

Human Sperm Coating Antigen From Seminal Plasma Origin

A. IBORRA, C. MORTE, P. FUENTES, V. GARCÍA-FRAMIS, P. ANDOLZ, AND P. MARTINEZ

Iborra A, Morte C, Fuentes P, García-Framis V, Andolz P, Martínez P. Human sperm coating antigen from seminal plasma origin. AJRI 1996; 36:118-125 © Munksgaard, Copenhagen

Sperm surface glycoproteins may be involved in sperm-zona pellucida recognition. Some of these coating proteins are of seminal plasma origin and their expression may change in the process of capacitation and acrosome reaction. Sperm specific monoclonal antibodies (mAb) define the presence and role of sperm membrane associated proteins. We have isolated a monoclonal antibody (SEM-12) specific for human sperm that shows, by indirect immunofluorescence, a discontinuous distribution of the antigen on the head and tail surfaces of non capacitated sperm. This antigen is also present in human seminal plasma as detected by ELISA. The antigen is detectable in sperm of goat, ram, and mouse. Two proteins in the range of 80-84 kDa have been isolated by affinity chromatography with SEM-12 mAb. The same result is obtained by immunoprecipitation. This antibody inhibits sperm motility and acrosome reaction (spontaneous and A_{23187} ionophore induced).

INTRODUCTION

Freshly ejaculated sperm is not able to undergo fertilization¹ and does not respond to acrosome reaction by inducers, or after incubation *in vitro* in an appropriate medium. In most mammalian species, sperm acquire this ability several hours after mating or after incubation in an appropriate medium, in the absence of seminal plasma components. The necessary events, collectively termed as capacitation, prepare the sperm to respond to an inducer of the acrosome reaction or, in the absence of an inducer, to undergo an acrosome reaction spontaneously. Because the plasma membrane is directly exposed to the capacitation environment, it is not surprising that very significant changes take place in this membrane during capacitation. Some authors² postulate that the removal or alteration of coating material from the sperm surface constitutes an important part of capacitation. Evidence supporting this view has been accumulated.³ The coating molecules are known to be removed or altered during capacitation; they include the so-called decapacitation factors all of seminal plasma origin, and the acrosome-stabilizing 125-129 kDa of epididymal origin.

Capacitated sperm bind firmly to the surface of the zona pellucida before penetrating it. Sperm-zona binding is mediated by the interaction between the zona and sperm surface molecules. Several functions for sperm plasma membrane have been proposed. Mouse sperm Gal-transferase has been reported as an integral membrane component and is restricted to a plasma membrane domain overlying the intact acrosome.⁴ The ligand within the zona pellucida to which Gal-transferase binds is

Key words:
Glycoproteins, zona pellucida,
seminal plasma origin

A. IBORRA
C. MORTE
P. FUENTES
V. GARCÍA-FRAMIS
P. MARTINEZ

Unitat d'Immunologia, Institut de Biologia Fonamental, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

P. ANDOLZ
Laboratorio de Investigaciones Seminales, Barcelona, Spain

Presented at the Sixth International Congress of Reproductive Immunology, Washington, DC, July 19-23, 1995.

Address reprint requests to A. Iborra, Unitat d'Immunologia, Institut de Biologia Fonamental, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain.

Submitted July 31, 1995; accepted August 10, 1995.

likely to be ZP3, a glycoprotein that possesses sperm-binding activity.⁴ Gal-transferase could contribute to sperm-egg binding in a nonspecies-specific manner, serving as an accessory gamete adhesion molecule. Sperm surface Gal-transferase acts in a lectin-like capacity during sperm-egg binding by forming stable complexes with its N-acetylglucosamine-binding site in the zona pellucida. After the acrosome reaction, Gal-transferase is redistributed to the lateral sperm head where it loses affinity for other ZP glycoproteins.

Some investigations have focused on the fusion between the sperm and egg plasma membranes.⁵ A protein on the guinea pig sperm surface called fertilin (PH-30 antigen) has high homology with the family of integrin ligands, the disintegrins, in its β -subunit; the α -subunit contains putative fusion peptide analogous to the fusion peptides of viruses. The results indicate that the disintegrin domains binds to the egg plasma membrane and that this binding is required for membrane fusion.

By using somatic cell hybridization technique to produce monoclonal antibodies (mAb) of predefined specificity, a number of hybrid cell clones have been developed against sperm from a variety of species, including man.⁷ These mAb have helped in the identification, isolation, and characterization of sperm specific antigens having a role in reproductive processes.⁸ Some sperm antigens show high specificity when tested with other tissues. SP-10, an intra-acrosomal protein sperm specific conserved among mammalian species including primate and man, remains associated with sperm head after the acrosome reaction.^{9,10} We have a panel of anti-human sperm mAbs,^{11,12} that are reactive to fresh human sperm and to sperm from other species.¹³ VIC-1 mAb has shown a high specificity for sperm; it does not react with other human tissues tested. This antibody reduces the acrosome reaction and the release of acrosin. It recognizes an antigen of 95 kDa (FA-2) that is located in the acrosome of human sperm.

A protein family named spermadhesins includes some zona binding molecules present in sperm and seminal plasma (AWN and AQN). These heparin binding proteins are located on the anterior part of sperm head and they interact with zona glycoproteins by a carbohydrate recognition mechanism.¹⁴ One of our anti-human sperm mAbs (SEM-12) recognize an antigen on sperm membrane and strongly react with seminal plasma. The purpose of this study was to investigate the distribution of the antigen recognized by SEM-12 mAb during capacitation. We characterized antigen binding to SEM-12 mAb and the effect of SEM-12 mAb on the acrosome reaction.

MATERIAL AND METHODS

Immunization and Hybridoma Production

Human sperm was obtained by masturbation from healthy donors after 3–4 days of sexual abstinence. To generate

anti-human sperm monoclonal antibodies (mAb), BALB/c female mice were immunized by intraperitoneal injection of 10^7 sperm/mouse with Freund's complete adjuvant on day 0, and Freund's incomplete adjuvant on days 15 and 30. Animals showing the highest antibody titers by ELISA were selected for the fusion and immunized without adjuvant 3 days after fusion.

On the day of fusion, mice were sacrificed and immune spleen cells were fused with mouse myeloma cells NS-1 (at ratio 10:1) in HAT selective medium by polyethylenglycol procedure.^{15–17} About 15 days after fusion, when the growing hybridomas showed antisperm antibody activity, detected by ELISA cloning of the positive wells by limiting dilution was performed. The positive clones were recloned.

BALB/c mice were injected intraperitoneally with the hybridoma cells, 10 days after pristane treatment, to obtain ascitic fluid with good antibody concentration. The immunoglobulins obtained from the ascites were purified by affinity chromatography using protein-A-Sepharose-CL-4B (Pharmacia, Uppsala, Sweden). The antibody peak was eluted with 0.1 M citric acid, pH 4, dialyzed, freeze-d, lyophilized, and stored before use.

ELISA for the Selection of Monoclonal Antibodies

The procedure for ELISA employed in our laboratory was described previously.¹⁸ Briefly, 100 μ l of washed intact human spermatozoa (10^8 cells/ml) were added to flat-bottom polystyrene plates. The plates were centrifuged for 10 min at 1,000g and then fixed with 0.25% glutaraldehyde. At the same time, 100 μ l of human seminal plasma (dilutions 1/100–1/100,000) in carbonate buffer, pH 9.6 were added to plates. Specific binding was avoided by incubating with 1% BSA (bovine serum albumin) in PBS 0.05% Tween-20 (PBS-T) for 1 h at room temperature.

One hundred microliters of SEM-12 mAb (0.1 mg/ml) were incubated for 2 h. The plates were washed three times with PBS-T, and goat anti-IgG from mouse conjugated to horseradish peroxidase was added and kept for 2 h at 37°C. The wells were washed again and the MBTH-DMAB (0.8 mM 3-methyl-2-benzothiazolinone hydrazone hydrochloride; 40 mM 3-dimethylaminobenzoic acid) substrate, 3 mM in H_2O_2 , was incubated for 20 min. The reaction was stopped with 2 M sulphuric acid, and the optical density was read at 620 nm by an "Anthos 2001" plate reader.

Sperm Capacitation and Acrosome Staining

Sperm was selected by swim-up in Ham F-10 medium containing 5 mg/ml of bovine serum albumin. A 200 μ l aliquot from the sperm suspension (10^7 cells/ml) was incubated with 200 μ l of mAb at a concentration of 1 mg/ml. An aliquot of 200 μ l of sperm was assayed in 200 μ l of Ham F-10 medium as a control of spontaneous acrosome reaction. Then, aliquots were allowed to capacitate for a period of 6 h in 5% CO_2 at 37°C. At this moment the acrosome reaction was induced by addition of 10

$\mu\text{M A}_{23187}$ ionophore for 15 min. The acrosomal status was analyzed by the triple staining technique,¹² and the spermatozoa were examined with bright-field microscopy at $\times 400$. At least 400 spermatozoa were classified according to the pattern defined. It is considered reacted acrosome live sperm the pattern at light brown postacrosomal regions with unstained acrosomes.

Progressive motility was evaluated every hour during capacitation with/without mAb and was observed under phase-contrast microscopy ($\times 400$). We evaluated progressive motility in terms of motile/immotile sperm. Vitality was examined by orange acridine stain and it was observed under Zeiss epifluorescence microscope.

Indirect Immunofluorescence

For the indirect immunofluorescence technique,¹³ we used 200 μl of sperm suspension. We added 200 μl of the mAb and sperm was then incubated for 60 min at 37°C; it was centrifuged at 600g for 10 min. After two washings, the supernatant was removed and cells were incubated with 200 μl of FITC-conjugated goat anti-mouse immunoglobulin diluted 1/50 for 1 h at 37°C. Sperm was washed twice and an aliquot of sperm suspension was placed on slide and observed under Zeiss epifluorescence microscope.

Membrane Extracts

Membrane antigens were extracted from pools of washed semen pellets by the following procedures: detergent extraction (0.5% SDS) and acid extraction. For SDS extracts we added 250 μl of SDS 0.5% to pellet and boiled at 100°C for 10 min. After centrifugation at 10,000g for 15 min at room temperature, the clear supernatant was dialyzed against water, and lyophilized until use. For acid extraction,¹⁴ sperm was extracted overnight at 4°C with 2.5% (v/v) acetic acid, 10% glycerol, and 5 mM benzamidine, with gentle stirring. After centrifugation at 10,000g for 15 min at room temperature, the clear supernatant was dialyzed against water, and lyophilized until use.

Immunoprecipitation Procedure

To further confirm the antigenic specificity of the mAb, we performed the immunoprecipitation procedure as described in detail elsewhere.²⁰ Briefly, 10–20 μg of the mAb was added to 20 μl of protein A in an eppendorf and mixed with human seminal plasma or LIS-solubilized human sperm preparation. The reaction was incubated at 4°C overnight in an eppendorf shaker. The immune complexes were obtained by centrifugation (2,000 rpm for 10 min). The supernatants were discarded, and the pellets were washed three times with PBS. The washed beads were mixed with 50–80 μl SDS-sample buffer,²¹ heated in a boiling water bath for 10 min and centrifuged again; the supernatant was subjected to SDS-PAGE, and silver staining.

Immunoaffinity Purification of the SEM-12

Binding Antigen

The IgG purified from ascites fluid by use of a protein A-Sepharose 4B column (Pharmacia, Uppsala, Sweden), was coupled to CNBr-activated Sepharose 4B.²² The coupled gel was placed in a column, and chromatography was performed at room temperature by means of low-pH elution procedure. The immunoadsorbent column was loaded with 100 μl human seminal plasma or LIS-solubilized human sperm preparation (containing 300–500 μg of protein) and incubated for 3 h at room temperature. The binding proteins were dissociated and eluted at pH 2.8 (0.1 M glycine-HCl buffer) at room temperature. The eluted fractions were neutralized with 1 M KH_2PO_4 , dialyzed against in PBS, analyzed by SDS-PAGE, and stained by a silver stain technique.

RESULTS

We developed some anti-human sperm monoclonal antibodies (mAb) by hybridoma technology. For a large-scale production of the mAbs, ascites fluid in mice was produced; 2 mg/ml of the mAb ascites fluid was obtained. SEM-12 mAb was selected for detailed analysis. This antibody recognizes both sperm membrane proteins and soluble seminal plasma protein. The absorbance obtained by incubation of 10 μg of SEM-12 with spermatozoa (100,000 cells) is 0.35; the same concentration of antibody gives an absorbance of 0.86 when it is incubated with seminal plasma (1/100 diluted) (Fig. 1). SEM-12 mAb

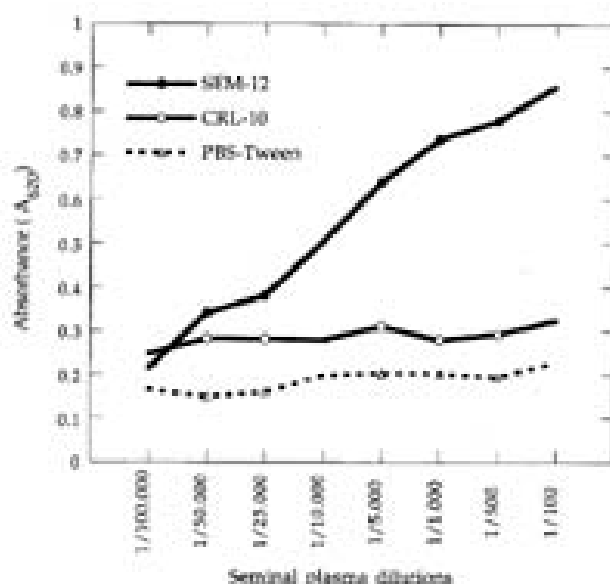


Fig. 1. Absorbances obtained by ELISA against several dilutions of human seminal plasma of two anti-human sperm mAbs (SEM-12, CRL-10) and control of PBS-0.05% Tween.

showed by ELISA positive reaction against sperm of several species (goat, ram, and mouse) (results not shown).

By indirect immunofluorescence technique (Fig. 2), SEM-12 binds predominantly to whole sperm cell surface. The antibody reacted with both noncapacitated and capacitated sperm; at similar percentage (55%) the antibody binds to head and acrosome regions before and after capacitation (30%). After induced acrosome reaction by ionophore, this percentage decreases (5%). Simultaneously a new binding pattern in the equatorial region appears (20%). The patterns above mentioned are shown in Figure 3. The control (myeloma supernatant) IgG₁ does not react with any of the sperm preparations.

The progressive motility was evaluated during *in vitro* capacitation, in presence/absence of SEM-12 antibody (Fig. 4a). SEM-12 monoclonal antibody inhibited sperm motility early in capacitation, and drastically after 5 h of incubation in HAM F-10 medium. In the presence of several mAbs, from our collection of anti-human sperm antibodies, progressive motility was preserved for VAC-4 mAb (midpiece and tail binding) but not for SEM-12 (Table I). SEM-12 mAb inhibited spontaneous acrosome reaction of the spermatozoa (50% of decrease). The effect of incubation with the SEM-12 antibody was apparent after ionophore treatment (100% of decrease) as it can be seen in Figure 4b.

SEM-12 mAb precipitated two protein bands of 80–84 kDa from human seminal plasma by immunoprecipitation procedure (Fig. 5). The same proteins were isolated from human seminal plasma preparations by SEM-12 antibody immunoaffinity column. The fraction eluted at 2.8 pH from the column contained two bands analyzed by electrophoresis and silver stained of 80–85 kDa. These bands were not

observed in the fraction eluted from the immunoaffinity column containing control myeloma IgG₁. The results for immunoprecipitation and affinity chromatography were not detectable when we used DOC and LIS-solubilized human sperm extracts.

DISCUSSION

SEM-12 mAb showed strong binding to seminal plasma. The ELISA described is a sensitive method and detects low concentrations of the antigen when it is diluted in seminal plasma (100 μ l of dilution 1/25,000 or 80 ng protein approximately). This mAb reacts with whole intact sperm as detected by indirect immunofluorescence. The mAb binding antigen is expressed in different regions of the cell according to his state of maturation and capacitation.

The antigen localization in ionophore acrosome induced human sperm moves from head/acrosome to equatorial region. No cells with equatorial region staining are found in non capacitated samples; the percentage of increase in this equatorial pattern (see Fig. 3b) is of 23.8% (Fig. 2) and this correlates with the percentage of live acrosome reacted sperm (20.6%, Fig. 4b).

Redistribution of sperm membrane proteins in live and motile spermatozoa after *in vivo* labelling of capacitated cells²¹ has been observed by Rochwerger et al. in a 37 kDa rat epididymal protein. The authors describe the migration of this protein from the acrosome to the equatorial region segment as a consequence of the acrosome reaction. This protein is involved in fusion of sperm plasma membrane with oocyte.

Herr et al.¹⁰ describe for SP-10, an acrosomal protein first identified in human sperm, a 22.4% of sperm with equatorial staining after induction of the acrosome reaction with calcium ionophore A₂₃₁₈₇. This percentage is similar to our results. The same laboratory confirmed in more recent publication by electron microscopy the SP-10 antigen localization. The immunogold labelling particles were detected in the equatorial segment and on the inner acrosomal membrane of acrosome-reacted sperm.²⁴

It is of interest that the ultrastructural localization of SP-10 was the same whether the ionophore A₂₃₁₈₇ or follicular fluid was used to induce the acrosome reaction. SP-10 is an antigen with a molecular weight of 45 kDa, different from SEM-12 antigen, but similar migration towards the equatorial region would suggest a possible involvement in sperm-zona binding for SEM-12 mAb.

A sperm surface protein from guinea pig, PH-20 antigen,²⁵ migrates to a new domain after the acrosome reaction. This antigen has been isolated from a monoclonal antibody to surface antigen. The mAb inhibits sperm binding to the zona pellucida of guinea pig oocytes. Localization of sperm surface molecules is important in determining the zona adhesion and sperm penetration through the zona pellucida. PH-20 antigen migrates to the

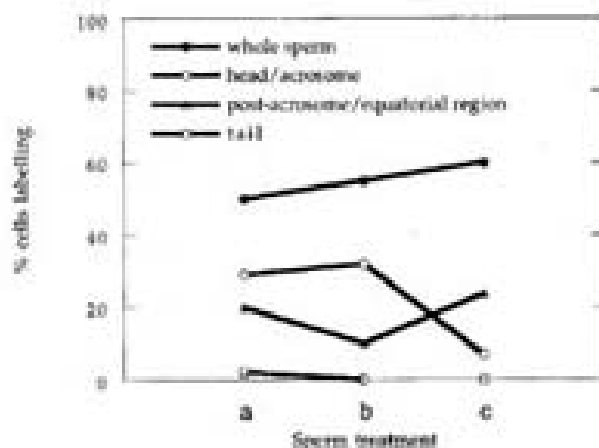


Fig. 2. Immunofluorescence labelling of SEM-12 mAb in sperm cells after capacitation and acrosome reaction: a) no capacitation, swim-up selected cells; b) capacitation after 6 h of incubation in HAM F-10 medium with 5 mg/ml of HSA; and c) 10 μ M A₂₃₁₈₇ ionophore induced acrosome reaction.

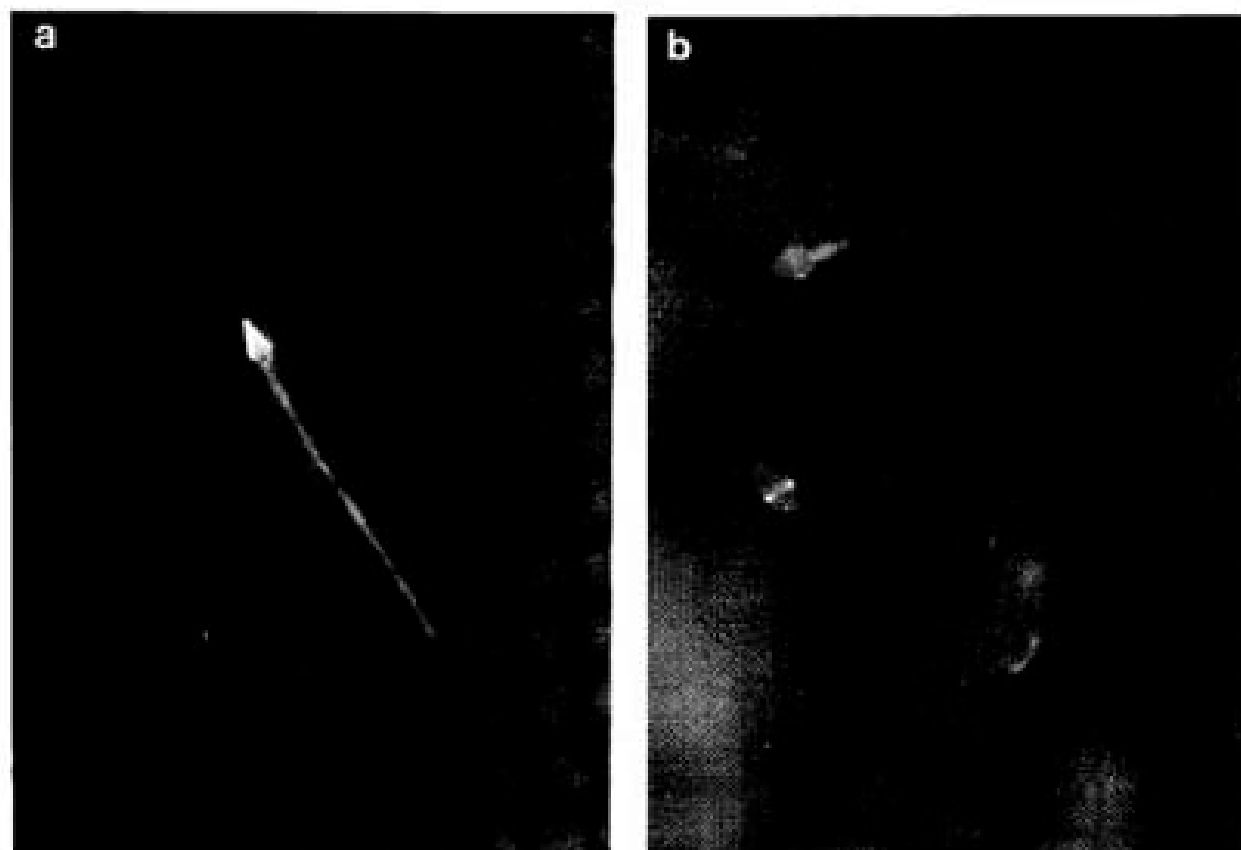


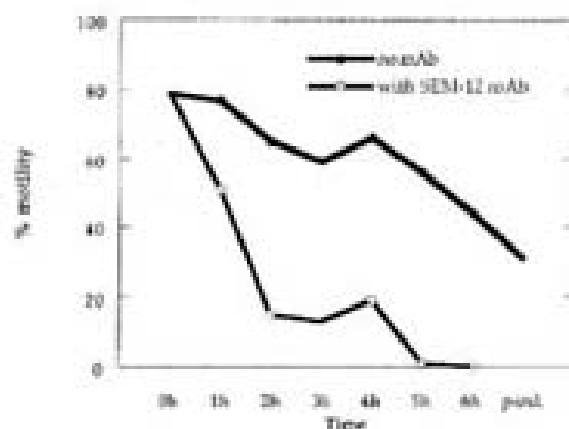
Fig. 3. Fluorescence micrographs obtained by indirect immunofluorescence technique with SEM-12 anti-sperm monoclonal antibody: a) whole sperm cell staining and b) equatorial region staining after acrosome reaction induced by ionophore.

posterior head surface to participate in binding the acrosome intact cell, and after acrosome reaction it migrates to the inner acrosomal membrane probably to participate in binding of acrosome reacted cells. Recent experiments indicate that PH-20 enables acrosome intact sperm to penetrate the cumulus barrier; the explanation for this is that PH-20 has a hyaluronidase activity.²⁶ It is present on the plasma membrane of mouse and human sperm.

The SEM-12 mAb described here has an inhibitory effect on capacitation, both on motility and on acrosome reaction. We have described previously some anti-human sperm mAbs that stain the tail and do not inhibit motility (VAC-4, STE-14); some other mAbs have a blocking effect on progressive motility, such as BIT-13 and BIO-9.¹¹ Monoclonal antibodies show different reactivities with ejaculated and capacitated sperm. The reactivity of mAb with seminal plasma suggests that the binding molecules are coating antigens. Gupta et al.⁷ describe mouse hybridomas that secrete monoclonal antibodies reactive with human sperm; some of them reacted with seminal plasma. SEM-12 antigen from seminal plasma is probably adsorbed

onto the sperm surface. Some authors describe factors in human seminal plasma that inhibit ionophore induced acrosome reaction.²⁷ Cross et al. investigate the effect of seminal plasma on the acrosome responsiveness in the acrosome reaction.²⁸ Seminal plasma inhibited the acrosome reaction after 12 h incubation but depending on how it is applied, seminal plasma can prevent or reverse the acrosome responsiveness and the acrosome reaction can also be induced. Some components such as caltrin,²⁹ from seminal plasma, that prevent the entry of external calcium into cells by binding calcium porters located over the acrosome, may become enhancer of calcium uptake. This transformation would be due to a conformational change induced by association/dissociation with specific components of seminal plasma. We have described the inhibition of acrosome reaction when the anti-human sperm SEM-12 mAb is added. This antibody covers the entire surface of the cell when is not yet capacitated (Fig. 3a). When SEM-12 mAb is preincubated with sperm the presence of antibody could prevent the relocalization from head and acrosome to the equatorial region, as well as the accessi-

(a)



(b)

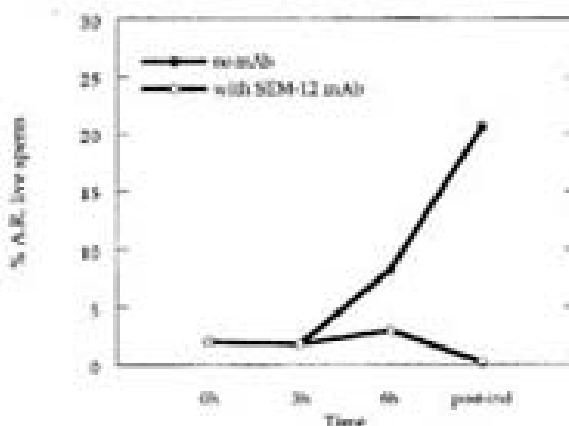


Fig. 4. Inhibitory effect of SEM-12 mAb on *in vitro* capacitation, (a) sperm motility and (b) physiological acrosome reaction percentages measured by triple stain technique (live acrosome reacted sperm).

bility for the decapitating molecules to the surface of sperm. This would be confirmed by the equatorial staining of capacitated sperm. The effect of anti-sperm monoclonal antibodies on acrosome reaction depends on the antigen to which they bind. We reported the inductive effect of CRL-10 mAb on acrosome reaction.¹² This antigen recognized by CRL-10 was not present in seminal plasma (see Fig. 1).

A group of low molecular weight glycoproteins, from seminal plasma, adhere to sperm membrane and show high affinity for zona pellucida glycoproteins.²⁰ They have a role in stabilizing the plasma membrane and acrosome and to promote survival in the female reproductive tract. This sta-

bilization would explain the inhibition effect of SEM-12 on acrosome reaction.

Some seminal plasma proteins that are coating sperm are from seminal vesicles origin^{21,22} and other molecules such as AWN spermadhesin are expressed in epididymal boar sperm.¹⁷ It has been reported the importance of epididymal sperm antigens in human infertility.¹⁹ In many mammalian species seminal plasma heparin binding proteins bind to spermatozoa at ejaculation and are regulatory capacitation factors. Calvete et al.¹⁴ hypothesize that AWN-1, synthesized in the rete testis, is the only spermadhesin found on the surface of epididymal sperm, and that it may be one of the factors contributing to the fertility activity of epididymal spermatozoa. In ejaculated sperm this glycoprotein is on the apical third of the acrosomal cap of spermatozoa, where porcine sperm initiate binding to oocyte zona pellucida. It is reported that some proteins secreted by male accessory glands that are coating the surface at ejaculation may play a role both in sperm capacitation and in gamete recognition in the pig.¹⁷ In mouse sperm membrane SEM-12 antigen is present at cauda epididymus, that is, in mature sperm. It is also present in capacitated sperm, although acrosome reacted sperm treated with ionophore shows less antigen binding (results not shown).

SEM-12 mAb was induced to fresh whole sperm and recognize two antigens (80-84 kDa); the results obtained by immunoprecipitation are confirmed by affinity chromatography. Monoclonal anti-sperm antibodies do not always recognize antigenic extracts.¹⁵ The binding of SEM-12 antibody is only detectable when the antigen is not denatured; for this reason we probably could not detect the antigens by western blot analysis with boiled seminal plasma samples and 0.3M LIS sperm membrane extracts. The epitope is probably lost after the process of extraction. Using LIS extracts no positive results were obtained by affinity chromatography or immunoprecipitation with SEM-12 mAb column (results not shown). To confirm the presence of this antigen in sperm membrane, we used two types of membrane extractions: SDS detergent and acetic acid extraction in the search of the antigens. The electrophoretic analysis of these extracts show the two bands (80-84 kDa) (results not shown). Wingate et al.¹⁷ describe several antigens that react with capacitated and non capacitated sperm. Among them a band of 80 kDa is found in sperm from infertile autoimmune men that

TABLE I. Motility Inhibition of Anti-Human Sperm Monoclonal SEM-12 After 5 Hours of Capacitation*

mAb	Progressive motility (%)
SEM-12	0
VAC-4	52
Control (no IgG)	64
Control (anti-RNAase mAb)	55

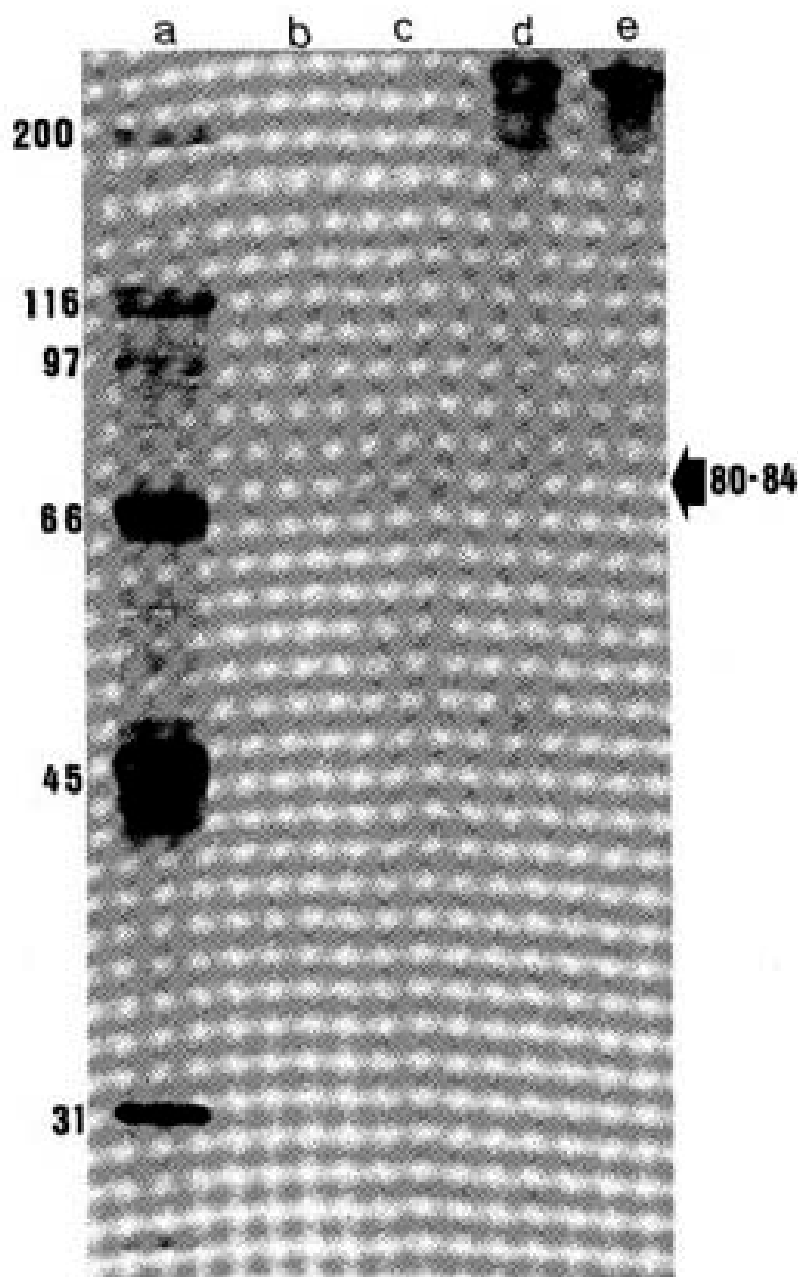


Fig. 5. Reactivity of SEM-12 mAb with human seminal plasma antigens: (a) molecular weight markers, (b) SEM-12 affinity chromatography without seminal plasma (control), (c) SEM-12 affinity chromatography, (d) immunoprecipitation, and (e) immunoprecipitation without seminal plasma (control). Two bands of 80-84 kDa are observed by both techniques.

react strongly with serum from infertile couples. Sperm from fertile men has this antigen but the expression increased after capacitation.

Acknowledgments

This research was supported by the CICYT Plan Nacional I&D (SAP95-0268).

REFERENCES

1. Chang MC. A detrimental effect of seminal plasma on the fertilizing capacity of sperm. *Nature* 1957; 179:258-259.
2. Williams CJ, Schultz RM, Kopf GS. Role of G proteins in mouse eggs activation stimulatory of acetylcholine on the ZP2 to ZP2f conversion and pronuclear formation in eggs expressing a functional ml muscarinic receptor. *Dev Biol* 1992; 152:288-296.
3. Yanagimachi R. Mammalian fertilization: The physiology of reproduction, 2nd Edition, Raven Press, New York, 1994, Chapter 5, pp. 189-317.
4. Fayer-Hinken RA, Caddle AB, Sizer BD. Galactosyltransferase activity is restricted to the plasma membrane of equine and bovine sperm. *Mol Reprod Dev* 1991; 28:74-78.
5. Wassarman PM. Profile of a mammalian receptor. *Development* 1991; 108:1-17.
6. Myles DG, Kimmel LH, Blobel CP, White JM, Primakoff

- P. Identification of a binding site in the disintegrin domain of fertilin required for sperm-egg fusion. *Proc Natl Acad Sci USA* 1994; 91:4195-4198.
7. Gupta SK, Fulgham DL, Alexander NJ. Characteristic of monoclonal antibodies reactive with human sperm and seminal plasma. *Indian J Exp Biol* 1990; 28:501-507.
 8. Coomrod SA, Westhusin MN, Naz RK. Monoclonal antibody to human fertilization antigen-1 inhibits bovine fertilization in vitro; Application in immunoneutralization. *Biol Reprod* 1994; 51:14-23.
 9. Freeman AJ, Wright RM, Flickinger CJ, Herr JC. Tissue specificity of the acrosomal protein SP-10: A contraceptive vaccine candidate molecule. *Biol Reprod* 1994; 50:615-621.
 10. Herr JC, Flickinger CJ, Homyk M, Klotz K, John E. Biochemical and morphological characterization of the intra-acrosomal antigen SP-10 from human sperm. *Biol Reprod* 1990; 42:181-193.
 11. Garcia-Franis V, Morte C, Andolz P, Martinez P. Characterization and regional binding of human sperm monoclonal antibodies. *Hybridoma* 1994; 13:317-321.
 12. Garcia-Franis V, Marrocell R, Marquez C, Benet J, Andolz P, Martinez P. Inhibition by anti-sperm monoclonal antibodies of the penetration of zona-free hamster oocytes by human spermatozoa. *Immunol Cell Biol* 1994; 72:1-6.
 13. Garcia-Franis V, Martin-Lunas E, Bosca A, Andolz P, Bielsa MA, Martinez P. Characterization of monoclonal antibodies specific for human sperm: Effect of CRL-10 on acrosome reaction. *Res Immunol* 1994; 145:533-539.
 14. Töpfer-Petersen E, Sinowatz F, Sanz L, Calvete JJ, Amselgruber W. Role of spermadhesins in gamete interaction. Proceedings of the XIV World Congress on Fertility and Sterility, Caracas, 1992.
 15. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975; 256:495-497.
 16. Benet-Rubinat JM, Martinez P, Lepp W, Egozcue J, Andolz P, Bielsa MA. Detection of induced anti-sperm antibodies by an improved enzyme-linked immunosorbent assay. *Int J Fertil* 1991; 36:48-56.
 17. Talbot P, Chacon RS. A triple stain technique for evaluating normal acrosome reaction of human sperm. *J Exp Zool* 1981; 215:201-208.
 18. Francavilla F, Romano R, Santucci R, Calignani P, Barone A, Fabbrini A. Detection of sperm surface related antibodies by indirect immunofluorescence test on sperm suspensions. Indirect IFT on sperm suspensions. *Andrologia* 1987; 19:69-75.
 19. Dostalova Z, Calvete JJ, Sanz L, Töpfer-Petersen E. Quantitation of boar spermadhesins in accessory sex gland fluids and on the surface of epididymal, ejaculated and capacitated spermatozoa. *BBA* 1994; 1200:48-54.
 20. Ahmad K, Naz RK. Presence and possible role of c-ras and nuclear (c-fos and c-jun) proto-oncogenic products in pre-implantation embryonic development in mice. *Mol Reprod Dev* 1993; 36:297-306.
 21. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 220:680-685.
 22. Naz RK, Morte C, Garcia-Franis V, Kaplan P, Martinez P. Characterization of a sperm specific monoclonal antibody and isolation of 95-kilodalton fertilization antigen-2 from human sperm. *Biol Reprod* 1993; 49:1236-1244.
 23. Rochewerger L, Cuasnicu PS. Redistribution of a rat sperm epididymal glycoprotein after in vitro and in vivo-capacitation. *Mol Reprod Dev* 1992; 31:34-41.
 24. Foster JA, Klotz KL, Flickinger CJ, Thomas TS, Wright RM, Castillo JR, Herr JC. Human SP-10: Acrosomal distribution, progressing, and late after the acrosome reaction. *Biol Reprod* 1994; 51:1222-1231.
 25. Primakoff P, Hyatt H, Myles DG. A role for the migrating sperm surface antigen PH-20 in guinea pig sperm binding to the egg zona pellucida. *J Cell Biol* 1985; 101:2239-2244.
 26. Liu Y, Mahan K, Lathrop WF, Myles DG, Primakoff P. A hyaluronidase activity of the sperm plasma membrane protein PH-20 enables sperm to penetrate the cumulus cell layer surrounding the eggs. *J Cell Biol* 1994; 125:1157-1163.
 27. Wingate EW, Patrick RT, Mathur S. Antigens in capacitated spermatozoa eliciting autoimmune response. *J Urol* 1993; 148:1331-1337.
 28. Cross NL. Multiple effects of seminal plasma on the acrosome reaction of human sperm. *Mol Reprod Dev* 1993; 35:316-323.
 29. San Agustín JT, Landy HA. Bovine seminal plasma constituents modulate the activity of caltrin, the calcium-transport regulating protein of bovine spermatozoa. *J Biol Chem* 1990; 265:6860-6867.
 30. Faery RV, Barber PJ, Jones R. Characterization of low Mr zona pellucida binding proteins from boar spermatozoa and seminal plasma. *Mol Reprod Dev* 1992; 33:108-115.
 31. Dostalova Z, Calvete JJ, Sanz L, Hettel C, Kiedel D, Schoneck C, Einspanier R, Töpfer-Petersen E. Immunolocalization and quantitation of acidic seminal fluid protein (aSFP) in ejaculated, swim-up, and capacitated bull spermatozoa. *Biol Chem Hoppe Seyler* 1994; 375:457-461.
 32. Sanz L, Calvete JJ, Karlheinz M, Gabius HJ, Töpfer-Petersen E. Isolation and biochemical characterization of heparin-binding proteins from boar seminal plasma: A dual role for spermadhesins in fertilization. *Mol Reprod Dev* 1993; 35:37-43.
 33. Fichorova RN, Dimitrova E, Tsvetkov D, Penkov R, Tsvetkov H. Detection of antibodies toward epididymal sperm antigens: An obligatory step in evaluation of human immunologic infertility? *Am J Reprod Immunol* 1995; 33:341-349.
 34. Calvete JJ, Sanz L, Dostalova Z, Töpfer-Petersen E. Spermadhesins: Sperm-coating proteins involved in capacitation and zona pellucida binding. *Fertilitat* 1995; 11:35-40.
 35. Gupta SK, Fulgham DL, Alexander NJ. Sperm penetration affects reactivity of monoclonal antibodies. *J Reprod Immunol* 1990; 18:187-197.