA in PBS) (800 g, 10 min) and then resuspended in buffer A to an appropriate concentration. 5 μl of washed sperm were mixed with 50 μl washed bead suspension and incubated for 10 min. 15-20 μl of sperm-bead suspension was added to a glass slide

and a coverslip was placed on top. The slide was examined under phase contrast at 400X. At least 100 motile sperm were scored for each Ig class. The proportion of sperm with beads bound to their surface was determined and also the predominant location of bound beads (head, midpiece, tail, tail tips or all sites) was recorded.

Indirect IBT

Indirect immunological binding determinations of serum and seminal plasma were determined by following IBT protocol (WHO manual). Normal sperm were obtained from antibody-free donors and washed twice in buffer A. Then 25 µl sperm were incubated with 275 µl buffer A and 100 µl human serum (previously inactivated at 56 °C for 30 min) or seminal plasma (separated by centrifugation at 3000 rpm) for 1 h at 37°C. After incubation, the sperm were washed twice in buffer A (800 g, 8 min). The pellet was resuspended in buffer A. The subsequent steps were same as direct IBT. With each assay, a previously positive reacting serum and a previously negative reacting serum were run as controls.

Purification of human sperm surface antigens by affinity chromatography

Sperm antigens potentially involved in human fertility were enriched by immunoaffinity chromatography. Briefly, IgG-enriched fractions of immunoglobulins from donor BW serum were purified as material precipitatable with 50% ammonium

sulfate, followed by dialysis against the coupling buffer (0.1 M $NaHCO_3$, 0.5 M NaCl). For use as affinity ligands, these IgG fractions were immobilized on Sepharose 4B by cyanogen bromide activation (Ausubel et al., 1992). Approximately 87% of IgG was coupled to the beads. This material was packed as an immunoaffinity column of a bed volume of 7 ml in 10 ml disposable syringe.

Human sperm autoantigens were purified from an initial pool of 24 confirmed antisperm-negative human semen specimens that had been previously characterized with direct immunobead binding test, washed with PBS pH 7.4 three times and stored at -20°C. These specimens were resuspended, pooled in PBS, and centrifuged at 3000 rpm at 4°C for 10 min. 0.36 g cell pellet was resuspended to $5x10^8$ /ml in 16 ml ice cold TSM solution (0.002 M Tris-Cl pH 8.0, 0.14 M NaCl, 0.01% Methiolate, 0.5% Triton X-100, and 0.5% Na-DOC), and 16 ml cold lysis buffer (2% Triton X-100, 5 mM iodoacetamide, 0.2 U trypsin inhibitor/ml, 1 mM PMSF, prepared in TSM). The cell suspension was stirred 1 hr at 4°C and centrifuged 10 min at 4000 g to remove nuclei. For purification of membrane antigens, 6 ml of 5% NaDOC was added to post-nuclear supernatant, followed by centrifugation 2 hrs at 150,000 g. The Ab-Sepharose 4B was suspended in the membrane crude extract in a beaker and stirred gently overnight at 4°C. The Ag-Ab-Sepharose was loaded onto a 10 ml disposable syringe to make an affinity column. The bed volume is 7 ml. Following extensive wash with wash buffer (0.01 M Tris-Cl pH 8.0, 0.14 M NaCl, 0.1% Triton X-100, 0.01% Methiolate), Tris buffer pH 8.0 and Tris buffer pH 9.0 until the absorbance at 280 nm of the eluent reached baseline, the adsorbed

sperm autoantigens were eluted from the affinity column with Triethanolamine solution (50 mM Triethanolamine pH 11.5, 0.1% Triton X-100, 0.15M NaCl) at pH 11.5 and immediately neutralized by addition of 0.2 Vol of Tris-Cl pH 6.7. Eluted fractions were pooled, dialyzed and concentrated by Centriprep 10 concentrator (Amicon 4304) against PBS. The final protein concentration was estimated by the Bradford assay (Bio-Rad protein assay dye reagent 500-0006) in accord with suppliers instructions. The total amount of sperm autoantigens purified from the immunoaffinity chromatography was 335.8 μg (29.2 μg/100μl x 1150 μl).

Generation of hybridomas

Immunizations with purified human sperm membrane extract in mice

Two CSJF1/J female mice, "L" and "R" (8 weeks old), were successively immunized with the affinity purified solubilized human sperm antigens described above. Both CSJF1/J female mice were first immunized by intradermal injection of about 10 μg affinity-purified human sperm autoantigens, in 100 μl of 50:50 (v/v) PBS and Freund's complete adjuvant. Booster injections (i.p.) Containing15-22 μg antigen in 100-150 μl of 50:50 (v/v) PBS and Freund's incomplete adjuvant were administered on day 22. Eleven days after the booster injection, both mice were checked for the presence of antisperm antibodies in peripheral blood, and they both successfully generated a high titer of

antisperm antibodies screened by ELISA against crude sperm extract 2 μg/ml coated on a ELISA plate. Three weeks after the second injection, the mice were given intraperitoneally 15-30 μg of purified sperm antigens in 50-100 μl of PBS without adjuvant. 3-4 days before fusion, 38 μg purified antigens in 130 μl PBS was injected (i.p.) on "L" mouse, and 30 μg purified antigens in 100 μl PBS on "R" mouse.

Production of hybridomas by semi-solid methylcellulose medium and selection of monoclonal antibodies against human sperm antigens

Mouse hybridomas secreting monoclonal antihuman sperm antibodies were produced by fusion of spleen cells obtained from immunized CSJF1/J female mice with SP 2/0 mouse myeloma cells, following the general protocol of Campbell (1991). A single-step technique was used for selecting and cloning hybridomas (Davis et al., 1982). Briefly, on the fusion day, $1x10^8$ spleen cells from the immunized mice were recovered and washed in a serum free IMDM medium. SP 2/0 mouse myeloma cells were obtained as a gift from Dr. Neal Simon and cultured in IMDM containing 10% FCS at 37°C in 5% CO_2. Spleen cells were mixed with SP 2/0 mouse myeloma cells at 5 : 1 ratio and they were fused in the presence of 50% PEG 1500. The pellet from the fusion procedure was resuspended in 15 ml of solution A [this solution contained 53.3% v/v FCS (Hyclone A-1111), 2.66% v/v HAT stock solution (Sigma), $8x10^6$ freshly prepared mouse thymus cells/ml and 133 μg LPS/ml in IMDM]. 25 ml of 2% w/v methylcellulose stock solution

was then added and mixed. 1 ml aliquots of this suspension were dispensed into 35 mm diameter bacteriological Petri dishes. Two such dishes were placed inside a 100 mm diameter Petri dish, together with an open dish containing about 1 ml of water. The plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2 in air. After 7-10 days in culture, the plates were examined for the presence of visible colonies. Thereafter, the plates were checked repeatedly at intervals of 2-3 days. Colonies greater than 0.5 mm in diameter were removed with sterile capillary tubes and cultured in 24 or 96-well culture plates with each well holding 1 ml or 200 µl of IMDM supplemented with 20% (v/v) FCS and 1% (v/v) of HAT stock (100x). Smaller colonies were allowed to grow further before picking. When the suspension cultures reached a density of about 1 $x10^6$ cells /ml or higher, the supernatants were checked for the presence of antibodies reacting with crude sperm membrane extracts by the microplate enzyme-linked immunoassay (ELISA) following the general procedure of Short Protocols in Molecular Biology (1992). Total 48 positive hybridomas were chosen, expanded, and stored in liquid nitrogen. After subculturing, total 32 hybridoma cell lines were established and relevant culture supernatants were collected for preliminary studies.

Immunolocalization of mAbs on human sperm

The interaction of the 32 mAbs with antigenic sites on the human sperm surface was visualized and localized using the avidin-biotin-immunoperoxidase complex (ABC)

method, following the protocol of Vector Laboratories Inc. Myeloma Sp 2/0 supernatant and layer deletions with PBS instead were used as negative control. Briefly, samples of fresh normal human semen from antisperm-negative donors were studied as smears on microscope slides. Slides were not allowed to dry during any of the series of steps. A smear of human semen was fixed in 4% formaldehyde for 15-20 mins at room temperature, then incubated with 50 µl of mAbs for 1 h. After washing twice in PBS (5 min), 50 µl of biotinylated goat antimouse IgG (Sigma) at 1:100 dilution in PBS was incubated with the smears for 1 h. The smears were washed and then reacted for 1 h with 50 µl of (Vector) ABC reagent. After washing, the peroxidase label was developed by reacting smears for 10 min with DAB substrate in urea and H_2O_2 buffer (Sigma). The smears were then washed in distilled H_2O twice (5 min), dehydrated through a series of graded alcohols, cleared with Hemo-de (Fisher) and coverslipped using Permount. Slides were examined by light microscope and photographed using Fuji film 1600x.

Cross-reactivity of mAbs with human lymphocytes.

Human lymphocytes were isolated from peripheral blood, following the isolation method in "Human Peripheral Blood Lymphocytes". Briefly, 10 ml human blood was diluted 1 :1 in a Hank's balanced salt solution (Sigma), slowly placed onto 10 ml Histopaque-1077 density gradient (Sigma) and centrifuged at 400 g for 30 min at RT. The interface containing mostly of lymphocytes was harvested and washed with Hank's

balanced salt solution. The cross-reactivity was studied as human lymphocytes smears on microscope slides using ABC method, following the protocol of Vector Laboratories. The procedures were basically same as immunolocalizaton studies except the blocking endogenous peroxidase activity was blocked by putting slides in 0.3% Hydrogen Peroxide + methanol solution for 30 min before incubation with A+B solution (Vector ABC kit).

Results:

Detection of antisperm antibodies

BW is a donor with normal sperm concentration, motility, and morphology. However, high level of antisperm autoantibodies were detected on his sperm surface, in seminal plasma and serum.

The presence of antisperm antibodies on BW sperm surface was determined by direct IBT. The percentage of BW motile sperm bound by 3 different classes (IgA, IgG and IgM) of immunobeads and the predominant location of bound beads on sperm is presented in Fig. 1. 64% and 87% of BW motile sperm were bound by IgA and IgG immunobeads respectively. The localizations of IgA and IgG beads on BW sperm surface were similar with predominant binding sites at tail tips, principle tail and midpiece, other binding sites (head or entire sperm) were also seen but with a small proportion. The BW surface bound IgM is 7%.

With the indirect IBT detection, the level of seminal plasma derived IgA antisperm antibodies was higher than the level of IgG (Fig. 2) 60% of motile sperm were bound by IgA immunobeads after incubation with BW seminal plasma and relatively low level (39%) of IgG antisperm antibodies found in seminal plasma. No IgM antisperm

antibodies were detected. The localization of IgA and IgG beads were mainly at tail and midpiece.

Very high level of IgG antisperm antibodies was detected in BW serum by indirect immunobead assay after incubation with BW serum. 93% of motile sperm were bound by IgG immunobeads, 15% by IgA bead, and 4% by IgM beads. The results were shown in Fig. 3. The high titer of IgG antisperm antibodies in BW serum was further characterized with diluted BW serum. As seen in Fig. 4, at 1:700 dilution, 10.7% of sperm still had IgG beads bound. The distribution of IgG immunobeads was predominantly at sperm tail and midpiece, similar to the direct IBT binding patterns, but with a little elevated binding of IgG beads at the head region. IgA and IgM beads mainly localized at tail.

Purification of Human sperm surface antigens

The IgG fraction of BW serum was used to purify human sperm surface antigens by immunoaffinity chromatography. BW is a donor who shows high titer of antisperm IgG in serum by the immunobead binding test (IBT). Almost 100% of sperm have immunobead bound, and at 1:600 serum dilution 20% of sperm still bind to immunobead. To purify human sperm autoantigens, BW serum IgG was precipitated with 50% ammonium sulfate and coupled to activated Sepharose 4B. The human sperm detergent

crude extract was loaded on the affinity column. The elution profile of human sperm antigens on immunoaffinity chromatography with BW serum IgG bound to Sepharose 4B is shown in Fig. 5. The peak of elution fractions 4-20 were pooled. The pooled fractions were centrifuged by Centriprep-10 to concentrate the purified antigens in PBS, then the concentration was measured by Bradford assay. The total protein content of antigens purified from the immunoaffinity column was 335.8 μg (29.2 μg /100μl X 1150μl) . This material was utilized for the immunization of the mice.

Generation and selection of monoclonal antibodies (mAbs) against purified antigens

Monoclonal antibodies against affinity purified human sperm antigens were generated by a semi-solid methylcellulose HAT selection medium (Davis, 1982). From two mice immunized with affinity purified human sperm antigens, one thousand distinct visible colonies were picked and transferred individually to IMDM medium for further culturing. Hybridomas producing antibodies that reacted with sperm were detected by antibody binding to human sperm crude extract coated on the microtiter plates (ELISA). A total of 48 hybridomas secreted anti-human sperm antibodies. After further subculturing, 32 stable hybridomas were saved and studied for the immunolocalization study.

Immunolocalization using immunoperoxidase technique

The interaction of 32 mAbs with antigenic sites on human sperm surface membranes was localized using the avidin-biotin-immunoperoxidase complex (ABC) method. Myeloma SP 2/0 supernatant and layer deletions with PBS instead were used as negative control. With this indirect immunolocalization technique, distinctive staining patterns and intensity on sperm surface were observed for 10 mAbs. The typical regional binding on sperm with these 10 mAbs was shown in Fig. 6 & 7. mAb 143 stained whole sperm head surface with stronger signal at the equatorial and postacrosomal region, midpiece and tail. mAbs 59, 102, 45, 21, and 6 stained sperm in the patterns similar to mAb143 with different intensity. mAbs 25, 36, 40, and 41 only showed staining at sperm tails.

Cross-reactivity of mAbs with human lymphocytes

The cross-reactivity of these mAbs with human lymphocytes was also examined. Among them, six mAbs , to some extent, cross-reacted with human lymphocytes. The level of cross-reactivity with lymphocytes generally correlated with the level of reactivity with sperm. However, the remaining of four mAbs that only stained sperm tail did not show the cross-reactivity with lymphocytes.

Discussion:

The mammalian sperm is a highly differentiated cell with a very specialized function. Some molecules on sperm surface are immunogenic and can produce antibodies. Antisperm antibodies have been implicated as a contributing factor to infertility. The IBT is one common approach for detection of antisperm antibodies. This test determines the isotype of Ig bound, the percentage of sperm bound by antibodies, and the region of the sperm to which specific antibodies are bound (Hellstrom et al., 1988). With IBT we found a donor BW who had a high percentage of antisperm antibodies bound to the sperm surface and high antisperm antibody titers in serum and seminal plasma. Antibodies present on his sperm surface were both IgA and IgG isotype. Antibodies in his serum belonged predominantly to IgG isotype. Antibodies found in his seminal plasma were predominantly of IgA isotype. The regions of the sperm to which antisperm antibodies IgA and IgG were bound were mainly at tail and midpiece. Other parameters of BW sperm including cell concentration, motility and morphology were normal. This observation was consistent with the finding that no correlation between antisperm antibody positivity and sperm concentration, motility and morphology has been observed (Cerasaro et al., 1985). Various studies have suggested that antisperm antibodies of IgG or IgA classes can interfere with sperm binding and penetration of the human zona pellucida (Liu et al., 1992; Bronson et al., 1982) and the penetration of human sperm into zona-free hamster oocytes (Alexander et al., 1984; Haas et al., 1985), and the concentration of antisperm antibodies in reproductive tract secretions influence

the degree of fertility impairment (Bronson et al., 1984). It has been reported that the different immunoglobulin isotypes (IgA, IgG, IgM) might have different effects on immunological infertility. IgA, with tail localization, reduces mucus penetration (Jager et al., 1980; Clarke et al., 1988). IgG and IgM antibodies interfere with capacitation and the acrosome reaction (Bronson et al., 1984). When sperm are covered with specific IgG and IgA antibodies, in-vitro fertilization is significantly reduced (De Almeida et al., 1989). From the findings of other investigators, it is likely that BW donor is infertile due to the presence of a high titer of antisperm antibodies. If the antisperm antibodies from an infertile BW are used for the purification of sperm antigens, the targeted sperm autoantigens are likely involved in the reproductive processes. Based on this idea, sperm antigens were purified from sperm surface extracts by affinity chromatography against the IgG fraction of serum from donor BW. IgG fractions of the autoimmune serum were purified by 50% ammonium sulfate precipitation, immobilized on Sepharose as affinity ligands, and utilized to purify related sperm autoantigens from sperm membrane extracts. The purified antigens correspond to the sperm antigens that elicit antisperm antibodies associated with infertility.

One of our important goals is to identify sperm specific antigens that are involved in the fertilization processes. Natural human immune responses to sperm have been reported. The resulting polyclonal antibodies to human sperm can also provide resources for such an investigation (Naz RK & Menge A, 1994), but polyclonal antibodies against sperm have a limited value for analysis of the complex sequence of molecular events and

the diverse antigens thought to act in fertilization. Polyclonal antibodies are expect to interact with different antigens and produce complex effects on fertilization by a combination of mechanisms (Naz RK, 1988). With the advent of hybridoma technology, human sperm surface antigens playing an important role in the fertilization process may be specifically identified by the mAbs that are produced against the sperm immunogens purified from antisperm antibodies IgG class of BW serum. In order to make mAbs, two CSJF1/F female mice were immunized with the purified human sperm antigens successively, then the spleen cells of the immunized mice were fused with SP 2/0 myeloma cells to generate hybridomas. The traditional way to clone hybridomas is limiting dilution which may result in a loss of potentially valuable hybridomas, because slow-growing antibody-secreting clones may be taken over by fast-growing nonantibody-producing cells in culture (Davis, 1982). Therefore, one step semi-solid methylcellulose cloning method (Davis, 1982) was adopted to make hybridoma clones. The advantage of this technique is that it is easy to plate out large numbers of cells and to recover many independent hybridoma clones (Davis et al., 1982). After culturing and subculturing, the mAbs were screened by ELISA. thirty two stable hybrid cell lines which produce mAbs against human sperm antigens were established. These mAbs were the constitution of polyclonal BW serum IgG fraction that may be the natural Igs involved in immune infertility. This implicated that these mAbs may be the antisperm antibodies of immunoinfertile patients in vivo and may interfere with fertilization through the mechanisms of capacitation, acrosome reaction, or/and sperm-egg interaction. These mAbs could be a powerful tool for identification of specific sperm antigens that have a

role in the reproductive processes.

The sperm cell surface is highly differentiated and is known to undergo dynamic reorganization during the life of the sperm cell. Thus it should be expected that sperm surface antigens will bind antisperm antibodies at different regions on the sperm surface. Stereotypical, but not absolute, patterns of mAb binding should be expected. In this study, indirect immunolocalization by the ABC assay was used to examine the localization of sperm antigens targeted by individual mAbs. Among 32 isolated hybridomas, 10 mAbs positively localized on sperm surfaces with strong and distinctive staining patterns. The interactions of mAbs 143, 102, 59, 6, 21, and 45 with human sperm surface antigens are predominately localized at the equatorial, posterior acrosomal, midpiece and tail regions. mAbs 25, 36, 40, and 41 are immunolocalized only at the midpiece and tails. These data indicate spatial differentiation of the target antigens on localized regions of the human sperm cell. Several laboratories have reported antisperm antibody effects (Snell et al., 1996). For example, Naz et al. (1984) characterized a mAb against antigen(s) localized at postacrosomal part, midpiece and tail. Binding of this mAb to normal sperm led to reduced rates of penetration in the zona-free hamster ovum penetration system (Naz et al., 1984). The equatorial and posterior acrosomal regions have both been suggested as possible sites of initial sperm egg contact and fusion (Yanagimachi, 1976). Therefore, it is reasonable to expect that one or more of our 10 mAbs with different regional binding specificities may have selective effects on sperm functions. Possible effects need to be further examined by the sperm function assays.

The key components of an antisperm-directed contraceptive vaccine should be sperm specific antigens that are not present on somatic tissues. The cross-reactivity of 10 mAbs with human lymphocytes was determined. Among them, 4 mAbs that stained only the sperm midpiece and tails didn't cross-react with human lymphocytes. This suggested that they may be good candidates for the sperm function assays. However, the other 6 mAbs showed the cross-reactivity with lymphocytes. The observation of extensive cross-reactivity between mAbs against mammalian sperm and other somatic cells is consistent with those of other investigators (Anderson et al., 1987; Naz, 1988).

These mAbs should prove useful in the definition of structure and function of the sperm antigens and assist in understanding the role of autoantibodies to human sperm and the complex mechanism of fertilization at the molecular level.

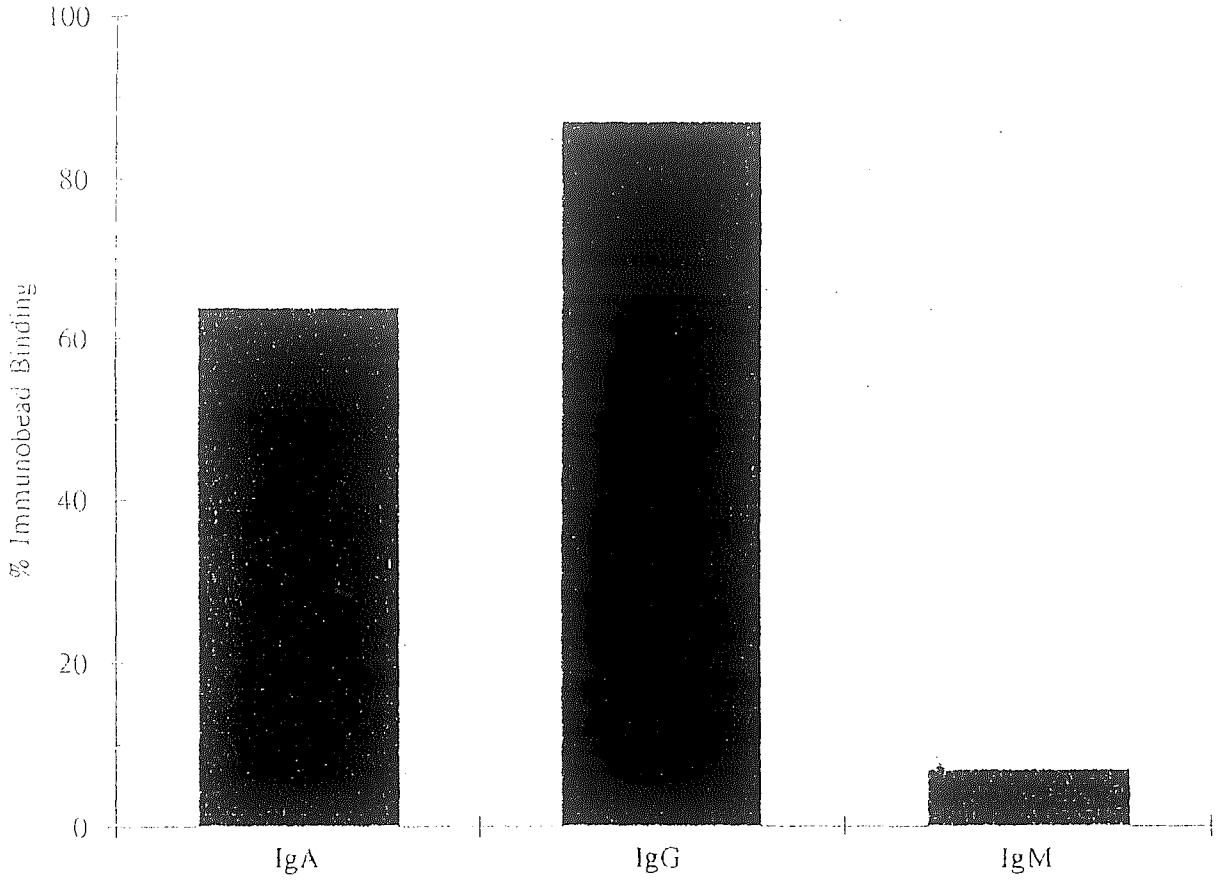

Fig.1 Direct IBT of BW Sperm

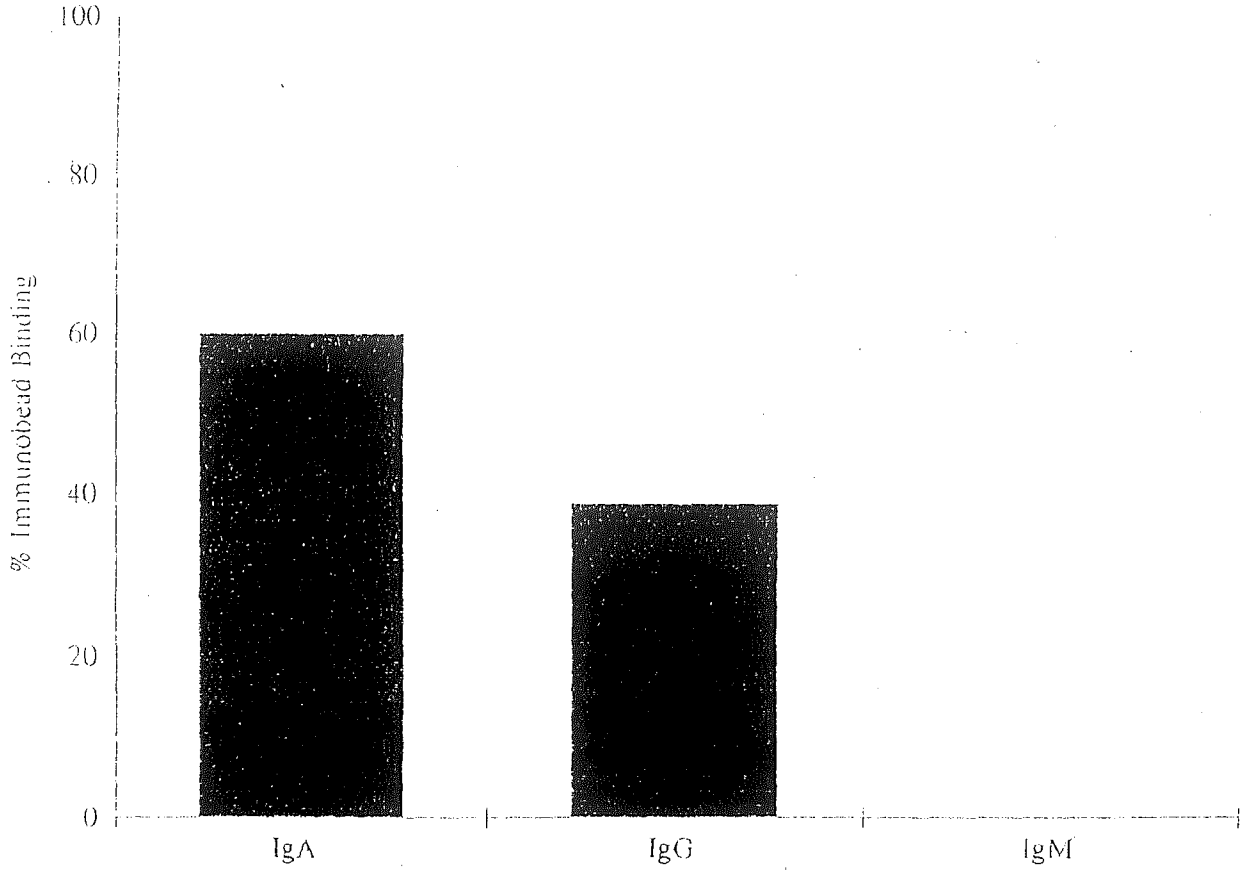

Fig2 Indirect IBT of BW Seminal Plasma

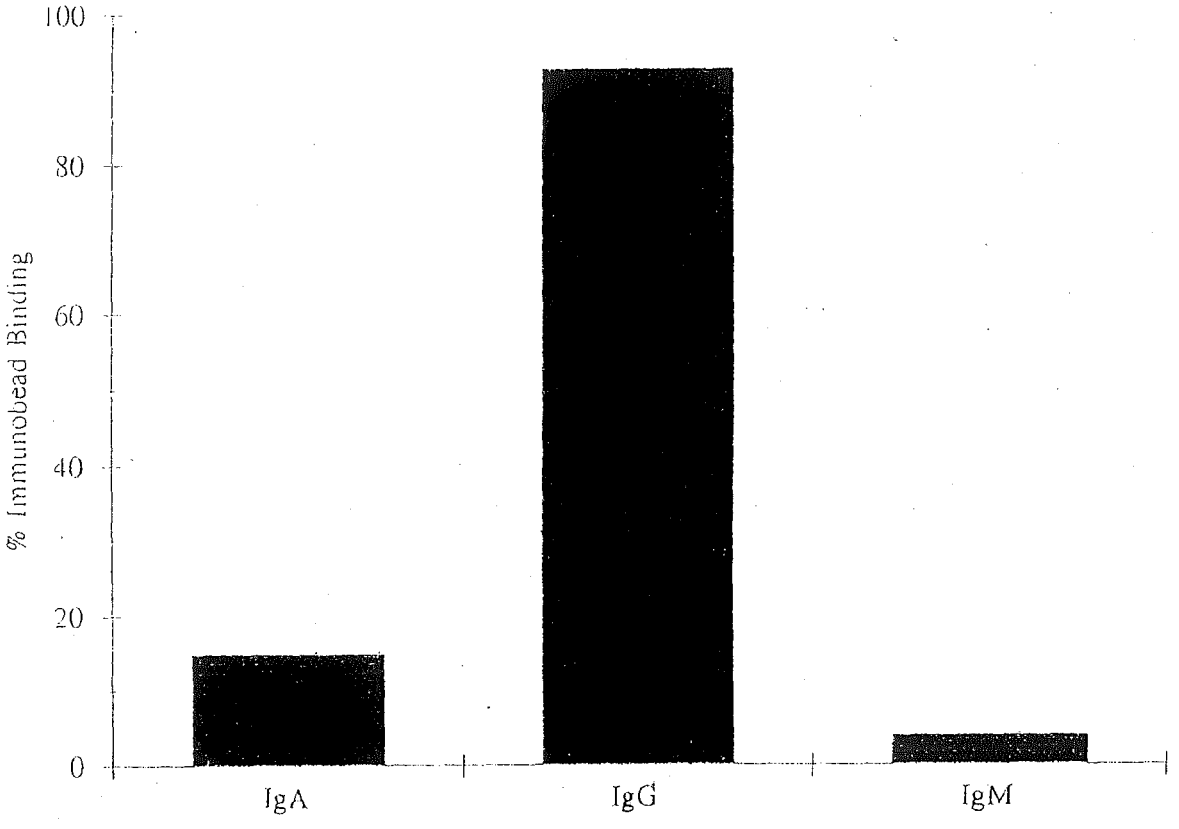

Fig.3 Indirect IBT of BW Serum

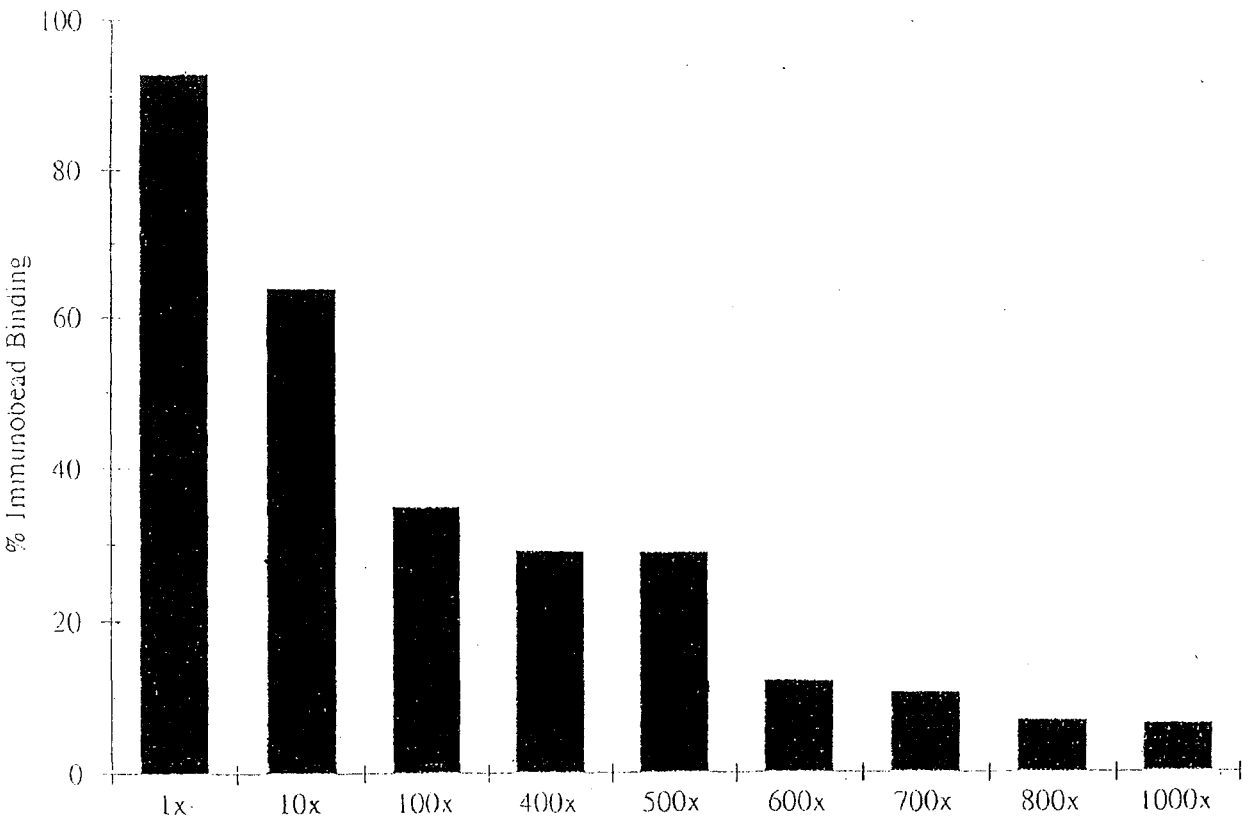

Fig.4 Indirect IBT of BW diluted serum IgG

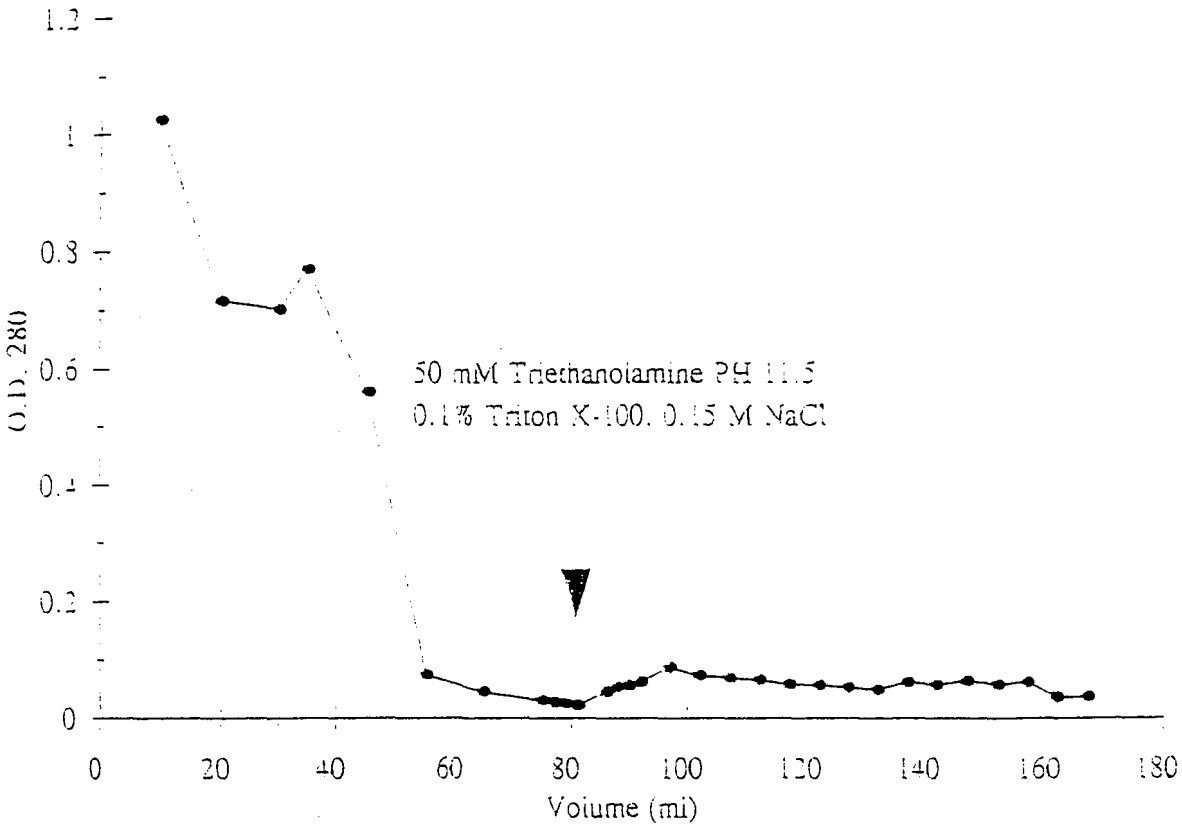

Fig. 5 Purification of human sperm antigens by using BW serum IgG coupled Sepharose 4B column. The column was sequentially washed with Wash buffer, Tris pH 8.0, and Tris pH 9.0 buffers. The specific antigens were eluted (arrow) with Triethanolamine solution pH 11.5. Total 71 ml of eluted peak was pooled. Flow rate = 0.25 ml/min.

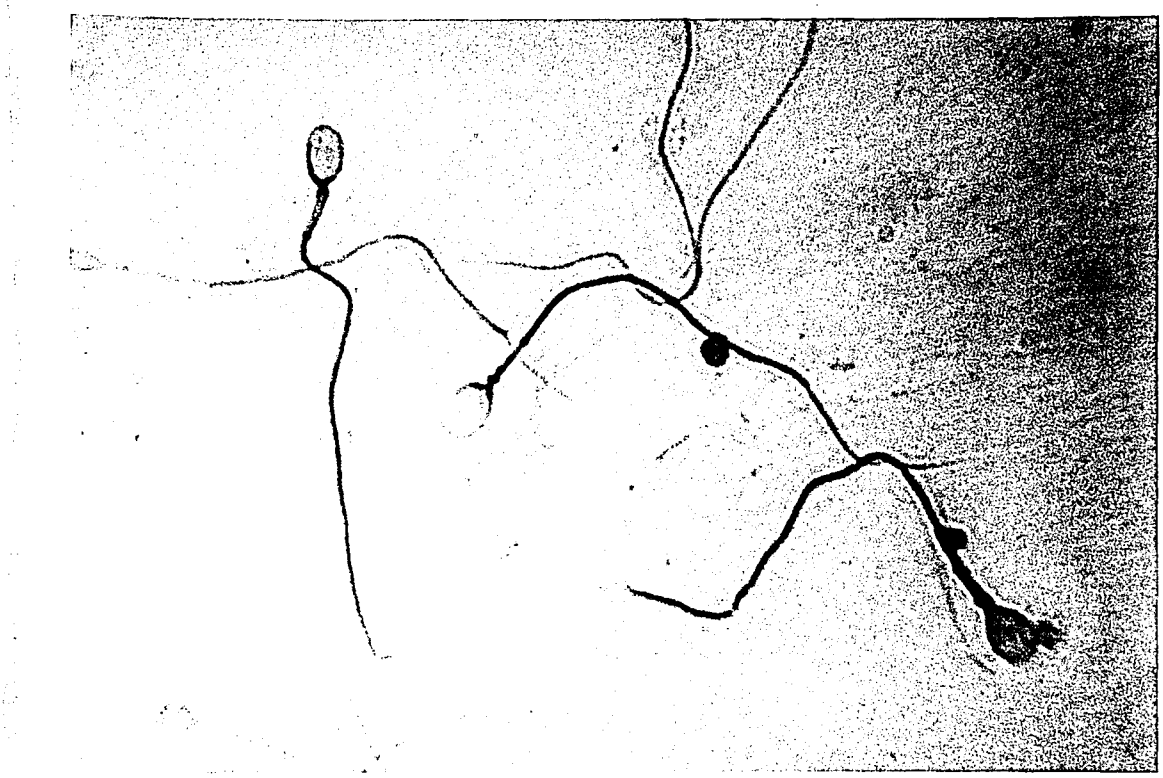

Fig. 6 Immunoperoxidase localization of human sperm with mAb 143.

mAb 143 stained sperm head surface mainly at equatorial and postacrosomal region, midpiece and tails. Light microscope.

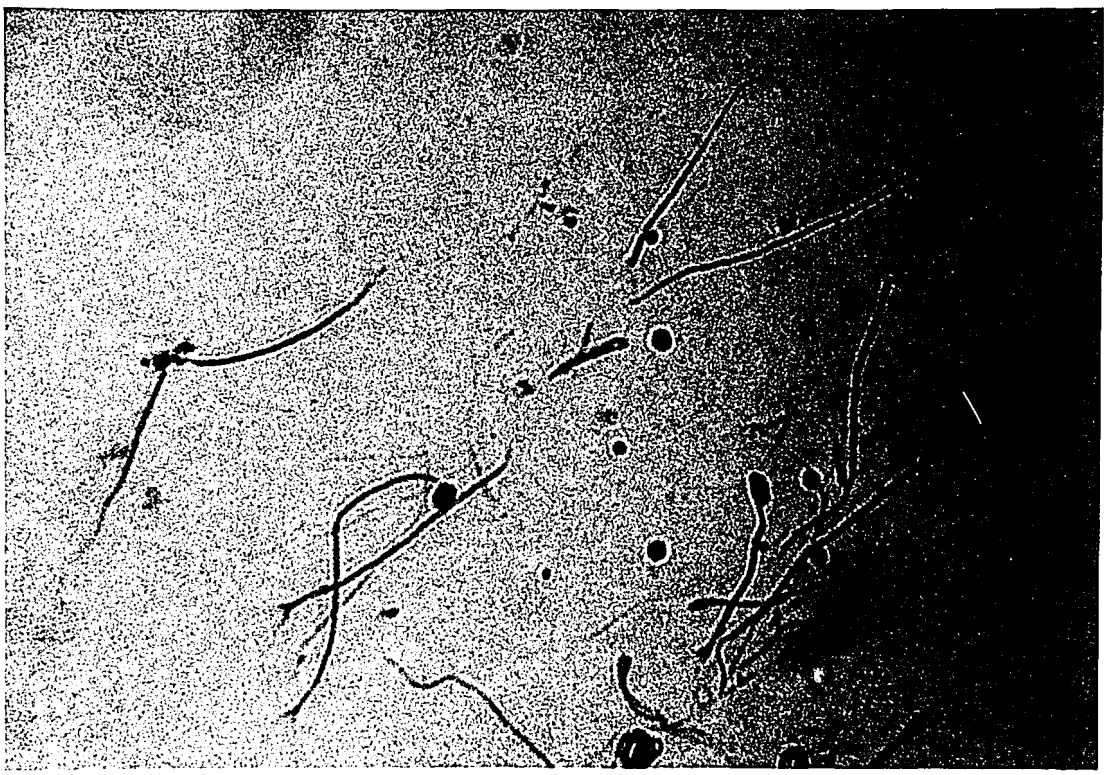

Fig. 7 Immunoperoxidase localization of human sperm with mAb 25. mAb 25 only stained sperm tails. Light microscope.

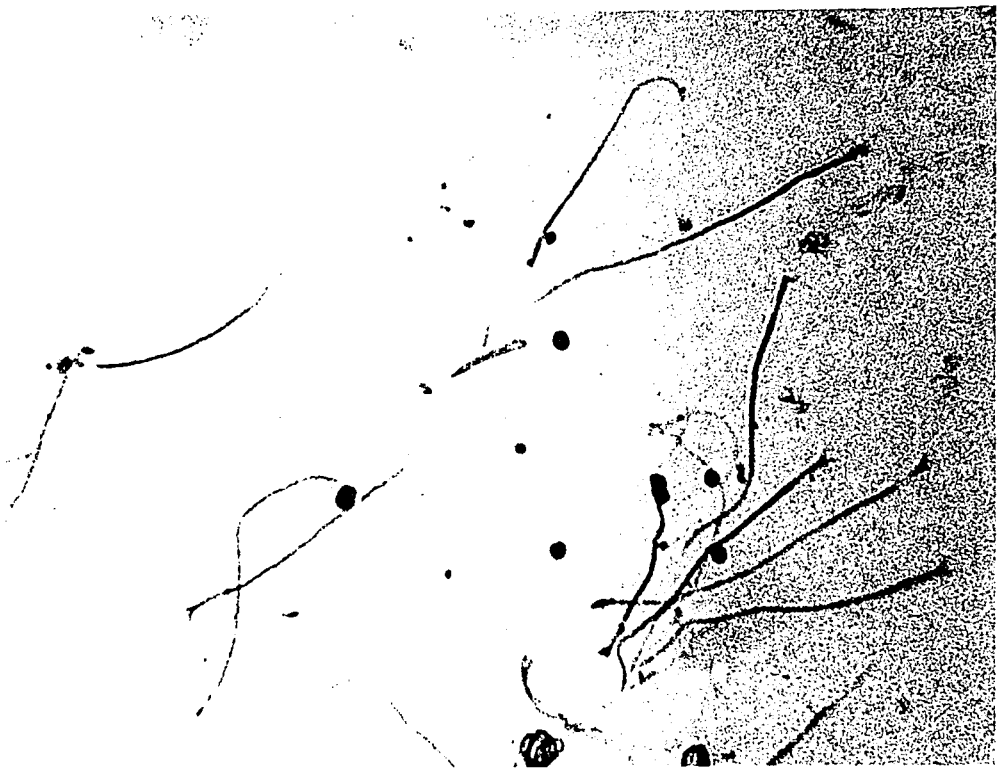

Fig. 7 Immunoperoxidase localization of human sperm with mAb 25.

mAb 25 only stained sperm tails. Light microscope.

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Vita

The author was born on March 14, 1963 in Beijing, People' s Republic of China. She is the child of Mr. Desan Tang and Ms. Meilan Liu.

The author received her high school education at the 7th High School of Beijing and enrolled at the Capital Institute of Medicine, P. R. C. in September, 1982. After receiving the degree of Bachelor of Medicine in Pediatrics in July, 1987, she worked in Beijing Children' s Hospital as a Pediatrician from 1987 to 1989. In September 1991, she started her master studies at Lehigh University.

In January 1988, she married Ming Xue, the child of Mr. Niangang Xue and Ms. Peiying Du. and has a daughter, Haley Robyn Xue.

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