Review Article

Immunocontraceptives: How far from reality?

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Abstract

Despite high expectations of safer, effective, economical, longer acting contraceptives, to date, there are no licensed contraceptive vaccines available in the market. Nevertheless, a role for vaccines undoubtedly exists as an aid to birth spacing and as a nonsurgical means of generating sterility. The research concerned in the area so far has been successful on the feline population, with room still for exhaustive studies on humans. The future of contraceptive vaccines holds great promise in terms of comfort, price, efficacy, rare complications, and possibly nonselective action on animal populations as well as on humans. This brief review deals with the basic aspects of immunocontraceptives along with the efforts done so far. There is a need for further research in aspects involving the rate of evolution of contraception resistance based on genetics, resistance phenotypes, or cross generation effects. Gonadotropin-releasing hormone and luteinizing-hormone have not been investigated in humans, as both reported impotency in animals; the follicle-stimulating hormone has been shown to cause oligospermia; zona pellucida has also not been studied in humans as it causes irreversible oophoritis, while the sperm has the potential for success in humans based on the data from immunoreproductive studies. Even as the position of the human chorionic gonadotropin vaccine looks hopeful, research on other possible targets continue with an eventual aim of discovering a vaccine that is more immunogenically effective.

Key Words: Immunocontraceptives, reproductive health, sperm antigens, zona pellucida antigens

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INTRODUCTION

The world's population surpasses the 6.891 billion mark and is increasing by one billion every twelve years.[1] Annually, worldwide, approximately 80 million women face unintended or unwanted pregnancies, and 45 million of these face abortions.[2] Undoubtedly, there is a call for a better method of contraception worldwide. About 48.2% of the couples practice family planning methods in India.[3] Overpopulation has been an overwhelming cry with limited resources for the country. The Government of India adopted the National Family Planning Program for development of newer technologies in this field.[4,5]

The Department of Biotechnology and the Indian Council of Medical Research, since the mid-seventies, are trying to develop birth control vaccines, which would be effective for both sexes.[6] Despite high expectations of safer, effective, economical, longer acting contraceptives, to date, there are no licensed contraceptive vaccines available in the market.[7] Nevertheless, the role of vaccines undoubtedly exists as an aid to birth spacing and as a nonsurgical means of generating sterility.[8]

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Indian scientists have made noteworthy contributions in exploring the possibilities to develop an effective and safe contraceptive vaccine. Achieving contraception by means of a vaccine is an original approach, which demands initiation of a specific antibody response against antigens critically involved in the process of mammalian reproduction.[5] This review deals with the basic aspects of immunocontraceptives along with the efforts done so far for humans.

The contraceptive vaccines lead to generation of a humoral and/or cell-mediated immune response against antigens that has a critical role to play in the reproductive process. These vaccines can be designed to inhibit (i) production of gametes (spermatozoa and oocyte), (ii) functions of gametes (obstructing fertilization), and (iii) the gamete outcome (pregnancy).[9]

Certain desirable properties of contraceptive vaccines make this approach a potentially attractive option for family planning programs both in developed and developing countries. Economic production, easy usage, tolerance, reversibility, less failures, and freedom from mechanical devices or exogenous hormones, make this approach an attractive option for family planning programs.[10] Moreover infrastructure for mass immunization in both the developed and most of the developing nations makes this concept an exciting proposition.[11]

The sperm was the first target for immunocontraception in 1899 when Nobel Prize winner Landsteiner and Metnikoff independently explored the antibody response to sperm injection, and later, in early 1900, its role in infertility was pronounced.[12,13] In 1937, Morris J. Baskin a surgeon from Denver was issued the US patent for a spermotoxic vaccine, which produced reversible sterilization in fertile women.[14]

MATERIALS AND METHODS

A systematic review aimed to provide an exhaustive summary of literature relevant to immunocontraceptive research on humans was done. A thorough search of literature from relevant articles was done by searching the citation indices, such as Medscape, Science Direct, Pubmed, Biomed Central, Cochrane database, and Web MD. The keywords used in the search were, 'contraceptive vaccines', 'immunocontraceptives', and 'research in humans'. Next, the titles and abstracts of the identified articles were checked for eligibility and relevance pertaining to studies done so far in the field of human need for contraceptive vaccines. The objective of the review was to bring forth various aspects of contraceptive vaccines and a brief compilation of the research done so far as well as its future prospects.

Targets and types of immunocontraception

Contraceptive vaccines can be broadly classified into three categories:[11]

- Vaccines targeting gamete production: The gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH), and luteinizing hormone (LH)
- Gamete function: Sperm antigens, zona pellucida (ZP) antigens
- Gamete outcome: Human chorionic gonadotropin (hCG) or into two categories based on,
- Hormone and receptor molecular targets (GnRH, LH, FSH, hCG)
- Gamete-associated molecular targets (sperm and ZP antigens).

Hormone and receptor molecular targets

Antibodies of appropriate specificity are able to block the action of hormones that are requisite for successful reproduction. Hence, if immunization using such hormones can provoke adequate titers of bioneutralizing antibodies in sexually mature individuals, the vaccinee becomes infertile ('immunocontraception') for as long as sufficient titers of antibodies are retained.[15]

The GnRH immunocontraceptive has undergone various veterinary trials to control feral animal populations by immunocastration.[16] In humans, a clinical trial has been conducted in postpartum women to prolong anovulation, also in healthy men, as well as in men with prostatic cancer.[17] The Phase I clinical trial in normal men for the FSH immunocontraceptive to assess immunogenicity and the effect on spermatogenesis has been completed. The prototype preparation was found to be only weakly immunogenic, with oligospermia (reduction in number) and oligoaesthenia (decreased motility), but no significant effect on the semen parameters.[18]

Anti-hCG vaccines terminate early pregnancy by preventing the maternal recognition of pregnancy.[19] Several types and formulations of hCG-based immunocontraceptives have been studied extensively in preclinical studies and clinical trials sponsored by: The National Institute of Immunology (Delhi, India), Population Council (New York, USA), and the World Health Organization (Geneva, Switzerland). A combined venture by the Population Council, New York, and the National Institute of Immunology (NII) has brought forth an anti-hCG vaccine, based on the complete beta subunit of the hormone (ß-hCG).[20,21] The other type of anti-hCG vaccine, built with the WHO Task Force's aid for the Vaccines for Fertility Regulation, is based on a portion (the carboxy-terminal peptide or CTP) of the beta subunit of the hormone (ß-hCG-CTP).[22-24]

The NII has supported research on a female contraceptive vaccine, based on β-hCG, which demonstrated for the first time that it was feasible to regulate fertility by such an approach.[5] They operated by preventing or interrupting pregnancy at the peri-implantation stage, probably by neutralizing the luteotrophic effect of hCG. The most advanced vaccine targeted the unique C-terminal peptide on the β-subunit of hCG, stimulating specific antibodies for hCG, and did not cross-react with the human luteinizing hormone (hLH).[7,25] Study for enhanced immunogenicity of a contraceptive vaccine using diverse synthetic carriers, with a permissible adjuvant for diverse population, has been done at the NII.[26]

These vaccines may also find applications in clinical situations that require inhibition of increased secretion of sex steroids, such as, uterine fibroids, polycystic ovary syndrome, endometriosis, and precocious puberty.[27]

Scientists at IISc, Bangalore, have immunized male monkeys with ovine FSH (oFSH) that has led to the generation of anti-oFSH antibodies. In females, FSH helps in the growth of ovarian follicles, and in males it regulates the growth of seminiferous tubules and spermatogenesis. Of late, it has been found to cause oligospermia in men. In India, three major programs on contraceptive vaccines, based on the beta-subunit of human chorionic gonadotropin (beta-hCG) for females, the ovine follicle stimulating hormone (oFSH) for males, and the riboflavin carrier protein for both sexes have been initiated.[5]

The contributions of Indian scientists toward the development of immunocontraceptives for women are considerable. The beta-human chorionic gonadotropin (beta hCG) vaccine developed by Talwar (1997) was the first contraceptive vaccine to be clinically tested in the world and found to be safe for use in women. Phase II trials using the hetero-species, dimer hCG vaccine showed high efficacy with one pregnancy in 1224 cycles.[28] The first ever contraceptive vaccine to be tested in a male was the heterologous follicle stimulating hormone (FSH) vaccine developed by Moudgal *et al.* (1997), which was proven to cause infertility in monkeys, and Phase I trials had unequivocally shown that the vaccine did not cause any ill-effects in men and also that some of the important semen characteristics were altered in a manner normally seen in infertile men.[29] Karande and Adiga (1997) took a very innovative approach to use an evolutionarily conserved vitamin transport protein as a contraceptive vaccine, because this protein was vital for the survival of the fetus. Animal studies showed that the vaccine was effective in reducing fertility in both sexes of mice and monkeys. The site and mechanism of action of the riboflavin carrier protein (RCP) vaccine, however, differed between the sexes.[30] A biodegradable system using microspheres for delivery of the vaccine has been reported (Singh *et al.* 1995).[31]

The gamete outcome vaccine targeting the hCG molecule has undergone successful Phase I and II clinical trials in women and demonstrated both efficacy and lack of immunopathology. The study is in progress to increase its immunogenicity and efficacy not only as a birth control vaccine, but also for its clinical applications in various hCG producing cancers.[11]

Hitherto several forms of hCG vaccines have successfully undergone both phase I and phase II clinical trials in women, demonstrating encouraging outcomes. In 1994, Talwar *et al.* completed their study, reporting that women developed antibody titers that prevented pregnancy. Essential and pregnancy-specific factors (cytokines/chemokines/growth factors/others) can be potential targets for contraception. Apart from hCG, the leukemia inhibiting factor (LIF) and pre-implantation factor (PIF) are known to be unique and promising molecules. Multi-epitope vaccine combining factors/antigens involved in various steps of the fertilization process and establishment of pregnancy may assure immunogenic and efficacious human contraception.[32] The current research for humans is focused on delineating infertility-related epitopes (B-cell epitopes) and oophoritis-inducing epitopes (T-cell epitopes).[11]

Gamete-associated molecular targets

Some cell surface antigens are unique, tissue-specific, immunogenic, and accessible to antibodies:

- Zona-pellucida antigens: The zona pellucida (ZP) glycoproteins have been proposed as candidates for developing contraceptive vaccines by virtue of their critical role in fertilization.[33] The zona pellucida, an acellular gelatinous layer, surrounds the mammalian oocyte and pre-implantation embryo. This layer breaks away just before implantation by the uterine proteolytic activity or ovum's hatching mechanism or both. It can prevent conception without acting as an abortifacient.[34] ZP vaccines focus on what may be the ideal target – the translucent glycoprotein extracellular matrix, enclosing all mammalian ova that a sperm must penetrate to achieve fertilization. As the zona-specific antigens develop at the stage of the secondary follicle and persist till its shedding during implantation, there is ample opportunity for the specific circulating antibodies to bind with the zona antigens. Antibodies raised against zona can inhibit infertility, presumably by preventing sperm attachment and passage through the zona.[35]

Zona pellucida vaccines were being considered for human contraceptive use long before Kirkpatrick's research group adapted it for wildlife.[36] ZP plays key roles in folliculogenesis, fertilization, and early embryogenesis, comprising of potent cell-specific antigens. The induction of fertility requires high ZP antibody titers, which are difficult to maintain without inducing ovarian pathology, characterized by a premature loss of primordial follicles. As premature menopause will be a high price to pay for long-term contraception, such a move toward a vaccine cannot evolve until the cause of ovarian pathology has been resolved.[37]

Around the time it gained favor with wildlife biologists, researchers in quest of human relevance were becoming rather disheartened. Native pig protein was inappropriate for human application, and researchers were unable to bring up an effective recombinant or synthetic form of human ZP. Although the protein backbone of the molecule could be successfully synthesized by molecular biologists, they failed in synthesizing the carbohydrate components. On account of the lack of carbohydrates, the vaccine would trigger an immune response, but could not block fertilization.[36] The current research for human applicability is focused on delineating infertility-related epitopes (B-cell epitopes) from oophoritis-inducing epitopes (T-cell epitopes).[27] However, the techniques to overcome the observed oophoritis associated with the ZP-based contraceptive vaccines are yet to be fully defined.[7,38]

- Sperm antigens: Contraceptive vaccines targeting sperms are an exciting proposition, as anti-sperm contraceptive vaccines and genetically engineered human antibodies can be used as immunocontraceptives.[39] Sperms have both auto- and isoantigens, and hence, can produce antibodies in either sex. Anti-sperm antibodies (ASA) upset fertilization and fertility both *in vitro* and *in vivo*.[40] The presence of ASA in the in vitro fertilization medium blocks fertilization. Purposeful immunization of both sexes of various species,[41] including humans, with sperms or their extracts, triggers the development of ASA leading to infertility.[42] Up to 70% of vasectomized men produce ASA[39] and 2-30% of the cases of infertility may be associated with the presence of ASA in the male and/or female partner of an infertile couple.[43] Thus, sperms can generate an immune response that is capable of inducing a contraceptive state. Hybridoma, recombinant DNA (deoxyribonucleic acid) technologies, and diverse proteomic and genomic line of approaches have been used by several laboratories to search for sperm-specific antigens that can be used for contraceptive vaccine development.[44] Multi-epitope contraceptive vaccines and human single chain variable fragment (scFv) antibodies from immunoinfertile and vasectomized men may eliminate the concern related to inter-individual variability of the immune response.[39]

Much of the research study at present is being carried out on spermatozoa.[45] Anti-sperm contraceptive vaccines and genetically engineered human (single chain variable fragment (scFv)) antibodies can be used as immunocontraceptives.[36,46] Various methods of proteomics and genomics, hybrid cell line formation technology, substractive complementary DNA libraries, differentialdisplay method, and phage display technology, are used to obtain sperm-specific genes and proteins, their efficacy enhanced with the multi-epitope combination vaccine.[47]

Several novel sperm/testis cDNA/antigens involved in various stages of fertilization have been delineated, cloned, and sequenced by using these techniques. Important among them are FA-I,[48] YLP_{12},[49] CV20,[50] and TSA-1.[51] Others are LDH-C4 (lactate dehydrogenase isoenzyme specific for spermatocytes, spermatids and spermatozoa),[52] P10G,[53] A9D,[54] SP56,[55] Epididymal protein inhibitor (Eppin),[56] and Izumo.[57]

CONCLUSION

The development of contraceptive vaccines is still at the research stage. The WHO records suggest that over 120 million pairs still have an unmet need for contraception and about 45 million pregnancies are terminated every year worldwide. The future of contraceptive vaccines holds great promise in terms of comfort, price, efficacy, complications, and possibly non-selective action in animal populations as well as in humans. This possibility comes as a result of development in the technology of recombinant DNA and creating new microorganisms, which may express certain antigens. GnRH and LH have not been investigated in humans, as both have reported impotency in animals; FSH has been shown to cause oligospermia; ZP also has not been studied in humans, as it causes irreversible oophoritis, while the sperm has a potential for success in humans, based on the data from immunoreproductive studies. Even as the position of the hCG vaccine looks hopeful, research on other possible targets continue with an eventual aim of discovering a vaccine that is more immunogenically effective.

REFERENCES

1. World POPClock Projection. US Census Bureau. Available from: http://www.census.gov/main/www.popclock.html. [Accessed on 2011 Jan 1].
2. WHO/63 News Release. Nov 1, 2006. Available from: http://www.whqlibdoc.

who.int/press_release/2006/PR_63.pdf. [Last accessed on 2013 Feb 19].
3. Sharma RS, Rajalakshmi M, Jeyaraj DA. Current status of fertility control methods in India. J Biosci 2001;26:391-405.
4. Feng H, Sandlow J, Sparks A, Sandra A. Development of an immun ocontraceptive vaccine: Current status. J Reprod Med 1999;44:759-65.
5. Gupta SK. Status of immunodiagnosis and immunocontraceptive vaccines in India. Adv Biochem Engin/Biotechnol 2003;85:181-214.
6. Kumar TCA. Development of immunocontraceptives: An introduction. Hum Reprod Update 1997;3:299-300.
7. Ferro VA, Mordini. Peptide vaccines in immunocontraception. Curr Opin Mol Ther 2004;6:83-9.
8. Aitken RJ. Immunocontraceptive vaccines for human use. J Reprod Immunol 2002;57:273-87.
9. Gupta SK, Gupta N, Suman P, Choudhury S, Prakash K, Gupta T, *et al*. Zona pellucid-based contraceptive vaccines for human and animal utility. J Reprod Immunol 2011;88:240-6.
10. Jones WR. Contraceptive vaccines. Baillieres Clin Obstet Gynaecol 1996;10:69-86.
11. Naz RK. Contraceptive vaccines: Success, status and future perspective. Am J Reprod Immunol 2011;66:2-4.
12. Landsteiner K. To knowing that sera which specifi cally acts on blood corpuscles. Int J Med Microbiol 1899;25:546-9.
13. Mtchnikoff E. Etudes sur la resorption de cellule. Ann Inst Pasteur 1899;13:737-79.
14. Baskin MJ. Temporary sterilization by injection of human spermatozoa: A preliminary report. Am J Obstet Gynecol 1932;24:892-7.
15. Peter JD. How far from a hormone-based contraceptive vaccine? J Reprod Immunol 2004;62:69-78.
16. Ferro VA. Current advances in antifertility vaccines for fertility control and noncontraceptive applications. Expert Rev Vaccines 2002;1:443-52.
17. Puri CP, Gopalkrishnan K, Iyer KS. Constraints in the development of contraceptives for men. Asian J Androl 2000;2:179-90.
18. Talwar GP. Fertility regulating and immunotherapeutic vaccines reaching human trials stage. Hum Reprod Update 1997;3:301-10.
19. McLaughlin EA, Holland MK, Aitken RJ. Contraceptive vaccines. Expert Opin Biol Ther 2003;3:829-41.
20. Talwar GP, Hingorani V, Kumar S, Banerjee A, Shahani SM, Krishna U, *et al*. Phase I clinical trials with three formulations of anti-chorionic gonadotropin vaccine. Contraception 1990;41:301-16.
21. Thau R, Croxatto H, Luukkainen T, Alvarez F, Brache V, Sunbdaram K, *et al*. Reproductive Immunology. In: Mettler L, Billington WD, editors. Amsterdam: Elsevier; 1989. p. 237-44.
22. Stevens VC, Powell JE, Lee AC, Griffin PD. Antifertility effects of female baboons with C-terminal peptides of the beta-subunit of human chorionic gonadotropin. Fertil Steril 1981;36:98-105.
23. Stevens VC. Progress in the development of human chorionic gonadotropin antifertility vaccines. Am J Reprod Immunol 1996;35:148-55.
24. Stevens VC, Jones WR. Reproductive Immunology. In: Isojima S, Billington WD, editors. Amsterdam: Elsevier; 1983. p. 233-7.
25. Jones WR. Contraception. Baillière's Clin Obstet Gynaecol 1992;6:629-40.
26. Gupta A, Pal R, Ahlawat S, Bhatia P, Singh O. Enhanced immunogenicity of a contraceptive vaccine using diverse synthetic carriers with permissible adjuvant. Vaccine 2001;19:3384-9.
27. Naz RK, Gupta SK, Gupta JC, Vyas HK, Talwar AG. Recent advances in contraceptive vaccine development: A mini-review. Human Reprod 2005;20:3271-83.
28. Talwar GP, Singh O, Gupta SK, Hasnain SE, Pal R, Majumdar SS, *et al*. The HSD-hCG vaccine prevents pregnancy in women: Feasibility study of a reversible safe contraceptive vaccine. Am J Reprod Immunol 1997;37:153-60.
29. Moudgal NR, Sairam MR, Krishnamurthy H, Khan H. Immunization of male bonnet monkeys (M. radiata) with a recombinant FSH receptor preparation affects testicular function and fertility. Endocrinology 1997;138:3065-8.
30. Karande AA, Adiga PR. Early pregnancy termination in rats immunized with denatured thiamine carrier protein in pregnant rats by using monoclonal antibodies. Int J Biochem Biophys 1991;28:467-70.
31. Singh M, Singh O, Talwar GP. Biodegradable delivery system for a birth control vaccine: Immunogenicity studies in rats and monkeys. Pharm Res 1995;12:1796-800.
32. Lemons AR, Naz RK. Contraceptive vaccines targeting factors involved in establishment of pregnancy. Am J Reprod Immunol 2011;66:13-25.
33. Gupta SK. Update on zona pellucida glycoproteins based contraceptive vaccine. J Reprod Immunol 2004;62:79-89.
34. Covey DC, Moore DE. Current trends in antifertility vaccine research. West J Med 1985;142:197-202.
35. Shivers CA, Dudkiewicz AB, Franklin LE. Inhibition of sperm-egg interaction by specific antibody. Science 1972;178:1211-3.
36. Kirkpatrick J, Turner JW, Liu IK, Fayrer-Hosken R, Rutberg AT. Case studies in wildlife immunocontraception: Wild and feral equids and white-tailed deer. Reprod Fertil Dev 1997;9:105-10.
37. Sacco AG. Immunocontraception: Consideration of the zona pellucid as a target antigen. Obstet Gynecol Annn 1981;10:1-26.
38. Gupta SK. Update on zona pellucida glycoproteins based contraceptive vaccine. J Reprod Immunol 2004;62:79-89.
39. Naz RK. Development of genetically engineered human sperm immunocontraceptives. J Reprod Immunol 2009;83:145-50.
40. Shaha C, Suri A, Talwar GP. Identification of sperm antigens that regulate fertility. Int J Androl 1988;11:479-91.
41. Edwards RG. Immunological control of fertility in female mice. Nature 1964;203:50-3.
42. Baskin MJ. Temporary sterilization by injection of human spermatozoa: A preliminary report. Am J Obstet Gynecol 1932;24:892-7.
43. Ohl D, Naz RK. Infertility due to antisperm antibodies. J Urol 1995;46:591-602.
44. Naz RK. Applications of sperm antigens in immunocontraception. Frontiers in Bioscience 1996;1:87-95.
45. Naz RK, Rowan S. Update on male contraception. Curr Opin Obstet Gynecol 2009;21:265-9.
46. Naz RK. Status of contraceptive vaccines. Am J Reprod Immunol 2009;61:11-8.
47. Naz RK. Antisperm vaccine for contraception. Am J Reprod Immunol 2005;54:378-83.
48. Zhu X, Naz RK. Fertilization antigen-1: cDNA cloning, testis-specific expression, and immunocontraceptive effects. Proc Natl Acad Sci USA 1997;94:4704-9.
49. Naz RK, Zhu X, Kadam AL. Identification of human sperm peptide sequence involved in egg binding for immunocontraception. Biol Reprod 2000;62:318-24.
50. Naz RK, Zhu X, Kalam AL. Cloning and sequencing cDNA encoding for a novel human testis-specific contraceptive vaccinogen: Role of immunocontraception. Mol Reprod Dev 2001;60:116-27.
51. Santhanam R, Naz RK. Novel human testis-specific cDNA: Molecular cloning, expression and immunological effects of the recombinant protein. Mol Reprod Dev 2001;60:1-12.
52. 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;15:1761-6.
53. O'Rand MG, Beavers J, Widgren E, Tung K. Inhibition of fertility in female mice by immunization with a B-cell epitope, the synthetic sperm peptide, P10G. J Reprod Immunol 1993;25:89-102.
54. Lea JA, van Lierop MJC, Widgren EE, Grootenhuic A, Wen Y, van Duin M, *et al*. A chimeric sperm peptide induced antibodies and strain-specific reversible infertility in mice. Biol Reprod 1998;59:527-36.
55. Hardy CM, Mobbs JK. Expression of recombinant mouse sperm protein sp56 and assess ment of its potential for use as an antigen in an immunocontraceptive vaccine. Mol Reprod Dev 1999;52:527-36.
56. O'Rand MG, Widgren EE, Sivashanmugam P, Richardson RT, Hall SH, French FS, *et al*. Reversible immunocontraception in male monkeys immunized with Eppin. Science 2004;306:1189-90.
57. Inoue N, Ikawa M, Isotani A, Okabe M. The immunoglobin superfamily protein Izumo is required for sperm to fuse with eggs. Nature 2005;434:234-8.

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