

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 Metchnikoff 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 bionutralizing 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 oligoaesthesia (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 (β -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 (β -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, subtractive complementary DNA libraries, differential display 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] YLP₁₂,^[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.

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