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Human Sperm-Specific Peptide Vaccine That Causes Long-Term Reversible Contraception[1]

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ABSTRACT

A novel dodecamer peptide sequence, YLP_{12}, was identified on human sperm that is involved in oocyte binding. We investigated its immunocontraceptive effects in a murine model. A vaccine was prepared by conjugating the synthetic YLP_{12} peptide with the binding subunit of recombinant cholera toxin. Vaccination of female mice by i.m. or intranasal routes without any additional adjuvant induced a sperm-specific immune response in serum and the vaginal tract that caused a long-term contraceptive state. Fertility was fully regained when antibody reactivity diminished at 305–322 days. The contraceptive effect was also completely reversed voluntarily by intravaginal administration of the peptide. Antibodies affected fertility at the prefertilization stage by inhibiting sperm capacitation and the acrosome reaction, and sperm-oocyte binding. The peptide sequence is an epitope of a 50 ± 5-kDa membrane protein localized on the acrosome and tail of spermatozoa. Thus, the sperm-specific YLP_{12} is an attractive candidate for contraceptive vaccine.

acrosome reaction, immunology, male reproductive tract, sperm, testis

INTRODUCTION

The human world population is growing at a tremendous rate despite the availability of various contraceptive modalities and is now estimated to be greater than 6.2 billion people. Additional safe and less-expensive methods that require infrequent administration and long-lasting but reversible contraceptive effects are urgently needed. Contraceptive vaccines that can fulfill these criteria can provide a valuable alternative to currently available methods of family planning. A vaccine that targets sperm represents a promising prefertilization approach to contraception [1]. The inhibition of fertility after deliberate immunization with spermatozoa [2–4] and the presence of antisperm antibodies associated with involuntary infertility in humans [5–7] indicate a feasibility for the antisperm contraceptive approach. The molecular sequences involved in the sperm-oocyte zona pellucida interaction constitute attractive candidates for immunocontraception [8]. Several sperm antigens have been characterized; antibodies to some of them also affect fertilization in vitro, but only a few inhibit fertility in vivo [9–12]. The utility of a sperm antigen in the development of a contraceptive vaccine is contingent on its involvement in the fertilization process, its tissue specificity, and raising enough antibody titer in the female genital tract to inhibit sperm-oocyte interaction. To obtain Food and Drug Administration approval and to conduct appropriate multicenter fertility trials in a quality-controlled manner, recombinant or synthetic peptide molecules are required.

Recently, using the phage peptide display technique, our laboratory identified a novel dodecamer sequence, YLPVGGLRRIGG, designated as YLP_{12}, on human sperm that is involved in oocyte zona pellucida binding [13]. An extensive computer search of GenBank, the National Biomedical Research Foundation, and the Swiss sequence databases did not identify any known protein having a complete 12-amino acid sequence identical with the YLP_{12} peptide. The sera and seminal plasma of immunoinfertile men but not of fertile men have antibodies to this peptide, indicating its involvement in involuntary immunoinfertility in humans [14]. In the present study, we prepared a vaccine by conjugating the synthetic YLP_{12} peptide with the binding subunit of recombinant cholera toxin (rCTB), and examined its immunocontraceptive effects and tissue-specificity in actively immunized female mice. CTB has been successfully used as a carrier and as an adjuvant in several species of animals for enhancing mucosal immunoglobulin (Ig) A and systemic IgG [15–17].

MATERIALS AND METHODS

Vaccine Preparation

The dodecamer peptide, YLPVGGLRRIGG, designated as YLP_{12}, was synthesized by solid-phase synthesis using Fmoc chemistry supplied by Biosynthesis Inc. (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. 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. The purified peptide was coupled to the binding subunit of rCTB (List Biological Labs, Campbell, CA) by the two-step glutaraldehyde procedure [15]. Briefly, 10 mg of YLP_{12} peptide was dissolved in 200 μl of phosphate buffer (0.1 M pH 6.8) and 10 μl of 25% glutaraldehyde was slowly added. The mixture was incubated overnight at room temperature in the absence of light. Then, 2 mg of rCTB was added, and the mixture was incubated for another 24 h at 4°C. The mixture was dialyzed to remove the unconjugated peptide, and the coupling of peptide to rCTB was examined by the dot blot procedure using YLP_{12}-specific antibodies [13].

Active Immunization and Fertility Trial

Virgin CD-1, 10- to 12-wk-old female mice were immunized (designated as day zero) against the YLP_{12}-rCTB vaccine, rCTB alone, or PBS through i.m., intranasal (i.n.), or combined i.m. + i.n. routes (Table 1). Each animal received a total of four injections at 2-wk intervals. Each injection consisted of 30 (i.n.) to 100 μl (i.m.) of PBS containing 100 μg of vaccine, or rCTB, or PBS alone. In the i.n. group, the vaccine was delivered directly into the nostrils, and in the i.m. + i.n. group, half the vaccine (50 μg/30 μl) was delivered into nostrils and the other half was injected i.m. (50 μg/100 μl) (Table 1). Starting 2–3 wk after the last injection, the animals were bled biweekly by retro-orbital puncture to collect the serum, and the vaginas were rinsed with 50 μl of PBS to collect the vaginal washings in order to examine the antibody titer, as described be-

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TABLE 1. Fertility at 100–104 days after immunization with YLP_{12}-rCTB vaccine by various routes.

Group	Immunization route*	Mice (n)	Pups born (n)	Pups born per animal (mean ± SD)	Overall reduction in fertility
Trial I					
YLP_{12}-rCTB vaccine	Intramuscular	12	52	4.3 ± 3.4*	70.3%
rCTB alone	Intramuscular	12	174	14.4 ± 0.82	
PBS control	Intramuscular	12	175	14.6 ± 0.84	
Trial II					
YLP_{12}-rCTB vaccine	Intranasal	12	69	5.8 ± 2.8*	61.4%
rCTB alone	Intranasal	12	170	14.1 ± 1.2	
PBS control	Intranasal	12	174	14.5 ± 1.0	
Trial III					
YLP_{12}-rCTB vaccine	Intramuscular + intranasal	12	65	5.4 ± 2.7*	62.2%
rCTB alone	Intramuscular + intranasal	12	168	14.0 ± 1.1	
PBS control	Intramuscular + intranasal	12	172	14.3 ± 1.3	

* Significantly ($P = 0.001$ to 0.007) different versus respective control; others were nonsignificant ($P > 0.05$).

low. When the antibody titers peaked at 100–104 days (44–48 days after the last booster injection), the animals were mated overnight with male animals of proven fertility (two females with one male in each cage). The next morning, the mating was confirmed by the presence of a vaginal plug, and the mated animals were separated until they delivered pups. The number of pups delivered by each mated animal was counted, and pups were killed by CO_2 asphyxiation. Three different trials were conducted 5 mo apart using different batches of YLP_{12}-rCTB vaccine.

Fertility was defined as the mean number of pups born by the YLP_{12}-rCTB vaccine group or by the rCTB-immunized group divided by the mean number of pups born by the PBS control group, multiplied by 100.

Voluntary and Involuntary Regain of Fertility

Some animals showing high antibody titers and maximum effect on fertility were selected to examine the effect of disappearance of antibodies on the regain of fertility. Two approaches were taken. One was to voluntarily neutralize the antibodies by peptide administration. The second was to keep the animals for a longer period to let the antibodies involuntarily disappear with time.

In the first approach, to voluntarily neutralize the antibodies, peptide was administered (200 μg/50 μl PBS) via i.v. or intravaginal routes daily for 3 days. Peptide administration was conducted 6–7 wk after delivery of pups in the fertility trial (Table 1). Following the third peptide administration, the animals were mated, and the number of pups delivered was recorded. Sera and vaginal washings were collected prior to and at day 30 following the third administration in order to examine, if any, the booster effect of the peptide on the antibody reactivity.

In the second approach, the animals were kept for a longer time and the sera and vaginal washings were collected every 2–3 wk to examine the decline of antibody reactivity. All animals in this group showed a disappearance of antibodies at 305–322 days, compared to control levels (<2 SD units). The animals were then mated, and the number of pups delivered was recorded.

Analysis of Antibodies

ELISA. The presence and reactivity of antibodies (IgA and IgG) in serum and vaginal washings were analyzed against YLP_{12} peptide by using ELISA [13]. Each serum (1:50 dilution in PBS) and vaginal wash (1:10 dilution in PBS) was run in duplicate and the uncoated wells treated identically served as controls. IgA and IgG class antibodies were determined by using anti-mouse α- and γ-chain second antibodies (A4937 and A3438, respectively, from Sigma, St. Louis, MO). The absorbance reading was converted to standard deviation (SD) units by the following formula: SD unit = mean (test) − mean (control)/SD of control group [18]. The samples with SD units ≥ + 2 were considered positive.

Western blot procedure. Tissue specificity of peptide antibodies was determined by Western blot procedure. Eleven tissues (testis, kidney, liver, intestine, spleen, muscle, heart, lung, brain, ovary, and uterus) and cauda epididymal sperm were collected from mice (n = 2–3) and then homogenized in 0.1% Triton X-100 containing 10 mM PMSF and centrifuged at 3000 × *g* for 30 min. The supernatant was collected and acetone-precipitated, and the precipitates were dissolved in PBS, aliquoted, and kept at −70°C until use. The protein extracts (~50 μg/lane) were run in nonreduced slab SDS-PAGE (5%–15% gradient gel) [19], transferred to nitrocellulose membrane [20], the blot was incubated with YLP_{12} antibodies from vaccinated animals or immunoglobulins from control animals (10 μg/ml), and the reacted bands were detected as described elsewhere [13]. The YLP_{12} antibody and control immunoglobulin were purified from sera by using Protein G Plus/Protein A agarose beads (Oncogene Research Products/Calbiochem, Cambridge, MA) and used in this and in subsequent experiments.

Indirect immunofluorescence technique. Indirect immunofluorescence was performed on methanol-fixed noncapacitated and capacitated cauda epididymal murine sperm cells according to the procedure described elsewhere [13].

Immunoscanning electron microscopy. Scanning electron microscopy was performed as described elsewhere [21]. Briefly, cauda epididymal sperm were prefixed in 0.5% glutaraldehyde in PBS for 2–3 min, washed, incubated with BSA/control immunoglobulin/YLP_{12} antibody (10 μg/100 μl) from vaccinated animals, washed, and then incubated with gold-labeled goat anti-mouse antibody (Ted-Pella, Reading, CA). The attachment of gold particles was visualized with a stereoscan scanning electron microscope.

Immunobead binding. Immunobead binding was performed to examine the interaction of YLP_{12} antibody with live cauda epididymal sperm cells [22]. Swim-up cauda epididymal murine sperm (5×10^6/ml; noncapacitated or capacitated for 2 h, as described below) were incubated for 45 min at 37°C with BSA/control immunoglobulin/YLP_{12} antibody (10 μg/100 μl) in 5% CO_2 and 95% air; washed three times; and suspended in Biggers, Whitten, and Whittingham (BWW) medium containing 1% BSA. An 8-μl aliquot of sperm suspension was incubated with 2 μl of rabbit anti-mouse IgG beads (Irvine Scientific, Santa Ana, CA) in a humid chamber for 10 min, and the number of the sperm bound to the beads was recorded.

Acrosome reaction assay. Motile sperm cells (~90%–95% motility) were collected by swim-up procedure from cauda epididymides and were capacitated in the presence of various concentrations (2.5–10 μg/100 μl) of BSA/control immunoglobulin/YLP_{12} antibody for 2 h at 37°C in 5% CO_2 and 95% air in BWW-BSA medium [23]. The acrosome reaction was induced by incubating with calcium ionophore A23187 (Sigma) in a final concentration of 10 μM for 30 min [23]. The sperm were washed three times, fixed in 7.5% formaldehyde, spread over the poly-L-lysine coated slides, air-dried, and stained with 0.04% (w/v) Coomassie blue G-250 in 3.5% perchloric acid in order to analyze acrosome status [24]. The YLP_{12} antibody had no effect on motility percentage at any concentration tested compared to the control antibody or BSA. Greater than 80% motility was maintained throughout the experiment in all groups. After ionophore treatment, ~65%–70% motility was observed in all three groups, and ~70%–80% of sperm demonstrated the acrosome reaction in the BSA control medium. At least 250–300 sperm cells were counted in different fields for each sample. The experiment was repeated at least three times on different days using sperm from five mice.

Sperm-egg binding assay. Virgin CD-1 female mice, 8–10 wk old, were superovulated by i.p. injection of 7 IU of eCG (Sigma), oocytes were collected after 12 h of hCG administration, and cumulus cells were removed by using 0.1% hyaluronidase [25]. Motile sperm cells (~90%–95% motility) were collected by swim-up procedure from cauda epididymides, capacitated for 1.5 h, and then incubated for 1 h at 37°C in micro-

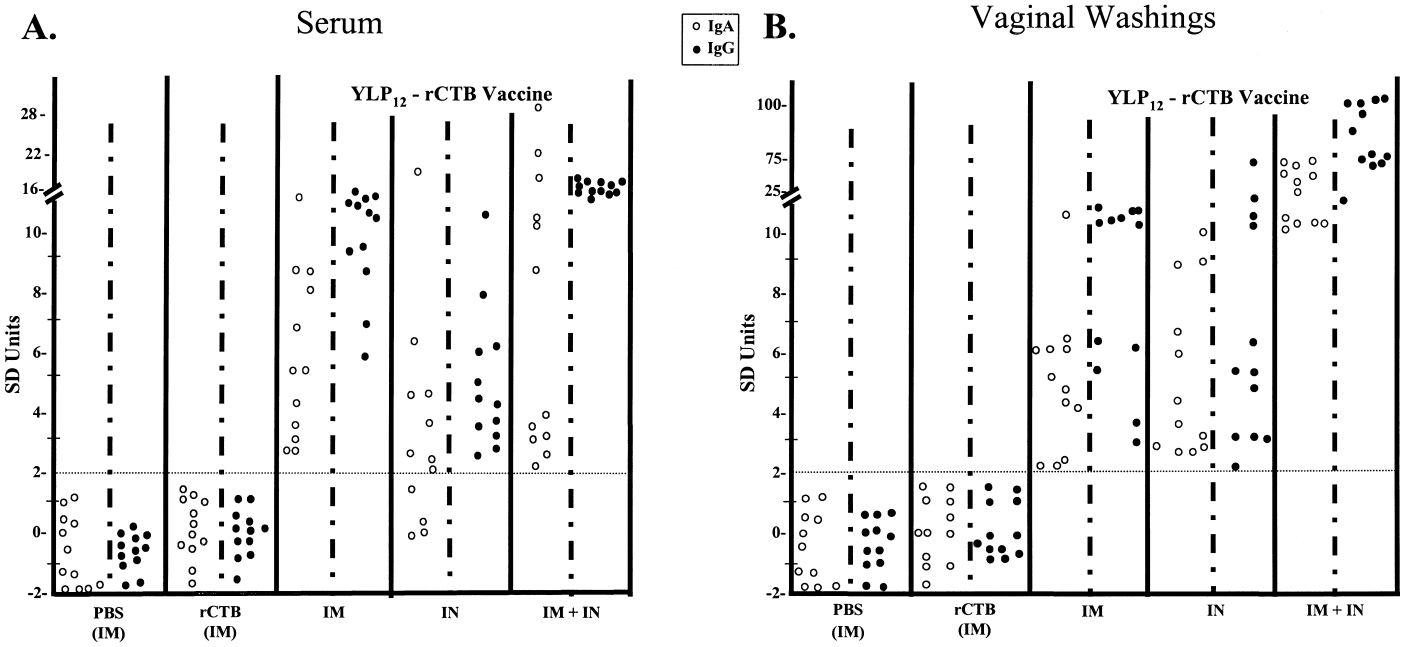

FIG. 1. Antibody reactivity with YLP_{12} peptide in serum and vaginal washings of mice immunized with the vaccine, rCTB alone, or PBS. The horizontal dotted line represents two SD units. The samples with SD units $\geq +2$ were considered positive.

drops of BWW-BSA medium under mineral oil with various concentrations (2.5–10 μg/100 μl) of BSA/control immunoglobulin/YLP_{12} antibody in a 5% CO_2 atmosphere [25]. The eggs (n = 4–10) were then added to the microdrops, the mixture was incubated for 20 min at 37°C in 5% CO_2, the eggs were washed off the loosely bound sperm, and the number of tightly bound sperm in a single plane of view were counted. The experiment was repeated at least three times on different days using eggs and sperm from three to five different mice. Incubation of sperm with YLP_{12} antibody did not affect the percentage of motile sperm at any concentration, compared to the control immunoglobulin or BSA control.

Statistical Analysis

Significance of difference was analyzed by one-way ANOVA. Post-hoc analysis was performed by using the Bonferroni test. Correlation between the antibody titer (SD units) and fertility (number of pups born) was analyzed by linear regression. A P value of < 0.05 was considered significant.

RESULTS

Vaccination of female mice with YLP_{12}-rCTB by various routes induced a contraceptive state resulting in a significant ($P = 0.001$ to 0.007) reduction in litter size. All vaccinated animals (100%) showed some degree of inhibition of fertility, with several animals having high antibody titers showing a complete block (Table 1). No animal in any group showed <38% reduction in fertility, compared with control animals. Vaccination via all routes inhibited fertility, with i.m. immunization showing the greatest reduction. Vaccination via the i.m. route caused an overall 70.3% reduction in litter size, the i.n. route caused a 61.4% reduction, and the i.m. + i.n. route caused a 62.2% reduction. Differences in percent inhibition of litter size among various groups were nonsignificant ($P > 0.05$).

Antibody reactivity started rising in animals after the last booster injection. Antibody reactivity peaked at 100–104 days after the first injection (44–48 days after the last booster injection), and the animals were mated. At that time, all vaccinated animals (100%) showed positive (>2 SD units) antibody reactivity against the peptide in serum and vaginal washings for the IgG class of antibodies (Fig. 1, A and B). For the IgA class, all animals (100%) in the i.m. group and the combined i.m. + i.n. group demonstrated positive reactivity in serum and vaginal washings. In the i.n. group, 75% (8 of 12) of animals showed positive antibody reactivity in the serum and 100% (12 of 12) of animals had positive titers in vaginal washings. The highest titers were obtained in the i.m. + i.n. group, with many animals showing antibody reactivity in the range of 25–105 SD units (Fig. 1, A and B). Antibody reactivity correlated ($r = 0.51$–0.74) significantly ($P < 0.01$) with a reduction in fertility, with vaginal IgA titers showing the highest degree of correlation.

To examine whether one could voluntarily and willingly reverse the contraceptive effect at a particular time, the purified synthetic peptide was administered in two groups of mice that had been previously immunized with the contraceptive vaccine, and which had shown a reduction in litter size. The administration of peptide by i.v. or intravaginal routes showed a regain of fertility, with intravaginal administration causing a complete reversal, with litter sizes in the range of fertile controls (Table 2). Intravenous or intravaginal administration of the peptide in control animals did not affect fertility. Peptide administration by either route did not cause a booster effect on antibody reactivity in either vaccinated or control groups (Table 2).

Animals showing the highest antibody reactivity and greatest reduction in litter size in each group were kept to examine reversibility. Antibody reactivity was monitored biweekly both in the serum and vaginal washings, and by Days 305–322, they had reached <2 SD units, as observed in controls. These animals regained fertility and delivered a litter size that was not significantly different from those of the control group (Table 3).

Antibodies raised after vaccination were tissue-specific and recognized a specific protein band of 72 ± 2 kDa in testis extract and a band of 50 ± 5 kDa in sperm extract. These specific bands were not observed in the protein extracts of 10 murine somatic tissues tested in the Western blot procedure (Fig. 2). Some nonspecific protein bands re-

acted in each tissue; however, the bands were recognized by both the peptide antibodies as well as the control immunoglobulins. Silver-stained SDS-PAGE of these tissue extracts showed several sharp and well-dissolved protein bands without any smear.

The YLP_{12} antibody reacted with the acrosome and tail regions of methanol-fixed capacitated or noncapacitated murine sperm with the indirect immunofluorescence and immunoscanning electron microscopic techniques (Fig. 3). With the immunobead binding technique, which used live murine noncapacitated or capacitated caudal sperm, the antibody showed binding to 75%–80% of the sperm membrane in the same regions.

The YLP_{12} antibody present in serum of vaccinated animals showing a complete block of fertility caused a significant and concentration-dependent inhibition (65%–70%) of sperm capacitation and acrosome reaction, and a complete block (100%) of sperm-oocyte binding in vitro (Fig. 4). Control immunoglobulin did not cause inhibition of sperm capacitation or acrosome reaction and did not affect sperm-oocyte binding. Immunoadsorption of the YLP_{12} antibody with the peptide (1:10 antibody:peptide ratio, w/w) completely abolished the inhibitory effect of the antibodies in both assays (data not shown). The YLP_{12} antibody did not affect the percentage of sperm motility in either of these assays, compared to the control immunoglobulin or BSA.

DISCUSSION

Active immunization with the peptide vaccine caused a significant reduction in fertility. Using 100 μg of the vaccine, which consisted of peptide and rCTB conjugated in a 5:1 ratio (w/w), the antibody titers raised were enough to cause an overall 62%–71% reduction in fertility compared to controls. A significant linear correlation between antibody reactivity and reduction in fertility was observed; animals having high antibody reactivity delivered fewer pups. This was especially true for IgA antibody reactivity in the vaginal tract. However, the correlation was not perfectly linear. Some animals had high antibody reactivity both in serum and the vaginal tract, but showed a lower reduction in fertility compared with other animals that had relatively less antibody reactivity. Systemic immunization via the i.m. route and mucosal immunization via the i.n. route raised circulating and local antibodies (both IgG and IgA) in vagina. No additional adjuvant was used for immunization. Recombinant CTB provided T cell carrier help and acted as an adjuvant to enhance the immunogenicity of the peptide.

The immunocontraceptive effect of the vaccine was reversible. The reversibility could be achieved either voluntarily or involuntarily. Voluntary regain of fertility could be achieved by intravaginal administration of the peptide. Involuntary regain of fertility was achieved when the antibody reactivity decreased to prevaccination control levels (<2 SD units), especially in the vaginal tract after 300 days. It appears that peptide administration transiently bioneutralizes the antibodies, which frees the sperm to fertilize the egg. Antibodies inhibited sperm capacitation, the acrosome reaction, and sperm-egg binding in vitro, and the inhibitory effects were abolished after the antibodies had been incubated with peptide (data not shown). Similar neutralization of the antibodies in vivo could abolish the contraceptive effect of vaccination. These findings also indirectly indicate that local antibodies in the genital tract may be more important than circulating antibodies in causing a contraceptive effect because the intravaginal administration, com-

TABLE 2. Effect of peptide administration on reversibility of immunocontraception.

Route of peptide administration (200 μg/mouse/day, × 3)	Mice[a] (n)	Antibody reactivity (SD units; mean ± SD)								Pups born (mean ± SD)	
		Before administration				After administration[b]					
		Serum		Vaginal wash		Serum		Vaginal wash			
		IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	Before administration	After administration
Vaccinated											
Intravenous	5	9.7 ± 2.7	8.5 ± 2.0	12.5 ± 4.0	6.0 ± 2.5	7.0 ± 5.7	5.0 ± 2.2	11.6 ± 3.7	5.3 ± 3.0	5.2 ± 3.1[a]	8.4 ± 3.2[a,b]
Intravaginal	7	9.5 ± 2.0	7.8 ± 2.2	13.0 ± 3.9	6.3 ± 2.6	7.15 ± 2.08	6.01 ± 1.05	10.4 ± 5.1	5.0 ± 2.8	6.0 ± 2.8[a]	12.8 ± 1.4[b]
Control											
Intravenous	5	<2	<2	<2	<2	<2	<2	<2	<2	12.3 ± 2.2[b]	11.9 ± 1.7[b]
Intravaginal	5	<2	<2	<2	<2	<2	<2	<2	<2	11.1 ± 2.6[b]	12.4 ± 2.1[b]

[a] Vaccinated via i.m. + i.n. routes.
[b] Examined 30 days after peptide administration.
[a,b] Vaccines with different superscripts are significantly different ($P < 0.05$ to <0.001) and with same superscripts nonsignificant ($P > 0.05$).

TABLE 3. Fertilty at 305–322 days after immunization with YLP_{12}-rCTB vaccine by various routes.

Group	Immunization route	Mice (no.)	Pups born (no.)	Pups born per animal (mean ± SD)
Group I				
YLP_{12}-rCTB vaccine	Intramuscular	6	48	8.0 ± 3.6[a]
rCTB alone	Intramuscular	6	65	10.8 ± 1.8[a]
PBS control	Intramuscular	6	72	12.0 ± 1.4[b]
Group II				
YLP_{12}-rCTB vaccine	Intranasal	6	66	11.0 ± 2.2[a]
rCTB alone	Intranasal	6	68	11.3 ± 1.6[a]
PBS control	Intranasal	6	74	12.1 ± 1.3[b]
Group III				
YLP_{12}-rCTB vaccine	Intramuscular + intranasal	10	119	11.9 ± 1.9[a]

[a] Versus [b] nonsignificant ($P > 0.05$).

pared with i.v. administration of the peptide, completely reversed the effect of the vaccine. Peptide administration by either route did not cause a booster effect on antibody titer. This could be expected because the peptide is only 12-mer, probably lacking a T cell epitope, and thus too small to be immunogenic without conjugation to a T cell carrier.

Vaccination was without any side effect. Animals seemed normal and did not lose weight. Also, no abnormality was seen in the pups born after involuntary or voluntary regain of fertility. This was expected because the antibodies raised after vaccination are tissue-specific, and they recognized a specific protein band of 72 ± 2 kDa in testis extract and a band of 50 ± 5 kDa in sperm extract, and no specific band in the protein extracts of 10 murine somatic tissues tested. YLP_{12} antibodies demonstrated similar tissue specificity with human tissues [13]. There was a difference in molecular mass between the testis (72 ± 2 kDa) and sperm (50 ± 5 kDa) antigens recognized by the YLP_{12} antibody. Similar differences in molecular mass between testis and sperm antigens were observed in humans [13, 14]. These findings suggest that the YLP_{12} peptide sequence is a part of a 72 ± 5-kDa protein that is synthesized during spermatogenesis in the testis, and later modified or cleaved during epididymal transit to form a 50 ± 5-kDa protein in the mature sperm cell.

The 50 ± 5-kDa protein, of which the YLP_{12} epitope is a part, was localized on the surface of acrosome and tail regions of spermatozoon. The YLP_{12} antibodies present in serum of vaccinated animals caused an inhibition of sperm capacitation and acrosome reaction, and a complete block of sperm-oocyte binding in vitro, indicating the mechanisms by which the antibodies inhibit fertility in vivo. There was no effect of YLP_{12} antibody on the percentage of sperm motility in either of these assays. However, it is possible that the antibodies affect velocity, amplitude of lateral head displacement, and beat frequency. These parameters have been reported to be important contributors to hyperactivation of sperm cells, which is considered an integral part of capacitation that precedes the acrosome reaction and sperm binding to the zona pellucida of the oocyte [26, 27]. This may be one of the mechanisms by which antibodies affect sperm function (i.e., capacitation, acrosome reaction, and sperm-oocyte binding).

Even very high titers of antibodies in both serum and in the vaginal tract caused inhibition of fertility by up to 70.3% after vaccination. In humans, normally, one egg is ovulated every cycle, whereas mice ovulate several eggs in each cycle. It is possible that even higher titers than were achieved in the present study are required for complete inhibition. Antibodies completely inhibited sperm-egg binding in vitro, indicating that high titers could simulate the similar situation in vivo. It is also possible that sperm cells have multiple antigens involved in egg binding. Also, using the whole YLP_{12} antigen, instead of a single 12-mer peptide epitope in a vaccine formulation, may further enhance the immunogenicity and efficacy of the vaccine. The hybridomas that secrete monoclonal antibodies (mAbs) against the YLP_{12} peptide has been prepared by fusing lymphocytes from vaccinated animals with mouse myeloma cells [28].

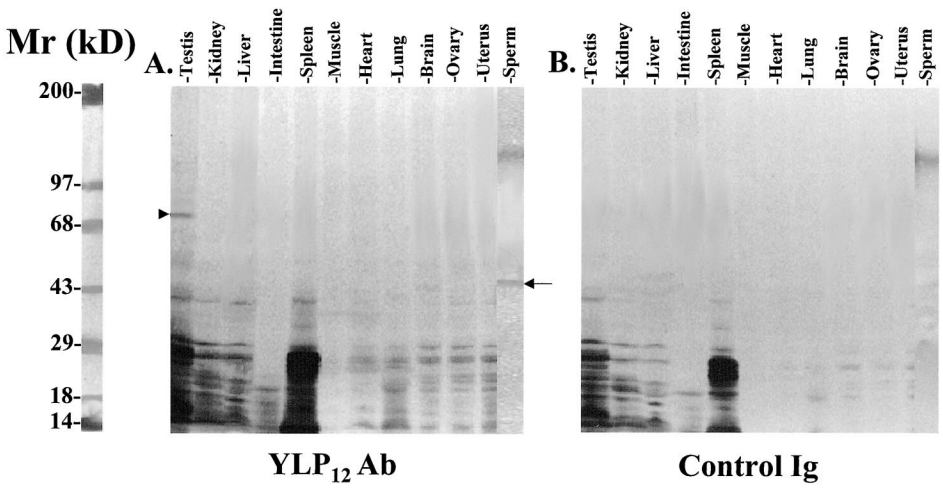

FIG. 2. Western blot indicating the testis/sperm specificity of peptide antibodies. YLP_{12} antibody (**A**) from vaccinated animals but not the immunoglobulin from PBS-control or rCTB-alone animals (**B**) recognized a specific band of 72 ± 2 kDa in the testis extract (arrowhead) and a specific band of 50 ± 5 kDa in the sperm extract (arrow). Antiserum from three different mice immunized with the vaccine was tested against four multiple tissue blots. Each antiserum showed a similar pattern.

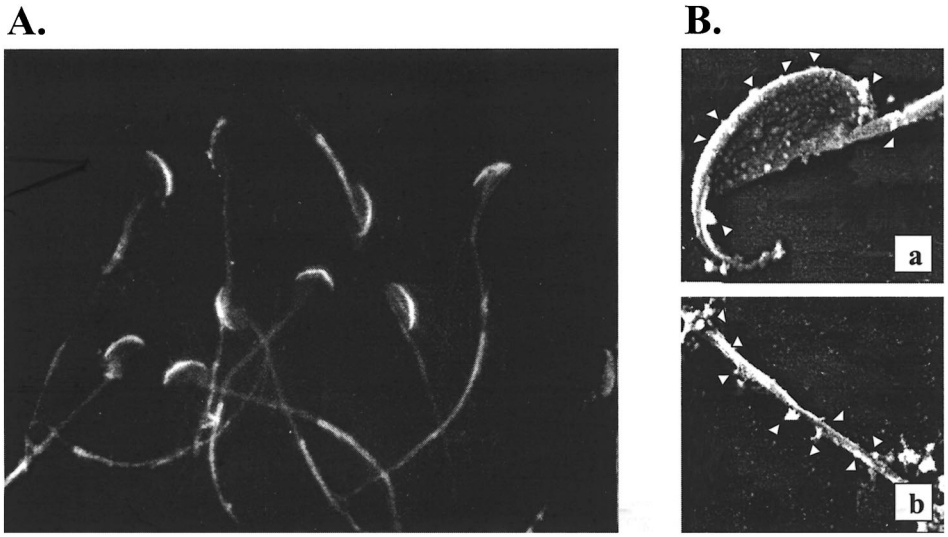

FIG. 3. Immunoreactivity of antibodies from vaccinated animals with sperm cells using indirect immunofluorescence (II) (**A**) and immunoscanning electron microscopy (IEM) (**B**). Antibodies showed binding to acrosome and tail regions of sperm cells in both these procedures as seen by fluorescence in II and gold particles in IEM. Gold particles were visible on the acrosome (**a**) and tail (**b**) of sperm cells (arrowheads) in IEM. Control immunoglobulins did not react with sperm in either procedure. Magnifications: **A** ×650; **B** (**a** and **b**) ×10 000.

The peptide-specific mAb binds to 72 ± 2 kDa in testis and 50 ± 5-kDa antigen in sperm extracts, and recognizes acrosome and tail regions of sperm cell in both humans and mice, as observed in the present study and a previous study [13]. These findings indicate that the YLP_{12} epitope sequence is evolutionarily conserved between humans and mice, and plays a role in fertilization. Using mAb as a probe, the cDNA encoding for the YLP_{12} antigen has been cloned from human testis-λgt11 expression library. It has an insert of ~1.2 kilobases, has been subcloned into p-Bluescript II SK+ at *Sma*1 site, and is presently being sequenced.

In conclusion, our findings indicate that vaccination with YLP_{12}-rCTB induces a sperm-specific antibody response in female mice that causes a contraceptive state by one or more mechanisms involving an inhibition of the fertilization process. The immunocontraceptive effect is long-lasting and reversible; with the disappearance of antibodies there was a complete regain of fertility. Reversibility could also be achieved voluntarily by intravaginal administration of the peptide. The utility of a sperm antigen in the development of a contraceptive vaccine is contingent on its sperm specificity, on involvement in the fertilization process, and on raising an immune response that is capable of inhibiting fertility in a reversible manner. YLP_{12} fulfills all these criteria. In addition to these parameters, the involvement of YLP_{12} antigen in human immunoinfertility makes it an attractive candidate for immunocontraception.

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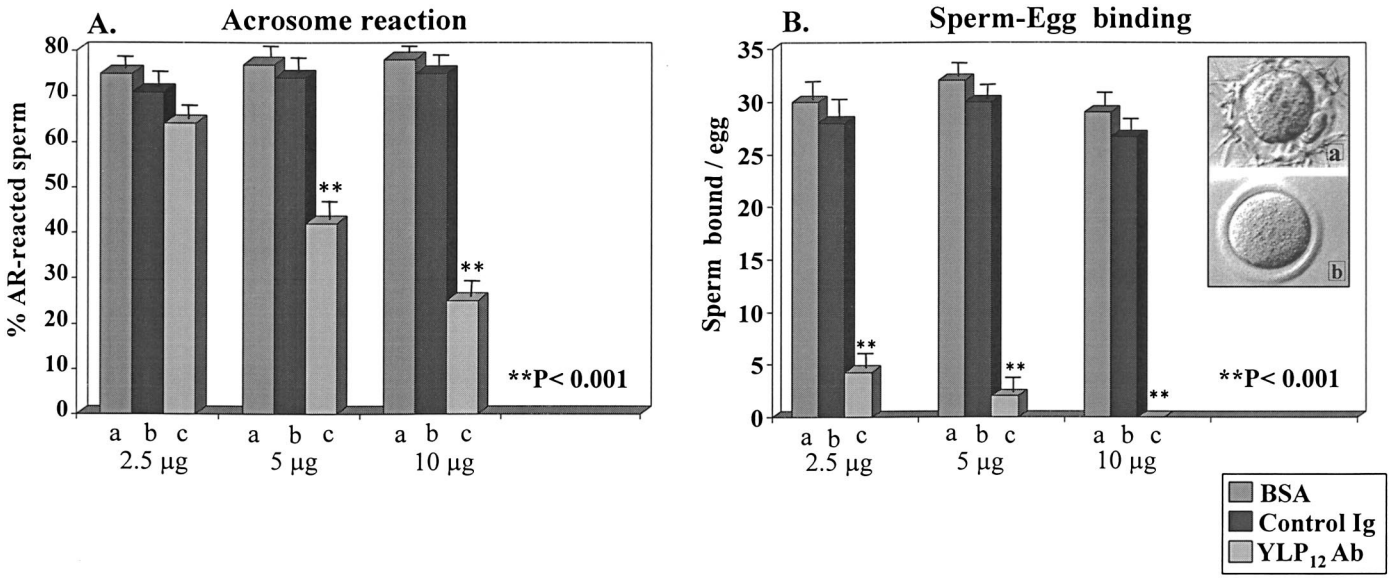

FIG. 4. Effect of antibodies from vaccinated animals on sperm capacitation and the acrosome reaction (**A**) and sperm-egg binding in vitro (**B**). YLP_{12} antibody caused a concentration-dependent inhibition of the percentage of acrosome-reacted sperm and a total block of sperm-oocyte zona pellucida binding. Incubation of antibodies with peptide (antibody:peptide ratio 1:10, w/w) overnight at 4°C neutralized the inhibitory effect of antibodies in both assays (data not shown). Inset: sperm treated with YLP_{12} antibody did not show binding to oocyte (**b**), and sperm treated with control immunoglobulin showed binding with >30 sperm binding per oocyte (**a**). Magnifications: **a** and **b** ×250.

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