

Antisperm Contraceptive Vaccines: Where We Are and Where We Are Going?

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Keywords

Contraception, infertility, sperm, vaccines

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Submitted February 16, 2011;
accepted February 27, 2011.

Citation

Naz RK. Antisperm contraceptive vaccines: where we are and where we are going? *Am J Reprod Immunol* 2011; 66: 5–12

doi:10.1111/j.1600-0897.2011.01000.x

This is a review of current status and future perspectives on the development of antisperm contraceptive vaccines (CV) and immunocontraceptives. The development of antisperm CV is an exciting proposition. There is a strong rationale and recent data indicating that this proposition can translate into reality. The search for novel sperm-specific antigens/genes, that can be used for CV, continues using various recent developing technologies. Various approaches of proteomics, genomics, reproductive biology, mucosal immunity and vaccinology and several novel technologies such as gene knockout technology, phage display technology, antibody engineering, differential display technique, subtractive hybridization, and hybridoma technology are being used to delineate sperm-specific antigens and construct CV. Various sperm antigens/genes have been delineated, cloned, and sequenced from various laboratories. Vaccination with these sperm antigens (recombinant/synthetic peptide/DNA) causes a reversible contraceptive effect in females and males of various animal species, by inducing a systemic and local antisperm antibody response. The efficacy is enhanced by combination vaccination, including peptides based on various sperm antigens. Several human novel scFv antibodies with unique complementarity-determining regions (CDRs), that react with specific well-defined fertility-related sperm antigens, have been synthesized. These human infertility-related antibodies may find application in the development of novel immunocontraceptives. Besides finding the novel sperm antigens, the present and future focus is on enhancing the immunogenicity, bioefficacy, and on obliterating the inter-individual variability of the immune response, and proceeding for primate and human clinical trials. Multi-epitope vaccines combining sperm proteins involved in various steps of fertilization cascade have been found to enhance the immunogenicity and bioefficacy of the contraceptive effect. The *in vitro* synthesis of infertility-related human scFv antibodies may provide unique once-a-month immunocontraceptives, the first of its kind, for human use. The multi-epitope CV and preformed engineered human antibodies of defined specificity may obliterate the concern related to inter-individual variability of the immune response.

Introduction

The population growth and unintended pregnancies are major public health issues worldwide. The world population has exceeded 6.891 billion and is increasing by 1 billion every 12 years.¹ In the USA alone, half of all pregnancies are unintended, which results in over one million elective abortions annually.^{2,3} These women use some type of contraceptive. An estimated 80 million women have unintended or unwanted pregnancies worldwide annually, and 45 million of these end in abortion.⁴ This calls for a better method of contraception that is acceptable, effective, and available both in the developed and in the developing nations. It should be non-steroidal, non-barrier, non-surgical, intercourse-independent, and reversible. Contraceptive vaccines (CV) have been proposed as valuable alternatives that can fulfill most, if not all, of the properties of an ideal contraceptive. As the developed and most of the developing nations have an infrastructure for mass immunization, the development of vaccines for contraception is an exciting proposition. The aim of this article is to review the current status and future prospective of CV targeting spermatozoa.

Discussion

CV that are being investigated target *Gamete production* [luteinizing hormone-releasing hormone (LHRH/GnRH) and follicle stimulating hormone (FSH)], *gamete function* [sperm antigens and oocyte zona pellucida (ZP)], and *gamete outcome* [human chorionic gonadotropin (HCG)].⁵ Vaccines targeting gamete production affect steroid production. They can be used as a substitute for castration of animals (feral, farm, and domestic) and for therapeutic purposes in clinical situations requiring inhibition of sex steroid secretion. Vaccines inhibiting gamete function are the preferred target. Although CV targeting ZP have a high contraceptive efficacy, they cause oophoritis, affecting sex steroids. For human applicability, the current research is focused on delineating infertility-related epitopes (B-cell epitopes) from oophoritis-inducing epitopes (T-cell epitopes) and modulation of immunogenicity by various carriers and adjuvants. The HCG vaccine, which targets gamete outcome, is the first CV that has undergone Phase I and Phase II clinical trials in humans.⁶ Presently, the focus is on increasing its immunogenicity and efficacy, and investigating its utility in HCG-producing cancer.

Rationale

Spermatozoa have drawn much attention for CV development. Spermatozoon can produce antisperm antibodies (ASA) in both men and women because of its auto- and isoantigenic properties. Up to 70% of vasectomized men produce ASA,⁷ and 2–30% of infertility cases may be associated with the presence of ASA in the male and/or female partner of an infertile couple.⁸ ASA affect fertilization and fertility through several mechanisms, including: inhibition of sperm capacitation, acrosome reaction, sperm-zona interaction and penetration, oocyte membrane binding and penetration, and pre-implantation embryonic development. Also, the deliberate immunization of male and female animals of various species,^{9–11} including humans,^{12,13} with sperm/testes induce ASA causing a contraceptive effect. Baskin¹² injected 20 fertile women, known to have at least one prior pregnancy, with their husband's semen. These women developed ASA and no conception was reported for up to 1 year of observation. These findings provide strong evidence that spermatozoa are capable of eliciting an immune response that can cause a contraceptive state. A US patent was issued for this spermatotoxic vaccine in 1937 (US patent number 2103240). However, the whole spermatozoon cannot be used for the CV development as it has several antigens that are likely to be shared with various somatic cells.¹⁴ Thus, only sperm-specific antigens may be used in the development of a successful CV. The utility of a sperm antigen for the CV development is contingent upon its sperm specificity, surface expression, involvement in fertilization/fertility, and ability to raise high antibody titers, especially in the genital tract. A sperm antigen that has these properties and is also involved in human immunoinfertility is a promising and exciting candidate for CV. Immunoinfertile patients with ASA have no other tissue pathology concomitant with the infertility, indicating probable sperm specificity of the molecule against which these antibodies are directed to. The sperm-ZP binding site is the most desirable target for antisperm immunocontraception.

Search for Candidates for CV

The mouse genome is 2.5 billion DNA letters long. It is about 14% shorter than the human genome, which is 2.9 billion letters long. The human genome

is filled with more repeat sequences than the mouse genome.¹⁵ The mouse and the human genomes each seem to contain approximately 30,000 protein coding genes. A majority of them are evolutionarily conserved, and some families of genes have undergone expansion/multiplication in the mouse lineage.¹⁵ The fertility field has exploded with the advent of gene knockout technology. Almost every month there is a report on a new gene knockout that has some effect on fertility. Using an extensive database search, we identified at least 164 genes in mice whose knockout affect male fertility.^{16,17} The majority of these knockouts did not show sperm specificity. These knockouts also demonstrated an effect on non-reproductive organs concomitant with an effect on some aspect of fertility (spermatogenesis/spermiogenesis/sperm function/fertilization/embryogenesis/mating behavior). Others did not demonstrate a surface expression, which is a must to make it accessible and amenable for antibody binding. Although these genes/proteins that are not expressed on the surface may provide targets for pharmacological inhibition for contraception, they are not suitable for CV development. We do not expect to find a large number of proteins that are exclusively expressed on the sperm surface, as sperm-zona binding is an exclusive event requiring highly specific, mostly species-specific, receptor(s)/ligands interaction. Spermatozoon does not bind and penetrate in to any other cell except the oocyte, indicating that the specific receptors/ligands are involved in an interaction between these two cells.

Besides gene knockout technology, several other methods such as hybridoma technology, subtractive hybridization, differential display technique, and phage display technique have been employed to delineate sperm antigens that play a role in fertilization/fertility and can be used for CV development. Our laboratory, using these technologies have delineated, cloned, and sequenced several novel sperm/testis cDNA/antigens that are involved in various stages of fertilization cascade and can be used for CV development. Notable among them are FA-1,¹⁸ YLP₁₂,¹⁹ CV,²⁰ and testis-specific antigen (TSA)-1.²¹ These antigens have sperm specificity in mouse and man, the species tested, and have surface expression. There are additional genes/proteins that have been reported from several other laboratories. These include LDH-C₄,²² P10G,²³ A9D,²⁴ SP56,²⁵ epididymal protein inhibitor (Eppin),²⁶ and Izumo.²⁷

Although a sperm protein involving in any step of sperm function and fertilization is a viable candidate, the antigen involved in sperm-zona binding site is the most attractive molecule for CV development. Our laboratory extensively investigated, using various technologies, the sperm proteins that are involved in zona pellucida (ZP) binding in humans. Human sperm proteins belonging to four major molecular regions, namely 95, 63, 51, and 14–18 kDa, were found to react with human zona pellucida proteins in the Western blot and immunoprecipitation procedures.²⁸ In these procedures, zona pellucida protein that reacted strongest with the sperm proteins belonged to the molecular region of 55 kDa (ZP3), besides weakly reacting proteins in the 110-kDa (ZP1/ZP2) and 14–18 kDa molecular regions. Three (95, 51, and 14–18 kDa) of the four molecular regions of sperm proteins that bound to the zona pellucida proteins also seem to involve o-phospho-L-tyrosine residues in their interaction. These proteins demonstrated the presence of phosphotyrosine residues, and the 51-kDa protein also showed autophosphorylating activity in the *in vitro* kinase assay. It was found that the 51 kDa protein is the FA-1 antigen that was delineated in our laboratory.²⁸

In a recent study, the yeast two-hybrid (Y2H) system was used to identify human sperm proteins that interact with human ZP3.²⁹ Human ZP3 cDNA was cloned into pAS2-1 yeast vector and used as bait to find reactive proteins in the human testis cDNA library. Six specific clones were obtained that were further confirmed for interaction using the mammalian two-hybrid system. These six clones showed homologies with several proteins in the GenBank database. Of these, the strongest ZP3-interacting protein, that shows 97% homology with ubiquitin associated protein-2 like (UBAP21), was tested in the hemizona binding assay. UBAP21 antibodies significantly ($P < 0.001$) inhibited human sperm-zona binding in this assay.²⁹ These cumulative findings indicate that there are at least 4–6 sperm proteins that interact/bind with zona pellucida in humans.

Vaccination Studies

Although there are over 400 published studies in the database examining the effect of sperm antibodies on sperm function and fertilization *in vitro*, there are only three studies examining the effect of antibodies *in vivo*.^{30–32} Two of them are from

our laboratory.^{30,32} For immunocontraception, the *in vivo* effect is important and relevant for the vaccine development. At higher concentration and using a longer period of incubation, almost any antibody/immunoglobulin can affect sperm function and fertilization to some degree *in vitro* non-specifically. It is pertinent to demonstrate the effect of an antibody on fertility *in vivo*. There are only limited studies that investigated the contraceptive effect of well-defined sperm antigens in actively immunized animals. The sperm antigens which have been examined for contraceptive effect include: FA-1,³³ YLP₁₂,³⁴ LDH-C₄,²² P10G,²³ A9D,²⁴ SP56,²⁵ 80 kDa HSA,³⁵ Eppin,²⁶ and Izumo.³⁶ Also, some additional proteins that cross-react with sperm, such as Bin1b, expressed in the epididymis, have been investigated.³⁷

Animal Models for Examining the Immunocontraceptive Effects

Finding a suitable animal model to examine the effect of a vaccine on fertility has been a major challenge for the reproductive immunologists working in the field of immunocontraception. Most of the active immunization studies have been carried out in the mouse model. A few of the studies have used rat, rabbit, and guinea pig as a model. The rabbit and guinea pig are ultra-sensitive models to examine the immunogenicity and antifertility effect and may not be suitable models for a vaccine to be tested in humans. Even injection of Freund's adjuvant alone, especially the complete, without any sperm antigen can cause some degree of antifertility effect in both the male and the female animals in these models. The mouse model is more acceptable, but it still has several concerns. No study has achieved 100% reduction after immunization with any of the antigens in the mouse model. The maximum reduction in fertility, after immunization with any antigen, is up to ~75%. It remains to be seen whether this reduction in fertility in the mouse model translates to a 100% reduction in humans. The female mouse ovulates several (over 20) eggs every cycle, and a woman ovulates typically one egg every cycle. Therefore, there are differences between the mouse and human. It is possible that ~75% reduction in fertility in the mouse model translates to a 100% block in humans. This may also be because of an inherent nature of the mouse model, where it is challenging to make mice completely infertile. How-

ever, after active immunization, one does find some mice that are totally infertile. Only a few, if at all, knockout/deletion of a single gene has shown complete infertility in male or female mice, without affecting any other cell/tissue function.^{16,17} Also, there are differences among various strains of mice.²⁴ The sperm from different strains of mice do not cross-fertilize oocytes from other strains. Using the same antigen, one can observe a different effect on fertility in different strains of vaccinated mice. It is pertinent to keep in mind that a mouse sperm antigen that shows an effect on fertility in vaccinated mice, its homologue/isologue may not necessarily affect fertility in humans. Although several genes may be evolutionarily conserved between mouse and man for sequence homology/identity, they may not be evolutionarily conserved for functional homology/identity. Mouse sperm do not bind to or penetrate human oocytes and *vice versa*. Thus, even if a sperm antigen shows a drastic reduction in fertility in the mouse model, its human homologue still needs to be tested in the human/subhuman primate model.

At the present time, no sperm antigen has undergone a phase I/II clinical trial in humans. Two studies have examined the effect of a sperm antigen vaccination in a non-human primate model. One study reported reduced fertility of female baboons after immunization with LDH-C₄.²² However, a study by another group found no effect on fertility in female monkeys after vaccination with LDH-C₄.³⁸ The reason for this discrepancy remains unclear. In another study, male monkeys were immunized with Eppin.²² After immunization, 78% of male monkeys, which developed high anti-Eppin antibody titers, became infertile, and in 71% of those monkeys fertility was recovered after immunization ceased. To maintain high antibody titers, booster injections with Freund's adjuvant have to be administered every 3 weeks for almost an entire duration (691 days) of the study. The potential immunopathologic effects of immunization were not investigated. These findings indicate that CV can work for both males and females. Sperm antigens have auto- as well as isoantigenic potentials.

For antisperm CV for males, ideally the vaccine should not affect spermatogenesis and sperm maturation or cause orchitis and epididymitis. The blood-testes barrier should remain intact. The circulating antibodies percolate into the male genital tract via rete-testis, vas deferens, seminal vesicle, and pros-

tate.³⁹ These antibodies in the semen are transferred to the female genital tract after intercourse and bind to sperm to exercise a contraceptive effect. Except for the few studies described above, most of the antisperm CV have been tested in female animals.

Local Genital Tract Immunity

One of the major challenges for the development of an effective antisperm CV is to induce high-titer antibodies locally in the genital tract, besides inducing high circulating titers in the serum. For an effective immunocontraceptive, both circulating and local antibodies are required. These antibodies have to be present throughout the various parts of the genital tract. The findings of immunofertility in humans and experimental data from active immunization studies in animal models indicate that ~10% of the circulating antibodies percolate from blood into the genital tract.⁸ The fertilization occurs at the ampulla-isthmus junction at the upper part of the genital tract. The antibodies can induce a contraceptive effect, by affecting sperm function through agglutination/immobilization/inhibiting sperm capacitation/acrosome reaction, when present in various parts of the genital tract. However, they must be present at the ampulla-isthmus junction if they are to exercise an effect at the sperm-zona/sperm-olemma binding and penetration sites. The female genital tract can also synthesize antibodies locally at several sites. However, our knowledge on the localization of various immune cells and components in various parts of the female genital tract is limited. Not much research has been performed in this area. Also, only a paucity of information is available on penetration of antibodies from circulation to different parts of the genital tract. Specific neutralizing immunoglobulins of the IgG isotype, when injected intravenously, have been shown to percolate into the vagina and protect female monkeys from vaginal challenge with human immunodeficiency virus (HIV)-1/simian immunodeficiency virus (SIV).^{40,41}

To understand the immune correlates of contraceptive protection and to develop effective vaccination strategies, an understanding of the mucosal immunology of the genital tract is vital. The female genital tract has immunological uniqueness and is different from other mucosal tracts. It has various components of mucosal immunity in different combinations from vagina to ovarian follicle, as examined in humans and animal models.^{8,42-45} Vagi-

nal mucosa is largely composed of stratified squamous epithelium with little or no organized lymphoid tissue, although plasma cells secreting IgA and IgG are present. Cervical secretions are rich in IgA, IgG, and secretory component. Endocervix has lamina propria with plasma cells that predominantly secrete IgA. Uterus is devoid of organized lymphoid tissues, but plasma cells secreting IgA and IgG are present. Oviducts have a few, if any, plasma cells in the submucosal tissues, although IgA and IgG have been detected in the secretions. It has been shown that the majority of immunoglobulins present in the genital tract of healthy fertile women enter via transudation from the circulation.⁸ Systemic immunization with sperm antigens induce not only circulating antibodies, but also antibodies in the vagina.³³ Also, the intranasal immunization with sperm antigens induce high-titer antibodies (both IgG and IgA) in the female genital tract besides inducing circulating antibodies.³⁴

Enhancement of Immunogenicity of CV

To increase the immunogenicity of CV, especially the immune response in the genital tract, various routes of delivery, adjuvants, carriers including multiple carriers, and virus-like particles, have been tried. The recent focus is primarily on two aspects: DNA vaccines and multi-epitope/multi-peptide vaccines.

DNA vaccines have been proposed as an exciting mode for vaccination and have several distinct advantages including easy manipulation, use of a generic technology, simplicity of manufacture, and chemical and biological stability.⁴⁶ DNA vaccines generally enhance a Th1 immune response. The cytokines produced after a Th1 immune response could inhibit gamete and embryo function as they express receptors for these cytokines.⁴⁷ In view of these findings, we conducted two studies to examine the effect of DNA vaccines based upon two sperm antigens, namely YLP₁₂⁴⁸ and FA-1.⁴⁹ Our data indicate that intradermal immunization of female mice using gene gun with the DNA vaccines, based on these sperm antigens, causes circulating and local immune responses resulting in immunocontraceptive effect. The effect was long-lasting up to at least 1 year of the observation period. The antifertility effect was augmented by using two in-frame CpG repeats or exogenous CpG oligodeoxynucleotide. Both Th1 and Th2 immune responses were induced

after DNA vaccination, with a preponderance of Th1 immune response. These are the first studies where contraceptive effect of sperm-specific cDNA vaccines was examined. To increase the efficacy of the vaccine, the constructs containing multiple repeats of the cDNAs, multiple repeats of in-frame CpG, and combined immunization of DNA and peptide vaccines are being investigated.

The present trend is to use synthetic peptides for CV development instead of whole recombinant molecules. Although synthetic peptides require conjugation with T-cell epitopes for immunogenicity, they are easy to synthesize in large amounts, are stable, and easy to manipulate. To enhance the immunogenicity, recently, multi-epitope/multi-peptide CV based on sperm proteins involving various steps of fertilization cascade,³⁶ and combination vaccines based upon sperm and zona pellucida antigens,⁵⁰ have been generated and examined for contraceptive effect in mice. The findings indicate the multi-epitope/multi-peptide vaccines enhance the contraceptive effect. Also, these vaccines are helpful in enhancing immunogenicity. The individuals who are low/non-responders to one antigen may respond to the other antigen in the multi-epitope/multi-peptide vaccine.

Passive Immunocontraceptive

There is variability of immune response among individuals after any vaccination. Incorporation of multiple sperm epitopes in combination vaccines and use of different carriers and adjuvants can obliterate this concern. Another approach to outstrip the variability is to use passive immunization employing preformed antibodies.⁵¹ The antibody therapies have been tried and proven to be successful against various infectious diseases, both in animals and in humans. Several of these antibodies have become treatment modalities in the clinics.⁵¹ The antibodies have to be of human origin if they are to be used for humans. The mouse monoclonal antibody can elicit strong anti-mouse antibody reaction; chimeric antibody can cause anti-chimeric response, and xenogenic complementarity-determining regions (CDRs) of humanized antibodies can also evoke an anti-idiotypic response, when injected into humans. Phage display technology has been widely used to obtain a variety of engineered antibodies, including single chain variable fragments (scFv) antibodies against several antigens. ScFv is an antibody fragment that plays a major role in the antigen-binding activity and is composed of

variable heavy (VH) and variable light (VL) chains connected by a peptide linker. The most widely used linker is (Gly₄Ser)₃. The affinity and stability of the scFv antibodies produced in bacteria are comparable with those of the native antibodies and are maintained by a strong disulfide bond. ScFv antibodies can be produced on a large scale using specially modified bacterial hosts and have an advantage over the whole immunoglobulin (Ig) molecule. ScFv antibodies lack the Fc portion that eliminates unwanted secondary effects associated with Fc and, because of its small size, they can be easily absorbed into the tissues as well as genetically manipulated.

Using the phage display technology and peripheral blood leukocytes (PBL) from ASA-positive immuno-infertile and vasectomized men, we have synthesized *in vitro*, for the first time, several human scFv antibodies that react specifically with well-defined sperm antigens that are involved in human sperm function.⁵² These clones are novel, have unique CDRs, and inhibit human sperm function in a concentration-dependant manner. Their contraceptive effect *in vivo* is being investigated. These scFv antibodies are very interesting because they are of *human* origin and related to *fertility/infertility*. At this time, no immunocontraceptive is available on the market. These antibodies may provide a novel once-a-month, the first of its kind, immunocontraceptive(s) for human use.

Conclusions

In conclusion, development of antisperm CV is an exciting proposition and may provide a viable alternative to other modalities of contraception. There is a strong rationale and recent data indicating that this proposition can translate into reality. During the last two decades, significant progress has been made in the field of antisperm immunocontraception. Several sperm antigens have been delineated, cloned, and sequenced that have function in sperm physiology and fertilization. Besides examining their role in immunocontraception, we have also gained knowledge regarding their development in the testes, gene regulation, and local and systemic immunogenicity pertinent to vaccine development. The utility of a sperm antigen in CV development is contingent upon sperm/testis-specificity, role in fertility, surface expression, and immunogenicity. The search for delineating such an antigen is extensively being performed in several laboratories. Finding such an

antigen will be a major breakthrough and will move the field forward. In contrast to other vaccines, such as against infections and cancer, a contraceptive vaccine will be used by healthy individuals. For a CV to be acceptable, it needs to be highly tissue-specific without any side effect and should provide close to full protection against pregnancy. It requires high bioefficacy and high tissue specificity. Also, the CV development is a multidisciplinary approach requiring expertise in several fields including vaccinology, immunology, molecular biology, cell biology, and reproductive endocrinology. This makes the development of CV more challenging than other vaccines. Antisperm CV need to be tested in primate models and human clinical trials. Both high systemic and local immune responses are required. Also, the inter-individual variability of immune response is a concern. The findings indicate that the multi-epitope vaccines can enhance the efficacy and obliterate the concern regarding inter-individual variability. This concern can also be addressed by the passive immunization approach using preformed human antibodies. Several antibodies are being tried as immunotherapeutic agents. At the present time, >100 antibodies are available in the market for various clinical conditions, including infectious diseases and cancer. Over 80% of these antibodies are genetically engineered.^{53,54} The human scFv antibodies that we have synthesized *in vitro* may provide useful, once-a-month immunocontraceptive. These human antibodies are sperm-specific and involved in human infertility. Their immunocontraceptive potential *in vivo* is presently being investigated.

Acknowledgements

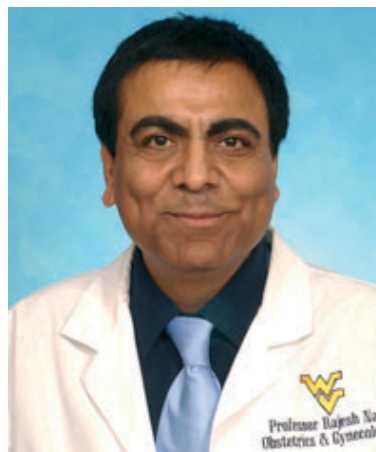
We sincerely thank Briana Shiley for providing excellent help in typing and preparing this manuscript. This work has been supported over the years by funds from the NIH and several other agencies.

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