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Contraceptive Vaccines

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Abstract

The world's population is growing at a tremendous rate, affecting growth and development. Apart from this population growth, unintended pregnancies resulting in elective abortions continue to be a major public health issue. In over half of these unintended pregnancies, the women have used some type of contraception. Thus, there is an urgent need for a better method of contraception that is acceptable, effective and available.

The contraceptive choices available to women at this time include steroid contraceptives, intrauterine devices, barrier methods, spermicides, natural family planning, male and female sterilisation, and recently available emergency contraceptives. Contraceptive vaccines (CVs) may provide viable and valuable alternatives that can fulfill most, if not all, properties of an ideal contraceptive. Since both the developed and most of the developing nations have an infrastructure for mass immunisation, the development of vaccines for contraception is an exciting proposition. The molecules that are being explored for CV development either target gamete production (gonadotropin releasing hormone, follicle-stimulating hormone and luteinising hormone), gamete function (zona pellucida [ZP] proteins and sperm antigens) or gamete outcome (human chorionic gonadotropin [hCG]). Disadvantages of CVs targeting gamete production are that they affect sex steroids and/or show only a partial effect in reducing fertility. CVs targeting gamete function are better choices. Vaccines based on ZP proteins are quite efficacious in producing contraceptive effects. However, they invariably induce oophoritis affecting sex steroids. Sperm antigens constitute the most promising and exciting targets for CVs. Several sperm-specific antigens have been delineated in several laboratories and are being actively explored for CV development. Antisperm antibody-mediated immunoinfertility provides a naturally occurring model to indicate how an antisperm vaccine will work in humans. Vaccines targeting gamete outcome primarily focus on the hCG molecule. The hCG vaccine is the first vaccine to undergo phase I and II clinical trials in humans. Both the efficacy and the lack of immunotoxicity have been reasonably well demonstrated for this vaccine. The present studies focus on increasing the immunogenicity and efficacy of this birth control vaccine.

The world population has exceeded 6.3 billion and is affecting growth and development in every sector.[1] In the first year AD the world population was 250 million, which increased to 1 billion by 1830. It took the next 100 years for the population to increase by 1 billion and at the present rate it will

take approximately 12 years to increase the population by 1 billion. Ninety-five percent of this growth is in the developing nations. Besides the population explosion, unintended pregnancies continue to be a major public health issue in the US. Each year, half of all pregnancies are unintended, which result in over 1 million elective abortions.[2] In over half of these unintended pregnancies, the women were using some type of contraception.[3] Thus, there is an urgent need for a better method of contraception that is acceptable, effective and available both in the developed and developing nations.

The contraceptive choices available to women at this time include steroid contraceptives (oral/injectable/implants), intrauterine devices (IUDs), barrier methods, spermicides, natural family planning, male and female sterilisation, and recently available emergency contraceptives.[4] Steroid contraceptives are the most frequently used reversible methods in the US by women <35 years old. All of these contraceptive modalities have some associated adverse effects and/or limitations. IUDs have a tendency to cause heavy menstrual bleeding, carry the possibility of expulsion and upper genital tract infection, and cannot be used in some gynaecological conditions. The most important properties of an ideal contraceptive method desired by women are that it is 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.[4]

A contraceptive vaccine (CV) has been proposed as a valuable alternative that can fulfill most, if not all, of the properties of an ideal contraceptive. CVs may be more acceptable than the currently available methods of contraception because of their high specificity, limited or no adverse effects, low cost and infrequent administration. Since both the developed and most of the developing nations have an infrastructure for mass immunisation, the development of vaccines for contraception is an exciting proposition.

Mammalian reproduction begins with the production of male and female gametes during spermatogenesis and oogenesis, respectively, followed by their unification. The production of gametes in the gonads is under the control of unique and specific hormones, namely gonadotropin-releasing hormone (GnRH) and gonadotropins (follicle-stimulating hormone [FSH] and luteinising hormone [LH]), which when bioneutralised with antibodies can result in the inhibition of gamete production. Both gametes, the spermatozoon and the oocyte, have antigens on the surface that are distinctive, cell-specific, immunogenic and accessible to antibodies. The binding of antibodies to these antigens can inhibit gamete function, resulting in the failure of fertilisation. Fertilisation is followed by embryogenesis, with the early embryo secreting several specific proteins, such as human chorionic gonadotropin (hCG), that have a vital role in the establishment and maintenance of pregnancy. These proteins are accessible to antibodies, and their immunoneutralisation can cause antifertility effects with a loss of early embryo. Several targets are being investigated in various laboratories for the development of CVs. These can be divided into three main categories: vaccines targeting gamete production, gamete function and gamete outcome (figure 1). The aim of this article is to review the current status, limitations (if any) and future perspectives of various CVs that are currently being investigated.

1. Vaccines Targeting Gamete Production

The decapeptide GnRH from the hypothalamus activates the secretion of FSH and LH from the anterior pituitary which regulate gametogenesis and the production of androgens and estrogens. Three sites/molecules have been explored for immunoneutralisation by antibodies, in order to block gamete production. These are GnRH, FSH and LH.

GnRH is a 'self' and low molecular weight peptide, and thus requires conjugation with a T-cell carrier to make it immunogenic. The GnRH conjugate vaccine causes a block in fertility of both male and female animals of several species.[5-9] The application of GnRH to immunocontraception in humans is limited by the fact that the vaccine, as expected, affects sex steroid production in addition to the

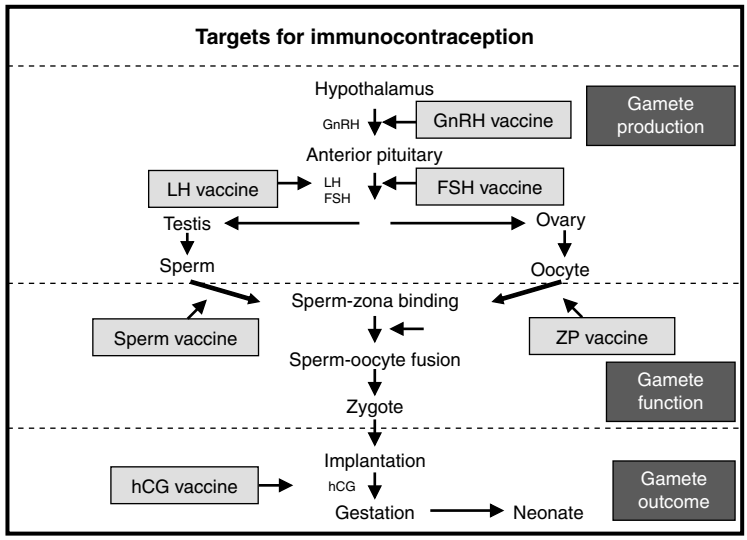

Fig. 1. Schematic model indicating various targets being explored for the development of contraceptive vaccines. These include targeting gamete production (gonadotropin-releasing hormone [GnRH], follicle-stimulating hormone [FSH] and luteinising hormone [LH]), gamete function (zona pellucida [ZP] proteins of the oocyte and sperm antigens) and gamete outcome (human chorionic gonadotropin [hCG]). Of these, the sperm cell is the most exciting target and the molecules involved in sperm-ZP recognition and binding are the most attractive candidates. The antibodies directed at this site will not interfere with hormone milieu, gametogenesis or female cyclicity.

inhibition of gamete production. Thus, the antifertility vaccine(s) based on GnRH will require androgen supplementation in males to maintain libido and secondary sex characteristics.[6] However, the GnRH vaccine might be useful in controlling farm and wild animal populations, and in regulating the fertility of domestic pets. It may also find applications in clinical situations where there is a need for the reduction of sex steroids, such as androgen ablation for prostate hypertrophy and carcinoma in men, and the reduction of excessive hormones in uterine fibroids, endometriosis, polycystic ovary syndrome and precocious puberty in women. Several GnRH vaccines, using different adjuvants and carriers, have undergone clinical trials in different countries sponsored by various pharmaceutical companies for these clinical purposes.[10-12]

FSH plays an essential role in the initiation of spermatogenesis at puberty in mammals. However, its role in the maintenance of spermatogenesis is not clear, as is evident from gene knockout studies in mice.[13] Immunisation of male rats and monkeys with FSH or its receptor causes a disruption of spermatogenesis, resulting in oligospermia.[14] A phase I clinical trial was conducted in humans; the data indicated that the FSH-based vaccine does not cause any immunopathological consequences in men.[15] The acceptance of FSH or FSH receptor-based CVs is hindered by the fact that immunisation with these molecules causes oligo- rather than azoospermia and the fertilising capacity of the remaining sperm in the semen is not totally blocked. To have a wider acceptability, a vaccine should provide near foolproof protection against conception. In females, FSH vaccination will cause loss of folliculogenesis, resulting in premature ovarian failure, and therefore this approach has not been extensively explored.

Immunisation with LH or its receptor can cause an inhibition of gametogenesis in both males and females of various species of animals.[16-18] Although long-term (5–7 years) immunisation with LH has been shown to have no adverse effect on pituitary function in monkeys, it is generally envisaged that immunisation with LH could potentially affect sex steroids. Therefore, it has not been widely accepted as a target for immunocontraception for

humans, though it might find applications in fertility control of domestic, wild and farm animals.

2. Vaccines Targeting Gamete Function

There are two targets that are being extensively explored in various laboratories: oocyte zona pellucida (ZP) proteins and sperm antigens.

2.1 Zona Pellucida Vaccine

The ZP is a unique extracellular matrix that surrounds the mammalian oocyte and preimplantation embryo.[19,20] Sperm must recognise, attach, acrosome react and pass through the ZP in order to fertilise the egg. The ZP represents an interesting target for contraception. It consists of three developmentally regulated, evolutionarily conserved and gamete-specific glycoconjugates, namely ZP1, ZP2 and ZP3, which have essential roles in fertilisation. Of the three, the ZP3 glycoprotein is the primary molecule involved in sperm recognition and induction of acrosome reaction in several species. Mice that are homozygous for an insertional mutation in the ZP3 gene lack ZP in the oocytes and are infertile.[21]

Antibodies raised against ZP3 or its peptide fragments block fertilisation *in vitro* in various species of animals, including humans.[22,23] Active immunisation of female animals of various species, including primates, with ZP3 or its peptide fragments induces infertility.[24-28] However, immunisation with ZP3 invariably causes ovarian dysfunction through a significant loss of primordial follicles from the ovaries. This adverse effect is observed even when the deglycosylated native or recombinant molecule is used for immunisation. This indicates that the effect is not due to antibodies raised against the carbohydrate moieties of the ZP3 molecule. It is hypothesised that the oophoritis induced after ZP3 immunisation is caused by activation of a cytotoxic T-cell response. Thus, if the ZP3-specific T-cell epitopes are deleted from the vaccine, infertility may be achieved without the loss of ovarian function. Indeed, immunisation of female mice with a 7-amino acid synthetic peptide, based on the mouse ZP3 protein sequence corresponding to amino acids 336–342, caused long-term reversible infertility without the induction of ovarian pathology.[27] Subsequently, through experiments involving adoptive transfer of CD4+ T cells, several T- and B-cell epitopes have been identified within the mouse ZP3 sequence.[29,30] Although these findings are interesting, the ovarian dysfunction induced after ZP3 immunisation is not always associated with T-cell infiltration in the ovaries. There are alternative explanations that involve antibody-mediated, rather than T cell-mediated, disruption of folliculogenesis.[23] Unless this serious adverse effect of premature ovarian failure is resolved, the ZP vaccine will not be an acceptable proposition for humans. However, the semi-purified vaccine, prepared from porcine oocyte ZP, designated as PZP, is being successfully used for controlling feral populations of dogs, horses, deer and elephants.[31-33] Interestingly, the PZP vaccine has been tried in over 112 different species including seals, sea lions and bears in 110 different zoos for control of animal populations (J.F. Kirkpatrick, personal communication, 18 January 2005). The vaccine has been found to work in almost all species except for some carnivores, canids and felids, where the vaccine did not show a consistent contraceptive effect.

2.2 Sperm Vaccine

Of all the potential targets, sperm has drawn the most attention, and a sperm vaccine represents an exciting proposition for contraception. The rationale and feasibility for the development of a sperm vaccine is provided by the following lines of evidence. Sperm have both auto- and isoantigenic potentials, and can therefore form antibodies in both men and women. Antisperm antibodies (ASAs) affect fertilisation and fertility both *in vitro* and *in vivo*. The presence of ASAs in the *in vitro* fertilisation medium blocks fertilisation by several mechanisms, such as the inhibition of sperm capacitation, acrosome reaction and sperm-zona interaction. Deliberate immunisation of male or female animals of various species,[34-36] including humans (both women and men),[37,38] with sperm or their extracts causes the development of ASAs, leading to infertility. Bas-

kin[37], in 1932, injected 20 fertile women, who had at least one prior pregnancy, with their husband's semen and these women developed antibodies and no conception was reported for up to 1 year of observation.[37] A US patent was issued for this spermatoxic vaccine in 1937 (US patent number 2103240). Another line of evidence is provided by studies of vasectomy and involuntary immunoinfertility. Up to 70% of vasectomised men produce ASAs,[39] and 2–30% of cases of infertility (depending upon the reporting infertility centre) may be associated with the presence of ASAs in the male and/or female partner of an infertile couple.[40] Thus, spermatozoa can generate an immune response that is capable of inducing a contraceptive state. However, the whole spermatozoon *per se* cannot be used for the development of a vaccine because of the presence of several antigens that are likely to be shared with various somatic cells.[41] Only those antigens that are sperm specific can be employed for a CV. The application of a sperm antigen in a CV is contingent upon its sperm specificity, surface expression, involvement in fertility and ability to raise enough antibodies to be capable of intercepting fertility. If an antigen is also involved in human immunoinfertility, it is an especially attractive candidate. The sperm-ZP binding site constitutes the most attractive target for immunocontraception.

For the last decade various laboratories, including this author's, have been using hybridoma and recombinant DNA technologies, and various proteomic and genomic approaches to search for sperm-specific antigens that can be used for CV development. Several antigens have been identified. Some of them have been further characterised and the complementary DNAs (cDNAs) encoding these antigens have been cloned and sequenced. Notable among them are the fertilisation antigen (FA)-1,[41] PH-20,[42] PH-30,[43] sperm protein (SP)-10,[44] SP-17,[45] testis-specific antigen-1[46] and contraceptive vaccinogen.[47] Active immunisation of female animals with some of these antigens has been shown to reduce fertility *in vivo*.[41,42,45] The FA-1 antigen and contraceptive vaccinogen are exciting molecules since they have receptor activity for ZP binding in humans.[41,47] The FA-1 antigen is clearly associated with immunoinfertility in men and women. The sera, seminal plasma and sperm of immunoinfertile and vasectomised men (but not fertile men), and the sera, cervical mucus and follicular fluids of immunoinfertile women (but not fertile women) have antibodies reactive with the FA-1 antigen.[41] On the basis of these findings, a clinical trial was conducted at the University of Michigan Medical School (Ann Arbor, MI, USA) to determine whether immunoadsorption with the FA-1 antigen would remove autoantibodies from the surface of sperm of immunoinfertile men, thus increasing their fertilising capacity. Indeed, the incubation of sperm from immunoinfertile men with the FA-1 antigen removed the autoantibodies and increased the amount of antibody-free sperm. As a consequence, the acrosome reaction rates increased significantly in 78% of the sperm samples after FA-1 adsorption. The intrauterine insemination of FA-1 antigen-adsorbed antibody-free sperm resulted in normal pregnancies and healthy babies, indicating that the antigen treatment does not have a deleterious effect on implantation or on embryonic and fetal development. This study is being extended to a larger number of immunoinfertile men and constitutes an exciting therapeutic modality using well defined sperm antigens.[48]

For US FDA approval for clinical use, and to conduct phase I and II multicentre fertility trials in a quality-controlled manner, the antigens have to be either recombinant or synthetic molecules. The cDNAs encoding human and murine FA-1 antigens have been cloned and sequenced.[49,50] The cDNA encoding murine FA-1 antigen was expressed and the recombinant FA-1 antigen was isolated and purified.[51] Vaccination of female mice with the recombinant FA-1 antigen caused a reversible contraceptive effect.[51] The peptide-based vaccines have some advantages over using the whole recombinant antigens. The peptides are well defined molecules that could be synthesised and purified in large quantities at relatively lower cost than the recombinant antigens. On the basis of these facts, several synthetic sperm peptides have also been investigated for contraception. Vaccination with sperm peptides has

caused varied degrees of contraceptive effects in animal models.[45,52] Recently, we identified a dodecamer peptide sequence on human sperm, designated as YLP_{12}, that is involved in binding to the complementary molecule, oocyte ZP3.[53] An extensive computer search in the GenBank, National Biomedical Research Foundation (NBRF) and Swiss Sequence bank databases did not reveal any known nucleotide/amino acid sequence having a complete identity or significant degree of homology with YLP_{12}, indicating that it is a novel sequence. Since we used a solubilised preparation of human oocyte ZP, which is glycosylated, as a probe to identify the YLP_{12} sequence, it is possible that it is a peptide mimetic of the carbohydrate moiety present on sperm that is involved in ZP binding. It has the amino acid sequence YLPVGGLRRIGG. The synthetic peptide based on this sequence significantly inhibited human sperm-human ZP binding in the hemizona assay, indicating its role in sperm-ZP binding in humans.

The involvement of the YLP_{12} peptide in human immunoinfertility has been examined.[54] Sera and seminal plasma from ASA-positive immunoinfertile men and antibody-negative fertile men were tested for immunoreactivity with the synthetic YLP_{12} sperm peptide using an ELISA technique.[54] Sera and seminal plasma from 73% of immunoinfertile men, and none from the fertile men, reacted with the YLP_{12} peptide. These findings indicate that the YLP_{12} sequence is immunogenic in humans and is involved in fertility/infertility. Since most infertile men and women are healthy individuals without any disease concomitant with infertility, the presence of antibodies to an antigen is indicative, though not confirmative, of the antigen's sperm specificity in humans. Thus, if an antigen, such as the YLP_{12} peptide or the FA-1 antigen, is involved in human immunoinfertility, extensive phase I clinical trials to investigate the immunopathological consequences in actively immunised subjects may not be absolutely necessary. These immunoinfertile men and women provide involuntarily vaccinated models that illustrate how a CV based on these antigens will work in humans.

To examine the contraceptive effect, a vaccine was prepared by conjugating the synthetic YLP_{12} peptide with recombinant cholera toxin B subunit (rCTB).[55] rCTB provided the T-cell epitopes to make the vaccine immunogenic. CTB has been successfully used both as a carrier and as an adjuvant for enhancing systemic and genital tract immunity. Vaccination of female mice with the YLP_{12}-rCTB conjugate by various routes (intranasal/intramuscular) induced a contraceptive state resulting in a significant reduction in litter size (overall up to 71%). All vaccinated animals showed some degree of inhibition of fertility, and the animals with high antibody titres, both in sera as well as vaginal washings, showed a complete block. Antibody reactivity was monitored biweekly and by days 305–322 it had completely disappeared from serum and vaginal washings of the vaccinated animals. Upon mating, the antibody-free animals delivered a normal litter size, indicating a regain of fertility. The immune response after vaccination was sperm specific, since the antibodies did not react with any other tissue/cell except the sperm and testis.

It was investigated whether the contraceptive effect could be reversed before the effect of the vaccine subsides, which takes 305–322 days. Administration of the YLP_{12} peptide by the intravaginal route, to neutralise the antibodies, resulted in the restoration of fertility.[55] Peptide administration did not cause a booster effect on the antibody titres. Thus, the contraceptive state after vaccination could be reversed voluntarily at any given time if desired. Besides immunocontraception, these findings may have implications in the treatment of immunoinfertility in humans.

3. Vaccines Targeting Gamete Outcome

Vaccines targeting gamete outcomes cannot be referred to as CVs; rather, these should be designated as birth control vaccines. Of the various hormones, cytokines and proteins involved in the establishment and maintenance of pregnancy, hCG has been extensively explored for immunocontraception for several reasons. Its synthesis and secretion oc-

curs only in pregnancy (or in some cancers) and it is not normally present in circulation in any significant amounts, making it a specific indicator of pregnancy. hCG plays a critical role in the establishment of pregnancy by sustaining the corpus luteum function in stimulating progesterone secretion, which maintains the endometrium's receptivity for implantation. The chemistry, structure and molecular biology of the hCG molecule have been thoroughly studied, making it an interesting and well defined target. As hCG travels via the circulation from the syncytiotrophoblastic cells to the ovaries, it is amenable to inactivation by circulating antibodies whose titres can be easily monitored.

hCG is composed of two subunits (α and β). The α subunit is common to three other pituitary hormones (LH, FSH and thyroid-stimulating hormone [TSH]), whereas the β subunit is reasonably specific to each hormone. Two approaches were investigated to develop a vaccine. One approach used the whole β subunit of hCG (β-hCG) as an immunogen,[56] and the second approach explored the 37-amino acid carboxyterminal peptide (CTP) of β-hCG. The 37-amino acid CTP sequence is highly specific to β-hCG and does not have any sequence homology with the β subunit of human LH.[57] There are advantages and disadvantages of both approaches. The advantages of the former approach are greater immunogenicity (as it has several antigenic determinants and is, thus, likely to elicit a response in a larger percentage of the population), and the higher affinity and better neutralisation capacities of the antibodies generated after immunisation. The disadvantage in choosing β-hCG, instead of CTP, is the partial cross-reactivity with human LH. However, it has been confirmed that the antibodies to β-hCG do not impair ovarian function. None of the baboons, marmosets or monkeys immunised with the β-hCG vaccine over several years showed disturbed ovulation or other adverse effects.[58] The female monkeys immunised with the β subunit of ovine LH generated antibodies that cross-reacted with monkey LH and monkey chorionic gonadotropin, but did not impair ovulation.[59] The vaccine did not elicit any autoimmune reactivity in immunised animals. It is hypothesised that LH is produced in surplus amounts and the partial neutralisation with the cross-reactive antibodies may not inhibit its biological activity. The female monkeys hyperimmunised with ovine β-LH (oLH) did not demonstrate any ovarian abnormality for up to 5–7 years of observation.[18]

The phase I clinical trials, conducted in women, using both the β-hCG vaccine[60] and the CTP vaccine[61] did not show any adverse effects. Both these vaccines produced low titres with the CTP vaccine being the weaker of the two. To increase the immunogenicity of the vaccine and to produce high affinity antibodies, β-hCG was non-covalently coupled to the α subunit of ovine LH to generate a heterospecies dimer vaccine. The vaccine underwent phase II clinical trials in three major hospitals in India. Women of reproductive age (20–35 years) and of proven fertility with at least two living children and an active sexual life were enrolled for the study. The subjects who developed antibody titres above 50 ng/mL (hCG bioneutralisation capacity) were protected from pregnancy.[62] The antibody response declined with time and fertility was regained when titres fell below 35 ng/mL. Those desiring to have a child could conceive and carry a pregnancy to term, delivering normal healthy babies.

Although the phase I and II clinical trials indicated safety, efficacy and reversibility, there are limitations/disadvantages of the β-hCG vaccine, including: (i) even after coupling to oLH, the vaccine did not produce antibodies in all women; (ii) antibodies were short lasting (30–60 days); (iii) it took 3 months to produce antibodies; (iv) long-term effects of vaccination need to be examined; and (v) the β-hCG vaccine is not a CV in a true sense. However, since hCG is produced by several tumours, the hCG-based vaccines are being investigated for their therapeutic utility in several cancers.[63-65] Immunisation with a β-hCG vaccine has improved survival in patients with metastatic colorectal cancer.[65]

4. Conclusions

Since both the developed and most of the developing nations have the infrastructure for mass im-

Table I. Advantages, disadvantages and current status of various contraceptive vaccines

Vaccine	Advantages	Disadvantages	Current status
Vaccines targeting gamete production			
Gonadotropin-releasing hormone	Applicable to both males and females Effective in several species	Affects sex steroids causing impotency, so not acceptable for humans Being a 'self' and small molecule, requires strong carriers and adjuvants to make it immunogenic	Several pharmaceutical companies have taken over for use in control of fertility in domestic pets, farm and wild animals to replace castration, and for noncontraceptive uses such as in prostatic hypertrophy and carcinoma
Follicle-stimulating hormone	Effective in inhibiting spermatogenesis in several species, including primates Can provide a male contraceptive	Partially effective; causes oligo- rather than azoospermia	Phase I clinical trials in humans indicated lack of any immunopathology; however, research is hindered because of incomplete efficacy
Luteinising hormone	Effective in both males and females	May affect sex steroids, so not acceptable for humans	May be applicable as a substitute for castration in domestic pets, farm and wild animals
Vaccines targeting gamete function			
Zona pellucida	Effective in inhibiting fertility in females of several species, including primates	Causes oophoritis affecting sex steroids and resulting in premature ovarian failure	Being successfully used for controlling populations of wild and zoo animals such as deer, horses, elephants and dogs Research being conducted to dissect out infertility effects from oophoritis-inducing properties
Sperm	Effective in causing reversible contraception in both males and females of several species, including humans Antisperm antibody-mediated immunoinfertility provides a model for how the vaccine will work in humans Provides an ideal target	No known disadvantages or adverse effects	No single recombinant/synthetic sperm antigen has shown a complete block of fertility in all the vaccinated animals. Multiple epitope vaccines are being investigated to increase the efficacy
Vaccines targeting gamete outcome			
Human chorionic gonadotropin (hCG)	First vaccine to undergo phase I and II clinical trials in humans demonstrating reversible efficacy and lack of immunopathology	It is not a contraceptive but rather an abortifacient Being a 'self' molecule it is difficult to achieve and maintain long-term high antibody titres for efficacy Variability of the immune response among vaccinated individuals	Current studies focus on increasing immunogenicity and efficacy of the vaccine – factors that are hindering phase III clinical trials Being tried for therapeutic purposes in hCG-producing cancers

munisation, the development of vaccines for contraception is an exciting proposition. Several targets are being explored for the development of CVs. These include targeting gamete production (GnRH, FSH and LH), gamete function (ZP and sperm) and gamete outcome (hCG) [table I]. CVs targeting gamete production have shown varied degrees of efficacy; however, they either affect sex steroids causing impotency and/or show only a partial rather than a complete effect in inhibiting gametogenesis. They may not therefore be suitable for use in humans in their present form. However, vaccines based on GnRH are being developed by several pharmaceutical companies as substitutes for castration of domestic pets, farm and wild animals, and for therapeutic anticancer purposes such as in prostatic hypertrophy and carcinoma. These vaccines may also find applications in clinical situations that require the inhibition of increased secretions of sex steroids, such as in uterine fibroids, polycystic ovary syndrome, endometriosis and precocious puberty.

CVs targeting molecules involved in gamete function, such as ZP proteins and sperm antigens, are better choices. Vaccines based on ZP proteins are quite efficacious in producing contraceptive effects, but they invariably induce oophoritis, affecting sex steroids. They are not acceptable for use in humans in their present form, although they are being successfully tested to control feral populations of dogs, deer, horses and elephants, and populations of several species of zoo animals. The current research for human applicability is focused on delineating infertility-related epitopes (B-cell epitopes?) from oophoritis-inducing epitopes (T-cell epitopes?). Sperm constitute the most promising and exciting target for CVs. Several sperm-specific antigens have been delineated in several laboratories and are being actively explored for CV development. At the present time, studies are focused on delineating appropriate sperm-specific epitopes, and increasing the immunogenicity (especially in the local genital tract) and efficacy of the vaccines. ASA-mediated immunoinfertility provides a naturally occurring model to indicate how a vaccine might work in humans. Vaccines targeting gamete outcome primarily focus on the hCG molecule. The hCG vaccine is the first vaccine to undergo phase I and II clinical trials in humans. Both efficacy and the lack of immunopathology have been reasonably well demonstrated for this vaccine. However, this vaccine is not a contraceptive in a true sense, but rather an abortifacient. Also, being a 'self' molecule, it is difficult to achieve high titres of antibodies in a large population that are long lasting and could have a birth control effect over a longer duration. At the present time, studies are focused on increasing the immunogenicity and efficacy of the vaccine.

One limitation that is common to all vaccines, including CVs, is to generate a protective immune response in 100% of vaccine recipients. Even if the protective immune response is generated, there are interindividual variations in the duration of immune response. This necessitates the development of a complementary simple diagnostic system to monitor periodically the generation of a protective immune response and its magnitude. The recipient of a CV has to be advised when the administration of a booster is required. Passive immunisation with preformed antibodies may be one way to overcome these limitations.[66]

The recent availability of recombinant technology, genomics, proteomics, bioinformatics, and carrier and adjuvant technologies, will help to overcome the limitations on the further advancement and development of CVs based on various targets. Besides contraceptive use in humans, and several species of domestic, farm, feral and zoo animals, these vaccines may find applications in anticancer therapies, the treatment of infertility and in various clinical situations requiring the modulation of hormone milieu. CV research is also a useful tool in understanding how a gene knockout/transgenic of a particular target molecule will function in humans if the vaccination can completely immunoneutralise that molecule.

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