ANTIBODIES TO HUMAN SPERM YLP$_{12}$ PEPTIDE THAT IS INVOLVED IN EGG BINDING INHIBIT HUMAN SPERM CAPACITATION/ACROSOME REACTION

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Recently, the authors reported a novel dodecamer peptide sequence, designated as YLP$_{12}$, on human sperm, that is involved in binding to zona pellucida (ZP) of human oocyte [10]. This unique sequence is present on the acrosomal region of the human sperm cell and is expressed only in human testis/sperm. The aim of the present study was to examine whether YLP$_{12}$ sequence is involved in capacitation/acrosome reaction. Swim-up sperm were capacitated with anti-YLP$_{12}$ Fab’ antibodies or control Fab’s (40 and 85 µg/mL) and then the acrosome reaction was induced with calcium ionophore. An average of 64–73% sperm underwent acrosome reaction when they were capacitated in the presence of 40–85 µg/mL of bovine serum albumin or control Fab’s. A significant ($p < .01$ to $< .001$) reduction (58–75%) in the percentage of acrosome-reacted sperm was observed when the sperm were capacitated in the presence of YLP$_{12}$ Fab’s. These data indicate that the YLP$_{12}$ peptide sequence is involved in sperm capacitation / acrosome reaction, and may find clinical applications in the diagnosis and treatment of male infertility and immunocontraception.

Keywords  acrosome reaction, antisperm antibodies, capacitation, human sperm, sperm antigens

Fertilization is a complex process requiring the spermatozoon to undergo a cascade of events before it can fuse with the oocyte plasma membrane. This chain of events includes capacitation, binding to the zona pellucida (ZP), acrosome reaction, penetration through the ZP, and fusion with plasma membrane of the oocyte, which subsequently cleaves, develops, and implants. These events are not understood clearly at the molecular level [13]. Capacitation is a physiological process that makes the sperm cell capable of fertilizing the oocyte. Capacitation is followed by an irreversible phenomenon called acrosome reaction that results in releasing several proteolytic enzymes. Although discovered in 1951 by Austin and Chang, the molecular basis of these two processes are not clearly understood.

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Recently we described a novel dodecamer sequence, designated as YLP\textsubscript{12}, on human sperm that is involved in binding to ZP of human oocyte [10]. This sequence was localized on the acrosomal region of the human sperm cell. The YLP\textsubscript{12} sequence is expressed only in the human testis and not in other somatic cells/tissues tested. The synthetic 12-mer peptide based on this sequence and its monovalent Fab’ antibodies specifically and significantly (\(p < .05\)) inhibited human sperm–ZP binding in the hemizona assay [10]. The aim of the present study was to examine whether the YLP\textsubscript{12} sequence is also involved in human sperm capacitation/acrosome reaction. This was investigated by examining the effects of the monovalent Fab' antibodies against the YLP\textsubscript{12} peptide on the human sperm capacitation/acrosome reaction in vitro. The long-term objective of the study is to examine the potential clinical applications of the YLP\textsubscript{12} peptide in the diagnosis and treatment of male infertility and in the development of a contraceptive vaccine.

MATERIALS AND METHODS

Synthetic YLP\textsubscript{12} Peptide

The peptide was synthesized by solid-phase synthesis using Fmoc chemistry (Biosynthesis, Lewisville, TX). Deprotection was achieved by 20\% piperidine in dimethylformamide, and the peptide was cleaved from the resin by 85\% trifluoroacetic acid (TFA). The peptide was then precipitated in methyl tert-butyl ether and purified by using reverse-phase high-performance liquid chromatography (HPLC). The fractions eluted with 0.5\% TFA in acetonitrile were dried in a speed vacuum, redissolved in water, and lyophilized. The peptide was water soluble and had >95\% purity level.

Antibodies to Peptide

The peptide was conjugated to tetanus toxoid (TT; Wyeth Laboratories, Radner, PA) using 1-ethyl-3-(3-dimethylaminopropyl) [12]. Antiserum was raised in sexually mature virgin female rabbits of the New Zealand white strain [5], and the antibodies were purified by using a protein A-Sepharose 4B column. The monovalent Fab's were prepared using pepsin by the method of Nisonoff and colleagues [11] as described elsewhere [9]. The Fab's were further immunoaffinity purified by using peptide–BSA–Sepharose 4B immunobeads [10].

Sperm Collection, Processing, and Acrosome Reaction Assay

Semen was collected from healthy, fertile men (\(n = 3\)) after 48–96 h of sexual abstinence, liquefied (37°C, 1 h), and centrifuged (700 g, 10 min). To obtain the swim-up sperm population, 500 \(\mu\)L of Ham's F-10 medium supplemented with 5 mg human semen albumin/mL was overlaid on top of the washed sperm pellet and incubated for 1 h at 37°C in 5\% CO\textsubscript{2} in air [10]. Swim-up sperm were analyzed for concentration, motility, and any contamination, and were capacitated with anti-YLP\textsubscript{12} Fab’ antibodies or control immunoglobulins from pre-immune serum/control rabbits (40 and 85 \(\mu\)g/mL) for 5 h at 37°C in 5\% CO\textsubscript{2} in air. After capacitation, 5 \(\mu\)M calcium ionophore A23187 (Sigma Chemical, St. Louis, MO) was added and the sperm were incubated for an additional 30 min for acrosome reaction [1]. After incubation, the sperm were washed twice with phosphate-buffered saline (PBS), the concentration was adjusted to 1 \(\times\) 10\(^6\)/mL, and a 20-\(\mu\)L drop of the sperm suspension was added onto each well of the immunofluorescence slides [8]. The sperm were then air dried, fixed in methanol for 30 min, and air dried again. Acrosome reaction was determined by incubating fixed-sperm
with fluorescein isothiocyanate (FITC) conjugated to *Pisum sativum* agglutinin (PSA) (ICN Biomedicals, Aurora, OH) for 1.5 h [2]. The slides were washed twice with PBS and a drop of mounting medium (PBS containing 90% glycerol, 0.1% sodium azide and 10 mg/mL of 1,4-diazabicyclo(2,2,2)octane) was added on each well, covered with a coverslip, and examined using a fluorescence microscope. At least 300 sperm were counted in different fields for each sample. For each concentration, the experiment was repeated at least 3 times on different days, including sperm from 3 different men.

**Statistical Analysis**

Significance of differences between antibody and control groups was analyzed by using unpaired and paired Student's *t* test. A *p* value of <.05 was considered significant.

**RESULTS**

To examine the effects of anti-YLP<sub>12</sub> Fab's on sperm capacitation/acrosome reaction, the antibodies were incubated with sperm during capacitation and then the percentage of sperm undergoing acrosome reaction was determined. Acrosome reaction was induced with the calcium ionophore A23187. Following treatment with the ionophore, an average of 64 to 73% sperm underwent acrosome reaction when they were capacitated in the presence of 40–85 µg/mL of BSA or Fab's from preimmune serum or control rabbits (Figures 1c and 2). A significant reduction (*p* < .01 to <.001) in the percentage of acrosome reaction was observed when the sperm were capacitated in the presence of anti-YLP<sub>12</sub> Fab' antibodies (Figure 2). At 40 µg/mL concentration of anti-YLP<sub>12</sub> Fab's, there was a 58% inhibition (Figure 2) and at 85 µg/mL concentration, there was a 75% inhibition of acrosome reaction (Figure 2). There was no effect of the Fab's on sperm motility and they did not cause agglutination of sperm.

**DISCUSSION**

The anti-peptide antibodies significantly inhibited human sperm acrosome reaction in a concentration–dependent manner. The effect was specific since (1) Fab's from preimmune serum collected from the same rabbits or from control rabbits injected with adjuvant alone did not affect acrosome reaction, (2) immuno-affinity purified antibodies were used in the present study, (3) monovalent Fab's rather than the whole intact antibody molecule were used to avoid nonspecific interaction mediated through Fc portion, and (4) immunoadsorption with the peptide decreased the inhibitory effect of the anti-peptide Fab's (data not shown).

The above findings indicate that the YLP<sub>12</sub> sequence is involved in human sperm capacitation/acrosome reaction. At the present time, it cannot be delineated whether it is involved in capacitation, in acrosome reaction, in both processes. YLP<sub>12</sub> peptide sequence is an epitope of a 72 ± 5-kD protein that develops in the testis during spermatogenesis [10]. The mechanism by which the antibodies to YLP<sub>12</sub> peptide inhibit capacitation/acrosome reaction is not clear. YLP<sub>12</sub> peptide sequence/72 ± 5-kD protein may constitute an enzymatic or nonenzymatic component that is vital for capacitation/acrosome reaction. It is also possible that it is involved in tyrosine and/or serine/threonine phosphorylation, since the phosphorylation of membrane proteins has been shown to have an important role in sperm capacitation/acrosome reaction [3, 4, 6–8].

In conclusion, the data indicate that the YLP<sub>12</sub> peptide is sequence is involved in sperm capacitation/acrosome reaction. Since it is also involved in human sperm–human zona binding
Figure 1. Epifluorescent photomicrographs indicating the fluorescence pattern of capacitated sperm before and after acrosome reaction. Before incubation with the ionophore, the capacitated sperm showed only a few spontaneously acrosome-reacted sperm (b, shown by arrow); most of them were acrosome-intact (shown by arrowhead). After incubation with ionophore, 64–73% of sperm underwent acrosome reaction in controls (c). Incubation with YLP₁₂ monovalent Fab' antibodies inhibited the acrosome reaction. The phase contrast picture of (b) is included in (a) for comparison. Magnifications: a–c, ×720.
[10], it would appear that this peptide sequence has a vital role in human sperm function. Thus, the synthetic YLP\textsubscript{12} peptide may find clinical applications in the diagnosis and treatment of male infertility and in the development of a contraceptive vaccine.

REFERENCES


