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REVIEW ARTICLE

Vaccines for immunological control of fertility

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Abstract Vaccines have been proposed as one of the strategies for population control. Immunocontraceptive vaccines can be designed to inhibit: (1) production of gametes (sperm and egg); (2) functions of gametes, leading to blocking of fertilization; and (3) gamete outcome (pregnancy). Immunization with gonadotropin-releasing hormone coupled to different carriers has shown curtailment in the production of sperm with concomitant infertility in various species. Immunization of nonhuman primates and men with ovine follicle stimulating hormone has also resulted in reduced sperm output. Various spermatozoa-specific proteins such as FA1, PH-20, LDH-C_4, SP-10, SP-17, sp56, SPAG9, and Izumo have been proposed as candidate antigens to develop contraceptive vaccines, which have shown efficacy in inhibiting fertility in different animal models. Immunization with zona pellucida glycoproteins-based immunogens also results in curtailment of fertility in a variety of species. However, ways to overcome the observed oophoritis associated with zona proteins immunization have yet to be discovered, a necessary step before their proposal for control of human population. Nonetheless, this is a very promising approach to control wildlife animal population. Phase II clinical trials of β-human chorionic gonadotropin-based vaccine in women have established the proof of principle that it is possible to inhibit fertility without any untoward side-effects by vaccination. Further scientific inputs are required to increase the efficacy of contraceptive vaccines and establish their safety beyond doubt, before they can become applicable for control of fertility in humans.

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Introduction

The increasing human population is an important driving anthropogenic factor, among others, responsible for increased atmospheric concentration of greenhouse gases (GHGs). The impact = population × affluence × technology (IPAT) model suggests that increasing population and economic growth in the coming decades will exacerbate GHGs [1], which may produce disruptive changes in global climate, putting our existence on Earth at stake. Hence, there is an urgent need for commensurate efforts to stabilize human population at a sustainable level. It is estimated that, by 2020, about 1.2 billion people, or 16% of the world's population, will be entering their child-bearing years. Furthermore, 90% of those entering reproductive age will be in the developing world, where there is a particularly pressing need for new contraceptives that are cheap, safe, reliable, convenient, reversible, and culturally acceptable. Hence, there is an urgent need for investment in efforts leading to the development of new forms of contraceptive.

In addition to growing concerns about the increasing human population across the globe, effective and humane management of wild and domestic animal populations is becoming a major issue [2]. For example, the elephant population in Africa is increasing at a significant rate. As a consequence of increased urbanization, conflict now exists between overlapping elephant habitats and land use for human habitation. Moreover, in a number of countries around the world, wild animals that act as vectors

or reservoirs for disease pose a major risk to both human health and agriculture. Wildlife managers have often used lethal means to control populations; however, growing public concerns over animal welfare issues make such approaches increasingly unacceptable. Immunocontraception for wildlife management is therefore taking on a new perspective, and many agencies charged with such management decisions are turning to fertility control as a potential humane solution for this problem [3]. Immunocontraceptive vaccines entail generating humoral and/or cell-mediated immune responses against hormones/proteins that are critical to reproduction, interference with the biological function of which will result in blocking of fertility. Contraceptive vaccines may result in either reversible or irreversible inhibition of fertility.

Targets for the development of immunocontraceptive vaccines

There are multiple points in the reproductive process that can be targeted for immunological intervention to achieve infertility (Fig. 1). These fall into three broad categories: (1) gamete production, (2) gamete function, and (3) gamete outcome. Gonadotropin-releasing hormone (GnRH) synthesized and secreted by the hypothalamus acts on the pituitary and regulates the production of luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH and FSH, in turn, act on the testes and ovaries, leading to the production of sperm and oocytes, respectively. Neutralization of GnRH, LH or FSH may interfere with the production of gametes and thereby inhibit fertility. Both male (spermatozoon) and female (egg) gametes have unique antigens against which immune response can be elicited, leading to blocking of fertilization. Post fertilization, the embryo synthesizes and secretes human chorionic gonadotropin (hCG), which helps in the rescue of corpus luteum and production of progesterone, which is crucial for establishment and maintenance of pregnancy. Neutralization of hCG by antibodies can interfere in implantation of the blastocyst. In the present review, an attempt is made to discuss the current status of various immunological approaches to contraception, current limitations, and future prospects.

Vaccines for inhibition of gamete production

To inhibit gamete production to lead to blocking of fertility, immunization strategies with respect to GnRH and FSH have been investigated, as summarized below.

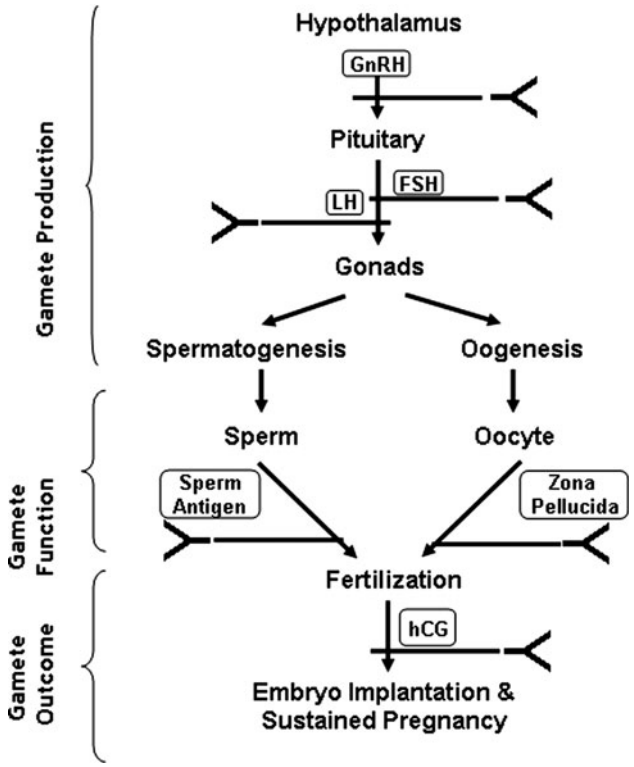

Fig. 1 Schematic presentation of the probable sites for immunocontraception. Antibodies against the respective targets may result in interference in their functions, leading to blocking of fertility

Gonadotropin-releasing hormone

Gonadotropin-releasing hormone, a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-GlyNH_2), is present in both males and females. There are three isomers of GnRH present in humans; however, the GnRH-I isoform plays a major role in the regulation of the hypothalamic-pituitary-gonadal axis [4, 5]. Active immunization with GnRH, coupled with various carrier proteins to facilitate generation of the immune response, led to blocking of fertility in various animal species [6–10]. Active immunization of white-tailed female deers (*Odocoileus virginianus*) led to an 88% reduction in fawning rates and failure to conceive; the blocking of fertility was reversible [9]. Immunization of dogs with synthetic peptide vaccine comprising GnRH with T-helper cell epitopes corresponding to F protein of canine distemper virus resulted in the generation of high anti-GnRH antibody titers and concomitant decrease in steroid hormonal profile, regression of testes, and decrease of spermatogenesis [11, 12]. An alternative approach that has been proposed is to deliver the GnRH agonist (Deslorelin) in a slow-release implant, which led to decrease in LH, testosterone, testicular volume, and semen output [13], and thus has potential as a long-term reversible antifertility agent for male dogs. GnRH-based vaccine, although a

feasible proposition for wildlife population control, may not find favor for fertility inhibition in humans, as it results in blocking of secretion of gonadotropins as well as gametogenesis.

In addition to the control of wild animal populations, GnRH-based vaccine has found two additional applications: (1) improving meat quality, and (2) in treatment of hormone-dependent prostate cancer. Peri- and postpubertal rams and boars accumulate androgen derivatives, androsterone and skatole, in their adipose tissue, which give an unpleasant odour to meat. Immunization with an anti-GnRH vaccine reduces testosterone and eliminates the taint, thus improving meat quality [14]. Vaccinated animals also showed better growth rate as compared with nonimmunized animals. Therefore, GnRH-based vaccine has good application in meat-producing industry and provides a humane treatment alternative to surgical castration [8]. Immunization of human subjects of prostate cancer with GnRH-based vaccine led to significant reduction in serum testosterone and prostate-specific antigen, with improvement in clinical parameters [15, 16].

Follicle stimulating hormone

Follicle stimulating hormone plays an important role in the growth of ovarian follicles in females, and seminiferous tubules and spermatogenesis in males. Immunization of male bonnet monkeys (*Macaca radiata*) with ovine FSH led to reversible blocking of fertility [17, 18]. Immunized animals showed impairment of spermatogenesis, resulting in oligospermia, decreased sperm motility, and normal libido. Based on these findings, a group of five human male volunteers were immunized with ovine FSH. The anti-ovine FSH antibodies reacted with human FSH and led to a marked reduction (30–90%) in seminal plasma transferrin, a marker for Sertoli cells and seminiferous tubule functions, and reduction in sperm count [19]. No significant changes in the levels of LH, testosterone or thyroid stimulatory hormone (TSH) were observed in the immunized humans. Further research is required to investigate the potential of FSH and/or its receptor as immunogens for blocking of fertility without any untoward side-effects.

Contraceptive vaccines targeting gamete functions

Spermatozoa- and egg-specific proteins offer excellent candidate antigens to develop contraceptive vaccine which will act at the prefertilization stage. In particular, those proteins that are involved in sperm–egg interaction and that are crucial for accomplishment of fertilization constitute the most attractive targets for immunocontraception. In order to qualify as candidate antigens for the development of contraceptive vaccines, they must fulfill the following criteria:

1. The antigen should be sperm/egg specific and absent in other somatic cells.
2. It should have fertility-associated function.
3. It should be accessible to antibodies generated subsequent to immunization.
4. It should be immunogenic in order to generate a robust immune response.

Contraceptive vaccines based on spermatozoa-specific antigens

Initial immunization studies in animals as well as humans with either sperm or its extracts resulted in the production of antisperm antibodies that led to infertility [20, 21]. These studies are supplemented by nature's experiment where the presence of antisperm antibodies in women and men is associated with idiopathic infertility [22, 23]. Encouraged by these observations, several laboratories across the world initiated programs to identify and characterize sperm-specific antigens that could fulfill the criteria to be candidate antigens for the development of contraceptive vaccines. These efforts led to the identification of various spermatozoa-associated antigens, such as PH-20 [24], sperm protein-10 (SP-10) [25], SP-17 (RSA) [26], sp56 [27], fertilization antigen-1 (FA-1) [28], sperm-associated antigen 9 (SPAG9) [29], etc. Immunization with most of these proteins led to variable degrees of contraceptive efficacy in various animal models [24, 26–30]. Immunization of nonhuman primates with testis-specific lactate dehydrogenase (LDH-C_4) showed variable results with respect to reduction in fertility [31, 32]. Immunization of male bonnet monkeys (*Macaca radiata*) with epididymal protein inhibitor (Eppin) led to a significant reduction in fertility [33]. Blocking of fertility was reversible, subsequent to the reduction in antibody titers against Eppin.

In addition to sperm-specific proteins/recombinant proteins, synthetic peptides have also been evaluated as immunogens, also resulting in variable blocking of fertility in various animal models [26]. Using a phage display library, a novel dodecamer peptide sequence YLPVGGLRRIGG (designated YLP_{12}) that binds to zona pellucida glycoprotein-3 (ZP3) has been reported [34]. Immunization of mice with YLP_{12} conjugated with recombinant cholera toxin B subunit (rCTB) elicited antibodies against YLP_{12} detectable in serum as well as vaginal washings. Immunized animals showed inhibition of fertility, which could be restored by intravaginal administration of YLP_{12} peptide or subsequent to decline in the antibody titers [35]. To enhance the contraceptive efficacy, a cocktail of synthetic peptides corresponding to different

sperm-specific proteins and coupled to different carrier proteins has also been used [36, 37]. However, these investigations also did not result in blocking of fertility in 100% of immunized animals.

Spermatozoa-associated immunoglobulin superfamily protein, Izumo, has been reported to play an important role in the fusion of the sperm membrane with the oolemma of the egg. Izumo-knockout mice are healthy but sterile. Izumo$^{-/-}$ mice produced normal-looking sperm which bind and penetrate the zona pellucida (ZP) but that were incapable of fusing with eggs [38]. Immunization of mice with recombinant Ig-like domain of Izumo [39] and synthetic peptide [37] led to reduction in fertility.

Contraceptive vaccines based on zona pellucida glycoproteins

The mammalian oocyte is surrounded by a glycoproteinaceous matrix, termed the ZP. The ZP matrix plays a crucial role during fertilization by serving as a species-selective substrate for sperm binding, an agonist for regulated exocytosis of the spermatozoon's acrosomal vesicle (acrosome reaction), and avoidance of polyspermy [40]. It also acts as a protective barrier around the oocyte and early stages of embryo development until implantation of the blastocyst into the endometrium takes place. The ZP matrix of mouse is composed of three glycoproteins, designated as ZP1, ZP2, and ZP3. In mouse, ZP3 acts as a primary sperm receptor and also induces the acrosome reaction [41, 42], whereas ZP2 acts as a secondary sperm receptor and helps in the maintenance of binding of acrosome-reacted spermatozoa to the oocyte [43]. However, recent studies have revealed that ZP matrix in mammals such as rats, hamsters, monkey, and humans is composed of four glycoproteins, designated as ZP1, ZP2, ZP3, and ZP4 [44–47]. The ortholog of the human ZP4 gene is present in the mouse genome as a pseudogene [48]. In human, in addition to ZP3, ZP4 also binds to capacitated acrosome-intact sperm and induces acrosome reaction [49–52]. Bearing in mind the critical role of ZP glycoproteins during fertilization, they have been proposed as candidate antigens for the development of contraceptive vaccines.

In addition to the functional relevance of ZP glycoproteins during reproduction, there is a variable degree of amino acid (aa) conservation in the zona proteins of various species, which has allowed heterologous immunization. For example, immunization with porcine zona proteins elicits antibodies that cross-react with human zona proteins [53]. Immunization with heat-solubilized porcine ZP led to blocking of fertility in female rabbits, dogs, and nonhuman primates [54–56]. However, the blocking of fertility was associated with follicular atresia and abnormal hormone profile. Subsequent immunization studies in nonhuman primates with purified porcine zona proteins incorporating adjuvants other than Freund's complete adjuvant also resulted in blocking of fertility with reduced adverse effects on ovarian functions [57–60].

In order to prevent contamination of other ovarian-associated proteins in the purified zona protein preparation from native source and to avoid batch-to-batch variation in the quality of the purified product, various groups have expressed the zona proteins from a variety of species using different expression systems. These recombinant zona proteins ensure the availability of the immunogen to carry out large-scale active immunization studies. Female marmosets (*Callithrix jacchus*) immunized with recombinant human ZP3, expressed in mammalian cells, showed high circulating antibody titers and long-term infertility [61]. However, in immunized marmosets, ovarian pathology characterized by depletion of primordial follicles was observed, in spite of the fact that recombinant protein employed for immunization studies is likely to be free from other ovarian-associated proteins. In another study, female cynomolgus monkeys (*Macaca fascicularis*) and baboons (*Papio cynocephalus*) were immunized with recombinant human ZP2, ZP3, and ZP4 (previously designated as ZPB) expressed in Chinese hamster ovarian (CHO) cells [62]. Animals immunized with ZP4 remained infertile for 9–35 months. During the time of high antibody titers, some immunized animals experienced disruption of the menstrual cycle, but eventually all the animals resumed normal menstrual cycles. Nonimmunized controls and animals immunized with ZP2 and ZP3 conceived before any of the ZP4-immunized animals, suggesting that ZP4 is a better candidate for curtailment of fertility as compared with ZP2 and ZP3.

The contraceptive potential of ZP4 (previously designated as ZPB/ZP1; as per the new classification it is designated as ZP4) was also confirmed by our group. The bonnet monkey (*Macaca radiata*) ZP4 (bmZP4) was expressed in *E. coli* [63]. Immunization of female baboons (*P. anubis*) and bonnet monkey with recombinant bmZP4 conjugated to diphtheria toxoid (DT) elicited high antibody titers associated with blocking of fertility [63, 64]. In female baboons, blocking of fertility was reversible, as the immunized animals became pregnant subsequent to decline in antibody titers [63]. However, bonnet monkeys immunized with bmZP4 failed to conceive subsequent to the decline in anti-bmZP4 antibody titers. Histological examination of the ovaries of the immunized monkeys revealed the presence of atretic follicles and degenerated oocytes [64]. Plausible reasons for the differential effect of immunization with bmZP4 in female baboons versus bonnet monkey are not clear at this stage.

Strategies to overcome oophoritis associated with ZP glycoproteins-based immunization

Studies with highly pure recombinant zona proteins have suggested that oophoritis observed subsequent to immunization with zona proteins may either be an intrinsic property of these proteins or be due to differential susceptibility to develop oophoritis of the employed experimental animal model. Employing a series of elegant experiments in a murine model and using ZP3 as a candidate antigen, it was established that the observed ovarian dysfunction is associated with the presence of "oophoritogenic" T-cell epitopes [65]. To make ZP glycoprotein-based contraceptive vaccine applicable to humans, various scientific groups have made efforts to delineate B-cell epitopes that are devoid of oophoritogenic T-cell epitopes [66–70]. The proof of concept was provided by an elegant study wherein female mice of eight different haplotypes were immunized with a synthetic peptide comprising minimal B-cell epitope of mouse ZP3 (335–342 aa; Phe_{336} substituted by Ala) synthesized collinearly with "promiscuous" T-cell epitope of bovine RNase (NCAYKTTQANK). Immunization with this peptide led to decrease in fertility. Oophoritis was not observed in immunized animals, suggesting that it is possible to achieve contraception without ovarian dysfunction [71].

Immunization of female marmosets with synthetic peptides corresponding to either human or marmoset ZP3, in contrast to recombinant ZP3, also did not result in ovarian pathology [61, 72]. Although antipeptide antibodies showed in vitro contraceptive efficacy, mating experiments did not reveal any significant reduction in fertility [72]. Female bonnet monkeys immunized with bmZP3 synthetic peptide corresponding to 324–347 aa residues and conjugated to DT failed to conceive despite having ovulatory cycles as judged by serum progesterone profile [73]. No ovarian pathology was observed in immunized animals. Reduction in fertility without ovarian pathology was also observed in white-tailed dear immunized with porcine ZP1 peptide (79–130 aa) and mice immunized with mouse ZP3 peptide (328–342 aa) [74, 75].

Enhancement of contraceptive efficacy of ZP-based epitopes

It is evident from the above that immunization with synthetic peptides corresponding to the B-cell epitopes of zona proteins results in no or minimal ovarian pathology. In order to enhance their contraceptive potential it may be interesting to use (1) multiple epitopes corresponding to a given zona protein, (2) epitopes from multiple zona proteins, or (3) combination of epitopes of zona proteins and sperm-specific proteins. Indeed, immunization with synthetic peptide encompassing B-cell epitopes of bmZP1 (251–273 aa) and bmZP3 (324–347 aa) separated by a triglycine spacer resulted in production of antibodies with higher contraceptive potential as compared with individual epitopes [76]. Similarly, a chimeric *E. coli*-expressed recombinant protein encompassing B-cell epitopes of bonnet monkey ZP2, ZP3, and ZP4 generated antibodies that showed significant inhibition of binding of human sperm to human zona in a hemizona assay [77]. Similarly, chimeric recombinant proteins encompassing B-cell epitopes of ZP protein and sperm antigens also have shown their ability to inhibit fertility [78, 79]. However, to date, in none of these experiments, 100% block of fertility has been reported in immunized animals.

ZP glycoproteins-based contraceptive vaccine for controlling wildlife population

Observed ovarian dysfunction often associated with ZP glycoproteins-based contraceptive vaccine must be resolved before these can be proposed as candidate vaccines for fertility control in humans. Furthermore, active immunization studies done in various animal models with zona-based contraceptive vaccines do not result in blocking of fertility in 100% recipients. Despite these shortcomings, ZP-based contraceptive vaccine has shown very promising results for controlling wildlife populations. Contraceptive vaccine based on native porcine ZP has been used to regulate the wildlife population of feral horses (*Equus caballus* [80]), captive zoo animals [81], and white-tailed deer (*Odocoileus virginianus*) [82]. This approach has been used for decades to manage entire population of wild horses at Assateague Island National Seashore, MD, USA and white-tailed deer inhabiting Fire Island National Seashore, NY, USA without showing any significant debilitating short- or long-term health effects in vaccinated animals [83–85]. This approach has also worked in African elephant (*Loxodenta africana*) [86]. Our group has shown that immunization of nondescript female dogs with *E. coli*-expressed recombinant canine ZP3 conjugated to DT generated good anti-ZP3 antibody titers. Immunized animals failed to conceive when mated with male dogs during estrus phase [87].

Alternate modes of ZP glycoproteins-based vaccine delivery

For effective control of wildlife populations, it is imperative that the vaccine delivery system should be efficient. To immunize feral horses and white-tailed deer, porcine ZP-based contraceptive vaccine has been remotely delivered by dart-gun approach, thereby avoiding the need to capture individual animals [88]. Alternately, zona

proteins can be delivered in slow-release devices, thereby increasing the duration of the immune response. Indeed, immunization of grey seals (*Halichoerus grypus*) with porcine ZP entrapped in liposome decreased fertility in 90% of immunized animals over a 5-year period [89]. Immunization of mice with ZP3 epitope, YLP_{12}, and YLP_{12}-ZP3 fusion peptide presented on Johnson grass mosaic virus-like particles without any adjuvant led to the generation of specific antibody responses and a significant reduction in litters in immunized mice [90]. Delivery of attenuated *Salmonella typhimurium*-expressing mouse ZP3 by oral route led to generation of antibodies in both the systemic circulation as well as the genital tract, accompanied by a decrease in fertility [91]. Host-specific live vectors such as ectromelia virus (a natural pathogen that causes mouse pox), cytomegalovirus (mouse-specific beta herpes virus), and myxoma virus have also been used as means of effective delivery of zona protein-based contraceptive vaccine [92–94]. These recombinant host-specific vectors may be released into the environment and transmitted from one animal to another, thereby leading to an effective means of controlling population of a given species. The major problem faced by this approach is that, by and large, recombinant virus has low infectivity as compared with nonrecombinant virus. The other concern about their use in controlling wildlife population is how stringent their host specificity is. If these recombinant virus lose host specificity, what will be the impact on the population of other species?

The potential of DNA vaccine to deliver zona-based contraceptive vaccines has also been explored by our group. We have shown that immunization of mice with DNA vaccine encoding either bmZP4 or canine ZP3 as a prime-boost strategy led to good antibody titers [95, 96]. Antibodies against bmZP4 inhibited binding of sperm to zona in the hemizona assay [95]. Recently, our group has shown that antibodies generated by a DNA vaccine encoding chimeric protein encompassing epitopes of human ZP3 and ZP4 led to a significant decrease in acrosomal exocytosis in capacitated human sperm induced by both recombinant human ZP3 and ZP4 [97]. Mice immunized with mouse ZP3 DNA vaccine and protein showed high reduction in fertility without T-cell inflammation [98]. Co-immunization resulted in reduced production of inflammatory cytokine, interferon (IFN)-gamma, and increased production of interleukin (IL)-10 and FoxP3 in CD4 T-cells. A significant correlation between normal follicular development and the inhibition of T-cell response in these immunized animals was reported [98]. These results suggest that co-immunization with DNA vaccine and protein can reduce fertility without interfering in normal follicular development.

Contraceptive vaccine blocking postfertilization events

One of the most extensively studied contraceptive vaccines in this category is based on hCG. This hormone can be detected as early as 7 days in the culture supernatant of the growing human blastocyst [99] and has been used as a marker for the establishment of pregnancy, as it is not detectable in nonpregnant healthy women. However, hCG is synthesized and secreted in a variety of nontrophoblastic and trophoblastic cancers [100]. It is composed of α- and β-polypeptide chains. The α-subunit of hCG is common to other gonadotropins such as LH, FSH, and thyroid stimulating hormone (TSH). The β-subunit of hCG is composed of 145 aa and has a unique extension of 30 aa at its carboxy terminus, known as the carboxy-terminal peptide (CTP). Of the remaining 115 amino-terminal aa of β-hCG, approximately 80% are homologous to those of β-human LH (hLH). Hence, β-hCG differs from β-hLH in about 51 positions, including the extra 30-aa tail piece present at the carboxy terminus.

To achieve immunocontraception targeting hCG, two different immunization strategies have been explored. One of these involves immunization with CTP of β-hCG, and the second approach is to use the whole β-hCG molecule. β-hCG has been used as an immunogen, based on the premise that it will have better immunogenicity than CTP and that antibodies thus generated will have greater hCG bioneutralization capacity.

β-hCG vaccine based on CTP

Immunization with the CTP of β-hCG, linked to different carrier proteins such as DT, generated antibodies that were devoid of cross-reactivity with hLH and that resulted in curtailment of fertility [101, 102]. Extensive preclinical safety studies revealed no serious health hazards, except that the CTP antibodies reacted with somatostatin-producing cells of the pancreas. Phase I clinical trials of CTP vaccine were conducted in Bedford Park, Australia under the auspices of the World Health Organization. Thirty young healthy sterilized (by tubal ligation) women divided into five groups were immunized with varying doses of the vaccine. During follow-up, no significant adverse reactions were observed. All immunized women generated antibody titers against hCG that would have potential contraceptive efficacy [103]. Based on these studies, CTP vaccine underwent a phase II clinical trial in Sweden, which had to be abandoned due to unacceptable adverse reactions in vaccinated women.

To enhance the immunogenicity of CTP corresponding to β-hCG, recombinant protein comprising a 37-aa CTP of β-hCG linked to heat-labile enterotoxin subunit B (LTB) with an intervening 9-aa linker was expressed in *E. coli*.

Immunization of mice with the purified recombinant LTB-CTP protein without any additional adjuvants led to generation of hCG-specific antibody responses [104]. In rabbits, a single injection of CTP of β-hCG cosynthesized with a T-cell epitope from tetanus toxoid (TT) and entrapped in polymer microspheres elicited strong antibody response with equivalent duration to that of a three-injection schedule delivered by water-in-oil emulsion [105].

β-hCG vaccine

Phase I clinical trials in women with β-hCG linked to TT were initiated after extensive studies related to optimization of immunogenicity, characterization of antibodies elicited subsequent to immunization, and safety aspects of such immunization in various animal models [106]. This study revealed that immunization led to generation of anti-hCG and anti-TT antibodies. However, the antibody titers and the duration of the immune response were variable among immunized women. Interestingly, administration of hCG in saline in the above immunized women did not result in increase of the anti-hCG antibodies titers, suggesting that endogenous hCG, if made, will not act as a booster. During these trials, no clinical, hematological or endocrine abnormalities were observed [107]. Subsequently, to increase the immunogenicity of β-hCG, this group proposed an immunogen composed of β-hCG annealed to the α-subunit of ovine luteinizing hormone (α-oLH). To avoid carrier-mediated suppression [108] β-hCG-α-oLH was coupled to either TT or DT. An alternative approach of using promiscuous T non-B cell peptides corresponding to TT, bovine RNAse, measles virus, human immunodeficiency virus-1 reverse transcriptase, and influenza virus hemagglutinin has also been proposed to avoid carrier-mediated suppression [109]. In landmark extended phase II clinical trials, women immunized with β-hCG-α-oLH-TT/β-hCG-α-oLH-DT generated bioneutralizing anti-hCG antibodies [110]. Immunized women with circulating bioneutralizing antibody titers above 50 ng/ml were protected against conception. Only one pregnancy was observed out of 1,224 cycles. Blocking of fertility was reversible, as women conceived when antibody titers declined to less than 35 ng/ml. Long-term follow-up of children born to vaccinated women showed normal full-term pregnancy leading to birth of healthy normal children [111].

These studies, for the first time, established the proof of principle that immunocontraceptive vaccine can block fertility in humans. However, at the same time, several shortcomings were also observed. Immunization did not result in generation of protective antibody titers (>50 ng/ml) in all vaccinated women. Furthermore, the duration of protective antibody levels was also highly variable amongst immunized subjects. Further efforts are required to enhance the immunogenicity of β-hCG-based contraceptive vaccine so that it elicits protective antibody titers for at least 6 months in 90–95% of immunized women.

To eliminate LH cross-reactive epitopes, various analogues of β-hCG have been prepared by using site-directed mutagenesis [112, 113]. Immunization of mice and rabbits with such mutant β-hCG generated antibodies which have low cross-reactivity with LH [113, 114]. Immunization of mice with chimeric DNA vaccine comprising β-hCG linked to three copies of molecular adjuvant C3d3 generated very good antibody responses. Furthermore, production of IL-4 and IL-10 was increased in immunized mice and the cytokine profile showed a Th2 bias of the immune response [115].

Current limitations and prospects of contraceptive vaccines

Various contraceptive vaccines proposed so far have not resulted in blocking of fertility in 100% of immunized subjects, which is highly desirable for application in humans. Furthermore, there is variability in the elicited immune response amongst immunized subjects, with some showing higher antibody titers for a longer period and others for a short duration. This will require periodical monitoring of antibody titers in immunized subjects to ensure that antibody levels are above the protective threshold. Hence, it is critical to find more potent adjuvants that are safe for human applications. On the other hand, contraceptive vaccines are an excellent proposition to control population of wildlife—a herd immunization approach, where a few pregnancies in immunized animals will not lead to ethical and legal issues. However, in this scenario, discovering novel ways for vaccine delivery will be a major challenge. Remote vaccine delivery by dart-gun approach and vaccination by oral route through baits are some of the interesting propositions.

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