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Antisperm Immunity for Contraception

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Andrology Lab Corner*

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Presently, there are only limited options available for family planning. Contraceptive modalities available to women include hormonal contraceptives (birth control pills, contraceptive patch, and hormone injections), natural methods (abstinence near ovulation and early withdrawal before ejaculation), intrauterine devices (IUDs; copper IUD, vaginal rings, diaphragm, and spermicidal combinations), and sterilization (Contraception online, 2004; Upadhyay, 2004; Harper, 2005). The contraceptive options available to men are vasectomy, condoms, and early withdrawal. Vasectomy is the most effective male contraceptive method currently available. However, vasectomy is a permanent procedure with a limited success rate of fertility reversal even after successful surgical reanastomosis (Silber and Grotjan, 2004). Therefore, for men who wish to father a child at a later time, condoms and withdrawal are the only contraceptive options. However, these two methods are either not readily acceptable or have high failure rates. Even with the available contraceptive methods, the world population exceeds 6.48 billion and will grow by 1 billion every 12 years at the present rate of population explosion (Anonymous World POP Clock Projection US Census Bureau, October 2005). In addition, unintended pregnancies continue to impose a major public health issue. In the United States alone, half the pregnancies are unintended, which results in more than 1 million elective abortions each year (Henshaw, 1998; Grow and Ahmed, 2000). In more than half of these unintended pregnancies, the women were using some method of contraception. The 2004 Institute of Medicine report indicates that between 1995 and 2000, more than one quarter of 1.2 billion pregnancies were unwanted (Institute of Medicine report, 2004). Thus, there is an urgent need for a better method of contraception that is reversible, nonsteroidal, nonbarrier, intercourse-independent, acceptable, and effective. The most important properties of an ideal contraceptive method desired by women are that it should be highly effective and safe, is inexpensive, has a prolonged duration of action, is rapidly reversible and easily accessible, requires infrequent administration, and can be used privately (Contraception online, 2004). A contraceptive vaccine (CV) can fulfill most of the properties of an ideal contraceptive. Since the developed nations and most of the developing nations have an infrastructure for mass immunization, the development of vaccines for contraception is an exciting proposition.

Various targets have been explored for the development of CVs. These broadly fall into three categories: vaccines inhibiting gamete production (gonadotropin-releasing hormone [GnRH], follicle-stimulating hormone [FSH], and leuteinizing hormone [LH]), gamete function (zona pellucida [ZP] proteins and sperm antigens), or gamete outcome (human chorionic gonadotropin [hCG]; Naz, 2005a; Naz et al, 2005). Advantages of GnRH-based vaccines are that they are effective in several species and can be used for both males and females. However, they affect sex steroids, causing impotency, thus, are not acceptable for human use. GnRH-based vaccines have been taken over by pharmaceutical companies for fertility control in domestic pets, farm animals, and wild animals, and for noncontraceptive purposes, such as prostatic hypertrophy and carcinoma (Ferro and Stimson, 1999; Simms et al, 2000). Advantages of FSH-based vaccines are that they can inhibit spermatogenesis in males of several species and can potentially provide a male contraceptive. However, FSH-based vaccines cause oligospermia rather than azoospermia. This incomplete efficacy has hindered further progress (Moudgal et al, 1997). The advantages of LH- or LH receptor–based vaccines are that they are effective in both males and females (Thau et al, 1987; Saxena et al, 2002). However, LH- or LH receptor–based vaccines affect sex steroids; therefore, they are not acceptable for humans. Disadvantages of CVs targeting gamete production are that they affect sex steroids and/

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or show only a partial effect in reducing fertility. Contraceptive vaccines targeting gamete functions are better choices. Vaccines based on ZP proteins are quite efficacious in producing contraceptive effects. However, they induce oophoritis, affecting sex steroids (Tung et al, 1999; Aitken, 2002). Vaccines based on ZP proteins have been successfully used for controlling wild and zoo animals, such as deer, horses, elephants, and dogs (Kirkpatrick and Turner, 2002). Sperm antigens constitute a promising and exciting target for CVs and, at the present time, no disadvantages are known. Vaccines targeting gamete outcome primarily focus on the hCG molecule. The hCG-based vaccines have undergone phase I and II clinical trials in women, which demonstrated efficacy and lack of immunopathology (Talwar et al, 1994). However, there was a variable immune response observed among vaccinated women. The present article will focus on the development of CVs based on sperm antigens.

Rationale for Sperm Vaccine Development

There is a strong rationale for the development of a sperm-based vaccine. The sperm cell is immunogenic in both males and females. Immunization of several species of animals and humans with sperm/testis preparations develop antisperm antibodies (ASA), leading to infertility (Menge, 1970; Allardyce, 1984). The following data provides a strong rationale for the development of a sperm CV for humans. In 1932, Baskin injected 20 fertile women, who had at least 1 previous pregnancy, with their husband's sperm (Baskin, 1932). These women developed ASA and no conception was reported for up to 1 year of observation. A US patent was issued for this spermatoxic vaccine in 1937 (US patent 2103240). More than 70% of men develop ASA after vasectomy (Liskin et al, 1983), and there is a limited success in the regain of fertility, even after successful surgical reanastomosis in vasovasostomy attributed to the presence of ASA (Silber and Grotjan, 2004). Up to 2% to 30% cases of infertility may be associated with the presence of ASA in the male or female partner of an infertile couple (Ohl and Naz, 1995). These ASA are causative factors of infertility, because disappearance of ASA cause regain of fertility (Bronson et al, 1984). These findings provide evidence that spermatozoa can generate an immune response in both men and women that can lead to a contraceptive state. However, the whole sperm cannot be used for the development of CV. There are numerous antigens present on the surface and internally in sperm that are shared with somatic cells. Thus, immunization with the whole sperm can cause immunopathological consequences in other tissues and organs. The usefulness of a sperm antigen is contingent on sperm-specific expression, surface expression accessible to antibody binding, and its role in fertilization/fertility. In addition, the sperm antigen, alone or after conjugation with an appropriate carrier protein, should be able to raise high titer and long-lasting antibody response in circulation and locally in the genital tract. If the sperm antigen is also involved in human immunoinfertility, then it is an especially attractive candidate. An ideal sperm antigen for immunocontraception should have tissue-specific expression on sperm surface, be involved in sperm-ZP binding, and be involved in human immunoinfertility.

Sperm Antigens

Various methodologies of genomics and proteomics have been used to delineate sperm antigens that have a role in fertilization/fertility and can be used for the CV development. Recently, using gene knockout technology, at least 93 novel testis/sperm genes/proteins have been identified that have a crucial role in various aspects of fertility (Naz and Rajesh, 2005a; Naz and Rajesh, 2005b). Some of these gene knockouts cause a defect in testis development and endocrine milieu, some in spermatogenesis, some in mating behavior, some in sperm structure/function/motility, and some in fertilization. The majority of these knockouts also demonstrated an effect on nonreproductive organs concomitant with an effect on fertility. We performed an extensive database analysis of these genes/proteins to examine how many of these have the characteristics required for the CV development, as discussed above. The knockouts of only a few genes/proteins induced a specific effect on fertility without a serious side effect. Further analysis of these genes/proteins indicated that majority are not expressed on the sperm surface, thus, are not amenable to antibody binding. Although these genes/proteins can provide ideal targets for pharmacological inhibition for contraception, they are not suitable for CV development. The gene knockout technology is a powerful approach to identify suitable novel targets, and the list of gene knockout mice is ever growing.

The molecules involved in sperm-oocyte membrane fusion have been actively examined for some time. Various candidates have been proposed, including DE, cluster of differentiation (CD)46, equatorin Sperad, and sperm acrosomal membrane-associated protein $(SAMP)_{32}$ (Stein et al, 2004). CD46 gene knockout mice do not show a defective sperm-oocyte fusion (Inoue et al, 2003). A *D*isintegrin *a*nd *M*etalloprotease (ADAM) family proteins have drawn a considerable attention because they have a putative fusion peptide ($ADAM_1$) and disintegrin domains ($ADAM_2$ and $ADAM_3$; Nishimura et al, 2004). However, $ADAM_1$, $ADAM_2$, and $ADAM_3$ gene knockout mice did not show a defect in sperm-oocyte membrane fusion, but did show an impairment in sperm-zona binding (Cho et al, 1998). CD9 present on the oocyte plasma membrane seems to be essential for fusion with the sperm cell (Le Naour et al, 2000). It was thought that integrins $\alpha 6$ and

β_1 present on sperm are involved in binding to oocyte CD9 for sperm-oocyte fusion (Almeida et al, 1995). However, gene knockout of these molecules did not inhibit fertility (He et al, 2003). Recently, a gene knockout was reported that is very interesting. The gene knockout mice of a sperm gene, designated as *Izumo*, are healthy, but all males are sterile (Inoue et al, 2005). *Izumo* is named after a Japanese shrine dedicated to marriage. The male mice produce normal-appearing sperm that bind to and penetrate the ZP but are incapable of fusing with the oocyte membrane. Human sperm also express Izumo protein. Izumo protein is not detectable on ejaculated sperm but becomes detectable after sperm cell undergoes acrosome reaction. Izumo antigen seems to be an interesting molecule, and its usefulness in the CV development needs to be investigated. Because it is not exposed until the sperm cell undergoes acrosome reaction, the antibodies have to be present at that particular time and space for binding to Izumo antigen. It is not clear at the present time whether the sperm-oocyte plasma membrane fusion event is a suitable target for immunocontraception (Naz et al, 2001).

Although several sperm genes/antigens have been delineated, cloned, and sequenced; and antibodies to some of these antigens affect sperm function/fertilization in vitro, only immunization with a few of them cause a contraceptive effect in vivo in any animal model. Notable among these are lactate dehydrogenase-C_4 (LDH-C_4; Goldberg and Herr, 1999), PH-20 (Primakoff et al, 1988), SP-17 (Lea et al, 1998), SP-10 (Herr et al, 1990), FA-1 (Zhu and Naz, 1997), and YLP_{12} (Naz et al, 2000). Most of these active immunization studies, except those related to the PH-20 antigen, were carried out in the mouse model. At the present time, no sperm antigen has undergone a phaseI/II clinical trial in humans. Two studies have examined the effect of sperm antigen vaccination in a nonhuman primate model. One study reported reduced fertility of female baboons after immunization with LDH-C_4 (O'Hearn et al, 1997). However, a study by another group found no effect on fertility in female monkeys after vaccination with LDH-C_4 (Tollner et al, 2002). The reason for this discrepancy is not clear. Recently, in an interesting study, male monkeys were immunized with an epididymal protein designated as epididymal protein inhibitor (Eppin; O'Rand et al, 2004). After immunization, 78% of monkeys who developed high anti-Eppin antibody titers became infertile, and 71% of those monkeys recovered fertility after immunization was stopped. To maintain high antibody titers, booster injections with Freund's adjuvant have to be administered every 3 weeks for almost the entire duration of the study, 691 days. The potential immunopathological effects of immunization were not examined. This interesting study indicates that anti-sperm CV can also be developed for men.

Antibodies to several sperm molecules inhibit sperm-oocyte interaction/fertilization in vitro. However, the active immunization with many of these molecules does not inhibit fertility in vivo. In addition, the gene knockouts of many of these molecules do not inhibit fertility. For example, although antibodies to fertilin/PH-30 inhibit fertilization in vitro (Stein et al, 2004), active immunization with fertilin/PH-30 does not affect fertility in vivo (Hardy et al, 1997). Similarly, although antibodies to sperm integrins $\alpha 6$ and β_1 inhibit sperm-oocyte fusion in vitro (Almeida et al, 1995), the gene knockouts of these molecules do not affect the fertility in vivo (He et al, 2003). These differences in in vitro and in vivo effects may be a result of 1) the class/subclass, valency, affinity, and kinetics of the antibodies generated in vivo; 2) the fact that antibodies have to be present in time and space to bind to the appropriate molecules; and/or 3) a possible redundancy of some of these molecules.

Another problem that the sperm vaccinologists are currently facing is finding an appropriate animal model to examine the efficacy of a sperm antigen. The most used animal model is the mouse. However, until now, no one has reported a 100% block in fertility after immunization with any single antigen in the mouse model. Even immunization with the whole sperm or their solubilized preparations does not cause a total block in fertility in mice, male or female. The maximum reduction in fertility after immunization with any antigen/sperm preparation is up to 70% to 75%. Very few, if any, knockouts of a single gene have made mice totally infertile. The recently reported *Izumo* gene knockout did make the male mice almost totally infertile (Inoue et al, 2005). It remains to be seen whether the 70% to 75% reduction in fertility in the mouse model translates to a 100% reduction in humans. The female mouse ovulates several (approximately 20–50) eggs every cycle and a woman ovulates mostly 1 egg every cycle. Therefore, there are differences between the mouse and human. It is possible that a 70% to 75% reduction in fertility in the mouse model translates to a 100% block in humans. It is possible that it is an inherent nature of the mouse model that it is difficult to make mice completely infertile. However, after active immunization or deleting a single gene, one does find a few mice that are totally infertile. There is no study at the present time that has examined the effect of immunization with more than 1 sperm antigen in the mouse model.

The phage display technology is a novel and innovative tool for delineating specific binding peptide sequences to various ligands and antibodies. It was first reported by George Smith in 1985 (Smith, 1985). This technology is currently being widely used in several laboratories. The peptide sequences are presented on the surface of filamentous phage to examine their interaction with specific ligands/antibodies. The DNA encoding any peptide sequence is incorporated into the genome of the phage cap-

sid protein, and the encoded peptide is expressed and displayed on the phage surface as a fusion protein. Recently, we used this technology to delineate the peptide sequences that are involved in human immunoinfertility, and the peptide sequences present on human sperm cells that are involved in binding to human ZP.

Using the antibody-positive sera from immunoinfertile men, seven clones that had dodecamer sequences in a random order, designated #2, #22, #69, #77, #81, #84, and #95, were identified in the phage display library (Naz, 2005b). These clones did not react with the antibody-negative sera from fertile men. The clones were sequenced (#2, PSALGRFTRGPL; #22, SLIFVTISSEWG; #69, LSLSLDLLTFRT; #77, PDIRHYFIQNRG; #81, GCRIVYRRPLHL; #84, RTAGFDIKLIDT; and #95, RIQYQAISTVSL) and the peptides were synthesized based on these 7 sequences and investigated for their immunoreactivity with sera from immunoinfertile and fertile men in the enzyme-linked immunosorbent assay. All of the 7 peptides, and especially 3 peptides (#22, #69, and #95), reacted strongly with the immunoinfertile sera, compared with the fertile sera. These 3 peptides reacted with 27% to 40% of the immunoinfertile sera for the IgG class, and two of these (#69 and #95) also showed a positive reaction with 27% of the sera for the IgA class. None of the peptides reacted positively with the fertile sera. These peptide sequences are novel, without any complete identity with any known sequence in the database. The sperm cell has several proteins on the surface, some are glycosylated. Many of these peptides may constitute partial or complete peptide mimetics of the carbohydrate epitopes present on sperm that are involved in immunoinfertility. The phage display technology seems to be a powerful tool to identify immunoinfertility-associated sperm antigens, both the peptide moieties as well as the carbohydrate mimetics of the peptide epitopes. In the past, several proteomic methodologies, immunologic techniques, and the two-dimensional gel electrophoresis/matrix-assisted laser desorption mass spectrometry have been used to delineate molecular identities of the sperm antigens that are involved in antisperm antibody-mediated immunoinfertility (Auer et al, 1995; Pillai et al, 1996; Shetty et al, 1999). Although a few interesting leads have been found, very few sperm antigens have been identified that are indeed involved in immunoinfertility. The FA-1 antigen was identified in our laboratory, and the complementary DNA (cDNA) encoding for the FA-1 antigen has been cloned and sequenced from the mouse (Zhu and Naz, 1997) and human testis (Naz and Zhu, 2002). The FA-1 antigen has been clearly shown to be associated with immunoinfertility (Naz et al, 1993). Antibodies to the FA-1 antigen are present in sperm, seminal plasma, and sera of immunoinfertile men, and in follicular fluid, cervical mucus, vaginal secretions, and sera of women. A clinical trial conducted at the University of Michigan Medical School indicates that the incubation of sperm from immunoinfertile men with the FA-1 antigen removes autoantibodies from the sperm surface, resulting in an increase in the antibody-free sperm, and enhanced acrosome reaction rates (Menge et al, 1999). The intrauterine insemination of FA-1 antigen-adsorbed antibody-free sperm caused normal pregnancies and healthy babies, indicating that the antigen treatment does not have deleterious effects on implantation or on embryonic and fetal development. Thus, the FA-1 antigen, along with other sperm antigens that are involved in immunoinfertility, may find application in immunotherapy of immunoinfertility, besides having application in the specific diagnosis.

Using the solubilized preparation of human ZP as a probe, a dodecamer sequence (YLPVGGLRRIGG), designated as YLP_{12}, present on human sperm, was identified using the phage display library (Naz et al, 2000a). An extensive computer search in the database did not reveal any known sequence with a complete identity or significant homology with YLP_{12}. Antibodies to synthetic YLP_{12} peptide inhibit human sperm-human zona binding. YLP_{12} recognizes the ZP3 component of human ZP. The YLP_{12} peptide sequence was localized primarily on the acrosome and tail regions of humans and murine sperm (Naz et al, 2000). This peptide is also involved in human immunoinfertility. To examine the immunocontraceptive potential, a vaccine was prepared by conjugating the YLP_{12} peptide with the recombinant cholera toxin B subunit (rCTB; Naz and Chauhan, 2002). The YLP_{12} peptide-rCTB vaccine caused a significant inhibition of infertility (up to 71%) in vaccinated female mice by raising a testis/sperm-specific immune response. The fertility reversed after approximately 10 months, when the antibodies disappeared from the circulation and genital tract. The fertility could also be reversed voluntarily at any given time by neutralizing the antibodies, which is done by administering the unconjugated peptide in the blood and/or in the vagina.

Besides antibodies, various cytokines can also affect sperm function and fertility, positively or negatively. For example, interferon-γ and tumor necrosis factor-α can negatively affect sperm motility and function (Naz and Kumar, 1991), and interleukin-6 can enhance sperm capacitation and acrosome reaction (Naz and Kaplan, 1994). Spermatozoa have receptors for many of these cytokines, such as interferon-γ and interferon-α (Naz et al, 2000b). These factors are present in the seminal plasma, and the levels are modulated to various degrees in infertility. Immunization with whole-sperm preparations or specific sperm antigens can raise many cytokines in addition to antibodies that can affect sperm function (Naz and Mehta, 1989). DNA vaccines have been extensively used to generate protective immunity against infectious agents and

tumors (Liu, 2003; Patil et al, 2005). DNA vaccination represents one of the recent advancements in vaccine technology. DNA vaccines have distinct advantages over the peptide vaccines because they have potential to generate both T helper cell subsets 1 and 2 immune responses. Based on these facts, a DNA vaccine was prepared by cloning YLP_{12} cDNA into a pVAX1 DNA vector. The female mice were immunized intraderminally with the YLP_{12} DNA vaccine via a gene gun. The DNA vaccination caused a long-term contraception in female mice (unpublished data).

The progress in the development of CVs against various targets, including sperm, has been hampered by the following factors: 1) difficulty in delineating the appropriate fertility-related antigen(s), 2) variability of the immune response among the vaccinated individuals, 3) attainment and maintenance of high titers of antibodies for bioefficacy, 4) time lag to achieve reasonably good antibody titers after the first injection, and 5) uncertainty regarding how long the antibody titer will remain in the circulation to exercise the contraceptive effects. The last 4 concerns are associated with the active immunization studies involving CVs. It is envisaged that these 4 concerns may be taken care of using the passive immunization approach (Naz and Rajesh, 2004). The passive immunization approach has been successful for protection against various immunologic and infectious diseases (Casadevall, 1999; Zeitlin et al, 2000). These studies have indicated that administration of homologous antibody preparations, especially the Fabs, are devoid of any side effect and immunopathological consequences. The bioefficacy of the passive immunization depends on the class/subclass of immunoglobulins and the route of antibody administration. Human IgG has a half-life of 21 days. Recently, there has been a growing interest to humanize the murine monoclonal antibodies or to raise human monoclonal antibodies against various sperm contraceptive epitopes (Isojima et al, 1987; Clayton et al, 1998; Norton et al, 2001). These antibodies, and additional antibodies that are being engineered, await trials in animal models and in humans for demonstration of efficacy, duration, and reversibility of the antifertility effects. The data from antibody therapies, including clinical trials in infectious diseases, indicate that it is an exciting, practical, viable, and durable proposition ready for experimentation.

In conclusion, a CV targeting sperm is an exciting proposition. The strong rationale and the progress made during the last several years indicate that it can culminate in a viable and successful reality. The immunologic approaches to contraception have been a priority of the World Health Organization since 1973, and were highly recommended by the Institute of Medicine in its 1996 report.

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References

Aitken JR. Immunocontraceptive vaccines for human use. *J Reprod Immunol.* 2002;57:273–287.

Allardyce RA. Effect of ingested sperm on fecundity in the rat. *J Exp Med.* 1984;159:1548–1553.

Almeida EAC, Huovila APJ, Sutherland AE, Stephens LE, Calarco PG, Shaw LM, Mercurio AM, Sonnenberg A, Primakoff P, Myles DG, White J. Mouse egg intergin $\chi_6\beta_1$ functions as a sperm receptor. *Cell.* 1995;81:1095–1104.

Anonymous World POP Clock Projection US Census Bureau. 2005. Available at: http://www.factfinder.census.gov. Accessed December 2, 2005.

Auer J, Pignot-Paintrand I, De Almeida M. Identification of human sperm surface glycoproteins by sperm-membrane specific autoantibodies. *Hum Reprod.* 1995;10:551–557.

Baskin MJ. Temporary sterilization by injection of human spermatozoa: a preliminary report. *Am J Obstet Gynecol.* 1932;24:892–897.

Bronson RG, Cooper G, Rosenfeld D. Sperm antibodies: their role in infertility. *Fertil Steril.* 1984;42:171–182.

Casadevall A. Passive antibody therapies: progress and continuing challenges. *Clin Immunol.* 1999;93:5–15.

Cho C, Bunch DO, Faure JE, Goulding EH, Eddy EM, Primakoff P, Myles DG. Fertilization defects in sperm from mice lacking fertilin beta. *Science.* 1998;281:1857–1859.

Clayton R, Cooke ID, Partridge LJ, Moore HDM. A combinatorial phage display for the generation of specific Fab fragments recognizing human spermatozoa and inhibiting fertilizing capacity in vitro. *Biol Reprod.* 1998;59:1180–1186.

Contraception online. Baylor College of Medicine, Houston, Texas. 2004. Available at: htttp://www.contraceptiononline.org/. Accessed December 2, 2005.

Ferro VA, Stimson WH. Antigonadotropin releasing hormone vaccines and their potential use in treatment of hormone responsive cancers. *Bio Drugs.* 1999;12:1–12.

Goldberg E, Herr JC. LDH-C_4 as a contraceptive vaccine. In: Gupta S, ed. *Reproductive Immunology*. New Delhi, India: Narosa Publishing House; 1999:309–315.

Grow DR, Ahmed S. New contraception methods. *Obstet Gynecol Clin North Am.* 2000;27:901–916.

Hardy CM, Clarke HG, Nixon B, Grigg JA, Hinds LA, Holland MK. Examination of the immunocontraceptive potential of recombinant rabbit fertilin subunits in rabbit. *Biol Reprod.* 1997;57:879–886.

Harper MJK. In search of a second contraceptive revolution. *Sex, Reprod Menopause.* 2005;3:59–67.

He ZY, Brakebusch C, Fässler R, Kreidberg JA, Primakoff P, Myles DG. None of the integrins known to be present on the mouse egg or to be ADAM receptors are essential for sperm-egg binding and fusion. *Dev Biol.* 2003;233:204–213.

Henshaw SK. Unintended pregnancy in the United States. *Fam Plann Perspect.* 1998;30:24–29.

Herr JC, Flickinger CJ, Homyk M. Biochemical and morphological characterization of intra-acrosomal antigen SP-10 from human sperm. *Biol Reprod.* 1990;42:181–189.

Inoue N, Ikawa M, Nakanishi T, Matsumoto M, Nomura M, Seya T, Okabe M. Disruption of mouse CD46 causes an accelerated spontaneous acrosome reaction in sperm. *Mol Cell Biol.* 2003;23:2614–2622.

Inoue N, Ikawa M, Isotani A, Okabe M. The immunoglobin superfamily

protein izumo is required for sperm to fuse with eggs. *Nature.* 2005; 434:234–238.

Institute of Medicine. *New Frontiers in Contraceptive Research. A Blue Print for Action.* Nass SJ, Strauss JF III, eds. Washington, DC: National Academies Press; 2004.

Isojima S, Kameda K, Tsuji Y, Shigeta M, Ikeda Y, Koyama K. Establishment and characterization of a human hybridoma secreting monoclonal antibody with high titers of sperm immobilizing and agglutinating activities against human seminal plasma. *J Reprod Immunol.* 1987;10:67–78.

Kirkpatrick JF, Turner J. Reversibility of action and safety during pregnancy of immunization against porcine zona pellucida in wild mares (Equus caballus). *Reprod Suppl.* 2002;60:197–202.

Lea IA, Van Lierop MJC, Widgren EE, Grootenhuis A, Wen Y, Van Duin M, O'Rand MG. A chimeric sperm peptide induces antibodies and strain-specific reversible infertility in mice. *Biol Reprod.* 1998;59: 527–536.

Le Naour F, Rubinstein E, Jasmin C, Prenant M, Boucheix C. Severely reduced female fertility in CD_9-deficient mice. *Science.* 2000;287: 319–321.

Liskin L, Pile JM, Quillan WF. Vasectomy safe and simple. *Popul Rep.* 1983;4:61–100.

Liu MA. DNA vaccines: a review. *J Int Med.* 2003;253:402–410.

Menge AC. Immune reactions and infertility. *J Reprod Fertil Suppl.* 1970; 10:171–185.

Menge AC, Christman GM, Ohl DA, Naz RK. Fertilization antigen-1 removes antisperm autoantibodies from spermatozoa of infertile men and results in increases rates of acrosome reaction. *Fertil Steril.* 1999; 71:256–260.

Moudgal NR, Murthy GS, Prasanna Kumar KM, Martin F, Suresh R, Medhamurthy R, Patil S, Sehgal S, Saxena BN. Responsivness of human male volunteers to immunization with ovine follicle stimulating hormone vaccine: results of a pilot study. *Hum Reprod.* 1997;12: 457–463.

Naz RK. Contraceptive vaccines. *Drugs.* 2005a;65:593–603.

Naz RK. Search for peptide sequences involved in human antisperm antibody-mediated male immunoinfertility by using phage display technology. *Mol Reprod Dev.* 2005b;72:25–30.

Naz RK, Ahmad K, Menge AC. Antiidiotypic antibodies to sperm in sera of fertile women that neutralize antisperm antibodies. *J Clin Invest.* 1993;92:2331–2338.

Naz RK, Chauhan SC. Human sperm-specific peptide vaccine that causes long-term reversible contraception. *Biol Reprod.* 2002;62:318–324.

Naz RK, Chauhan SC, Rose LP. Expression of alpha and gamma interferon receptors in the sperm cell. *Mol Reprod Dev.* 2000b;56:189–197.

Naz RK, Gupta SK, Gupta JC, Vyas HK, Talwar GP. Recent advances in contraceptive vaccine development. *Hum Reprod.* 2005;20:3271–3283.

Naz RK, Kaplan P. Interleukin-6 enhances the fertilizing capacity of human sperm by increasing capacitation and acrosome reaction. *J Androl.* 1994;15:228–233.

Naz RK, Kumar R. Transforming growth factor β_1 enhances expression of 50 kDa protein related to 2′–5′ oligoadenylate synthetase in human sperm. *J Cell Physiol.* 1991;146:156–163.

Naz RK, Leslie MH, Zhu X. Inhibition of murine sperm-oolemma binding by antibodies to an oocyte membrane (OM) antigen: implication in contraceptive vaccine development. *Am J Reprod Immunol.* 2001; 45:52–61.

Naz RK, Mehta K. Cell-mediated immune responses to sperm antigens: effects on mouse sperm and embryos. *Biol Reprod.* 1989;41:533–542.

Naz RK, Rajesh C. Passive immunization for immunocontraception: lessons learned from infectious diseases. *Front Biosci.* 2004;9:2457–2465.

Naz RK, Rajesh C. Novel testis/sperm-specific contraceptive targets identified using gene knockout studies. *Front Biosci.* 2005a;10:2430–2446.

Naz RK, Rajesh P. Gene knockouts that cause female infertility: search for novel contraceptive targets. *Front Biosci.* 2005b;10:2447–2459.

Naz RK, Zhu X. Molecular cloning and sequencing of cDNA encoding for human FA-1 antigen. *Mol Reprod Dev.* 2002;63:256–268.

Naz RK, Zhu X, Kadam AL. Identification of human sperm peptide sequence involved in egg binding for immunocontraception. *Biol Reprod.* 2000;7:21–26.

Nishimura H, Kim E, Nakanishi T, Baba T. Possible function of the ADAM1a/ADAM2 fertilin complex in the appearance of $ADAM_3$ on the sperm surface. *J Biol Chem.* 2004;279:34957–34962.

Norton E, Diekman AB, Westbrook VA, Flickinger CJ, Herr JC. RASA, a recombinant single chain variable fragment (scFv) antibody directed against the human sperm surface: implications for novel contraceptives. *Hum Reprod.* 2001;16:1854–1860.

O'Hearn PA, Liang ZG, Bambra CS, Goldberg E. Colinear synthesis of an antigen-specific B-cell epitope with a promiscuous tetanus toxin T-cell epitope: a synthetic peptide immunocontraceptive. *Vaccine.* 1997;15:1761–1766.

Ohl D, Naz RK. Infertility due to antisperm antibodies. *J Urol.* 1995;46: 591–602.

O'Rand MG, Widgren EE, Sivashanmugam P, Richardson RT, Hall SH, French FS, VandeVoort CA, Ramachandra SG, Ramesh V, Jagannadha Rao A. Reversible immunocontraception in male monkeys immunized with eppin. *Science.* 2004;306:1189.

Patil SD, Rhodes DG, Burgess DJ. DNA-based therapeutics and DNA delivery systems: a comprehensive review. *Am Assoc Pharm Sci J.* 2005;7:E61–E77.

Pillai S, Wright DR, Gupta A, Zhou G, Hull G, Jiang H, Zhang H. Molecular weights and isoelectric points of sperm antigens relevant to autoimmune infertility in men. *J Urol.* 1996;155:1928–1933.

Primakoff P, Lathrop W, Woolman L, Cowan A, Myles D. Fully effective contraceptive in male and female guinea pigs immunized with the sperm protein PH20. *Nature.* 1988;335:543–547.

Saxena BB, Clavio A, Singh M, Rathnam D, Bukharovich Y, Reimers T Jr, Saxena A, Perkins S. Modulation of ovarian function in female dogs immunized with bovine luteinizing hormone receptor. *Reprod Domest Anim.* 2002;37:9–17.

Shetty J, Naaby-Hansen S, Shibahara H, Bronson R, Flickinger CJ, Herr JC. Human sperm proteome: immunodominant sperm surface antigens identified with sera from infertile men and women. *Biol Reprod.* 1999;61:61–69.

Silber SJ, Grotjan HE. Microscopic vasectomy reversal 30 years later: a summary of 4010 cases by the same surgeon. *J Androl.* 2004;25:845–859.

Simms, MS, Scholfield DP, Jacobs E., Michaeli D, Broome P, Humphreys JE, Bishop NK. Anti-GnRH antibodies can induce castrate levels of testosterone in patients with advanced prostate cancer. *Br J Cancer.* 2000;83:443–446.

Smith G. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science.* 1985;228:1315–1317.

Stein KK, Primakoff P, Myles D. Sperm-egg fusion: events at the plasma membrane. *J Cell Sci.* 2004;117:6269–6274.

Talwar GP, Singh O, Pal R, Chatterjee N, Sahai P, Dhall K, Kaur J, Das SK, Suri S, Buckshee K, Saraya L, Saxena BN. A vaccine that prevents pregnancy in women. *Proc Natl Acad Sci U S A.* 1994;91:2532–2536.

Thau RB, Wilson CB, Sundaram K, Phillips D, Donnelly T, Halmi NS, Bardin CW. Long-term immunization against the beta-subunit of ovine luteinizing hormone (oLH beta) has no adverse effects on pituitary function in rhesus monkeys. *Am J Reprod Immunol Microbiol.* 1987;15:92–98.

Tollner TL, Overstreet JW, Branciforte D, Primakoff PD. Immunization of female cynomolgus macaques with a synthetic epitope of sperm-specific lactate dehydrogenase results in high antibody titers but does not reduce fertility. *Mol Reprod Dev.* 2002;62:257–264.

Tung KSK, Lou Y, Bagavant H. Zona pellucida chimeric peptide vaccine. In: Gupta S, ed. *Reproductive Immmunology*. New Delhi, India: Narosa Publishing House; 1999:303–308.

Upadhyay U. New contraceptive choices. *Population Reports Special Topics.* Series M, No. 19. Baltimore: Johns Hopkins Bloomberg School of Public Health, The INFO Project; 2004.

Zeitlin L, Cone RA, Moench TR, Whaley KJ. Preventing infectious diseases with passive immunization. *Microbes Infect.* 2000;2:701–708.

Zhu X, Naz RK. Fertilization antigen-1: cDNA cloning, testis specific expression, and immunocontraceptive effects. *Proc Natl Acad Sci U S A.* 1997;94:4704–4709.