The development of contraceptive vaccines.

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Abstract

The use of vaccination as a means of controlling fertility was established during the last decade with the publication of a successful phase II trial demonstrating the efficacy of this approach to family planning. However, only this one phase II trial has been completed despite a plethora of hormonal and gamete antigens that have been proposed as candidate vaccines. Improvements in the design and formulation of contraceptive vaccines are underway and will be a necessary prelude to further clinical trials.

Keywords: contraception, antifertility, vaccine, glycoprotein hormones, spermatozoa, oocyte.

1. Introduction

The United Nations has estimated that the world population passed 6,000 million on 12th October 1999, double the population in 1960, and it is predicted to reach 9,000 million in 2054 [1] (Figure 1). Such an alarming rise reflects the fact that, at a global level, 40% of couples of reproductive age do not currently practice any form of contraception [2]. Indeed, it is estimated that approximately one half of the one million new pregnancies each
day are unintended [3], and in the USA 46% of women have had at least one elective abortion by the end of their child-bearing years [4]. There is thus a clear need not only for increased use of contraception in general but also for a wider choice of contraceptive options.

Each form of contraception currently in use has some disadvantages. Sterilisation is usually irreversible, intrauterine devices are generally not recommended for nulliparous women because of an increased risk of pelvic sepsis and infertility, and barrier methods are felt obtrusive by some and usually additionally require a spermicide if they are to have a high degree of success [2]. Apart from sterilisation, the most highly efficacious methods are those based on hormonal contraception, but even these have some disadvantages. Combined oral contraceptives containing an oestrogen and a progestogen are associated with an increased risk of ischaemic [5] and haemorrhagic [6] stroke, mainly in smokers over 35 years of age, and oral contraception may also be associated with a small increase in the risk of breast cancer [7], although there is a decreased risk of ovarian and endometrial cancers [8]. One approach towards creating additional methods of contraception is the development of vaccines that will stimulate an immune response against key components of the reproductive process [9] (Figure 2). This would provide an extremely convenient form of contraception. That such an approach should work was originally indicated by the fact that either the female or male partner of some couples who are naturally infertile were shown to possess antibodies to spermatozoa [10-12]. The presence of these antibodies is not associated with any side effects apart from infertility.

The feasibility of using active immunisation to regulate fertility has now been established both in animal studies [13-16] and in clinical trials [17]. In addition to providing further contraceptive choice for the human species, many of the strategies that have been explored are also applicable for limiting fertility in other species, including livestock, feral and companion animals [13-16]. With respect to the development of vaccines for use in humans, a number of groups worldwide are carrying out research in this field, with the major activity to date based largely in India and in the USA.

2. The immune response to contraceptive vaccines

The part of an antigen recognised by the immune system either following natural infection or vaccination is referred to as the epitope. Antibodies are produced by B lymphocytes (B-cells), which, in order to be specifically activated, have to recognise B-cell epitope(s) on the antigen. The minimal essential component of a vaccine aimed at stimulating antibody production therefore needs to be an antigen which contains relevant B-cell epitopes, or a synthetic peptide either based upon or mimicking the amino acid sequence of such epitopes.
However, in order to fully activate the B-cells it is usually necessary to also recruit helper T-cells, and these usually recognise different epitopes on the antigen to those that are recognised by the B-cells. The T-cell epitopes, either as part of an intact protein or as synthetic peptides, are referred to as carriers when artificially linked to B-cell epitopes. Finally, even when the relevant B-cell and helper T-cell epitopes are present, the immune system usually requires an additional stimulus in the form of an adjuvant, a substance that enhances the immune response to the antigen. The requirement for adjuvant is likely to be even more stringent for contraceptive vaccines, as in most cases these are aimed at provoking an immune response against a ‘self’ antigen. Thus the immune system’s usual non-responsiveness (tolerance) to self antigens needs to be overcome. Finally, contraceptive vaccines would need to stimulate B-cells and helper T-cells in the absence of a response from potentially pathogenic cytotoxic T lymphocytes.

Neutralising antibodies operate either by stearic hindrance (for example, blocking binding of a hormone to its receptor or of a spermatozoon to an oocyte) or by mediating the rapid clearance of the antigen via the formation of antibody-antigen complexes. Complement-mediated cytotoxicity (for example of sperm coated with complement-fixing antibody) may also be an objective, although the presence of complement-inactivating proteins on the surface of sperm might frustrate this mechanism.

Although for some contraceptive vaccines the production of antibodies in the form of circulating IgG may be sufficient, in other cases local mucosal immune responses within the genital tract will be required [18,19]. Optimal vaccination schedules for genital tract immunity are under intensive investigation, especially given the importance of such studies to the prevention of sexually transmitted diseases. Intranasal immunisation appears to be a particularly effective route for the generation of antibody in the female reproductive tract [19].

3. Phase II clinical trials

*Human chorionic gonadotropin (hCG)*

To date, the only contraceptive vaccine which has successfully undergone a phase II clinical trial is based upon the hormone hCG [17]. Although produced ectopically by certain tumours [20], in healthy individuals hCG is only present in readily detectable amounts during pregnancy. It is initially secreted by the early blastocyst prior to implantation, and marmoset embryos which have been incubated *in vitro* with antibodies to hCG fail to implant [21]. Such observations would indicate that if sufficient levels of antibody can be elicited in the uterus then implantation of the fertilised egg would be prevented. Following implantation, hCG is produced by the trophoblast as it invades the endometrium to form the foetal placenta. The function of the placentally-derived
hCG is to maintain the corpus luteum in the ovary, a structure which produces the progesterone and oestrogens necessary to maintain the integrity of the endometrium in order that pregnancy may proceed [22].

hCG is a member of the glycoprotein hormone family, each member being a heterodimer consisting of an α-chain common to all the family members and a hormone-specific β-chain (Figure 3). The other members of the family are luteinising hormone (LH), follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH). Even though each carries a hormone-unique β-chain, there is extensive sequence homology between the β-chains of hCG and LH leading to the production of LH-cross-reactive antibodies following vaccination with the intact hCG β-chain [24]. However, the carboxy-terminal region of the hCG β-chain, referred to as the C-terminal peptide (CTP), is a unique feature of hCG.

The hCG vaccine used in the phase II clinical trial was constructed by combining the human CG β-chain with sheep α-chain to create a heterospecies dimer (HSD) [17]. This strategy endowed upon the β-chain a structure more akin to the native heterodimer than is the case for the isolated β-chain alone. Furthermore, the HSD is more immunogenic than the isolated β-chain and more faithfully mimics the native hormone in functional assays [24]. The HSD was conjugated to either tetanus toxoid (TT) or to diphtheria toxoid (DT) used as alternating carriers for the vaccine. In a small percentage of women if the same carrier was repeatedly used a phenomenon known as carrier-induced immune suppression was seen. By alternating the carriers this was avoided. The vaccine also contained alum as adjuvant, together with the sodium phthahyl derivative of lipopolysaccharide (LPS) for the first immunisation. The use of this LPS derivative led, on average, to a doubling of anti-hCG titres and increased the frequency of high responders. The first injection consisted of 150µg each of HSD-TT and HSD-DT adsorbed onto the alum, together with 1.0mg of the LPS derivative. Subsequent injections used the alternating sequence of 300µg of HSD-TT or HSD-DT adsorbed onto alum. The phase II trials were carried out in India, with informed written consent obtained from healthy females aged 20-35. Three primary injections were given at 6 week intervals, and boosters administered when necessary to maintain antibody titres above 50ng/ml of hCG bioneutralising specific antibody. Blood samples were taken every two weeks to determine the titres. One hundred and forty eight women completed the initial course of 3 injections and all individuals produced antibodies to hCG, with 80% of the women producing circulating antibody titres above 50ng/ml. Such antibodies had a high affinity for hCG (Ka approximately 10^{10}M^{-1}) and could inactivate hCG bioactivity in both in vitro and in vivo tests. In the trial only one woman with titres above 50ng/ml became pregnant, out of a total of 1,224 cycles followed. The average requirement for booster
injections to maintain contraceptive levels of antibody was every 3 months (Figure 4). Amongst those who had bioneutralising specific antibody below 50ng/ml there were 26 pregnancies. The HSD vaccine was therefore extremely successful in preventing pregnancy, but only in the moderate to high antibody responders. Although this has been the only phase II clinical trial of a contraceptive vaccine so far, the HSD trial was of extreme importance because it established the efficacy of a vaccine approach for protecting against undesired pregnancy [24].

4. Phase I clinical trials

hCG

An important question relating to the HSD vaccine is the bioneutralising capacity of the anti-hCG antibodies produced in the vaccinated individuals. The ability of the hCG β-chain-based vaccine to stimulate antibodies capable of neutralising increasing amounts of hCG in vivo was recently ascertained in women who were given daily intramuscular injections of increasing amounts of hCG from 500 to 15,000 International Units to simulate the rise in hCG seen during early pregnancy [25]. The women enrolled in this trial were selected on the basis of being incapable of becoming pregnant because they had previously undergone elective tubal ligation. Those women producing ≥40ng/ml hCG-specific antibody efficiently neutralised the injected hCG, preventing rescue of the corpus luteum and thereby permitting the normal menstrual cycle despite the presence of the exogenously injected hCG. This trial also addressed concerns that endogenous hCG might act as an undesirable booster for the vaccine. The findings demonstrated that in the absence of a carrier and an adjuvant, the injected hCG (i.e. equivalent to endogenous hCG) did not boost the anti-hCG response.

Some early versions of the hCG-based contraceptive vaccine employed the hCG β-chain in the absence of an α-chain. The β-chain was conjugated to a tetanus toxoid carrier, with alum as the adjuvant. Phase I clinical trials were initially carried out in India in the 1970’s and 1980’s, and then in Finland, Sweden, Chile, Brazil and the Dominican Republic under the auspices of the International Committee for Contraception Research (ICCR) of the Population Council [24]. These trials led to the development of the HSD discussed above, and no further trials of an hCG vaccine using the entire β-chain alone have been performed.

One approach to producing an hCG-based vaccine lacking the potential for cross-reactivity with LH is to use only the CTP segment. This strategy was employed for a World Health Organisation supported peptide vaccine which utilized the 37 C-terminal amino acids of the β-chain [26]. This peptide sequence was conjugated to diphtheria toxoid as a carrier, and the adjuvant consisted of squalene, muramyl dipeptide and Arlacel A. Phase I
clinical trials were conducted in Australia in the mid 1980’s and demonstrated that the vaccine was able to induce antibodies which bound to native hCG [27]. However, phase II clinical trials using a reformulated immunogen at a higher dose were abandoned due to an inflammatory reaction occurring at the injection site in some individuals [28]. Therefore the efficacy of a vaccine based solely upon the CTP has never been established, and it is known that the avidity of the antibodies in women immunised with CTP is lower than those in women immunised with intact hCG β-chain [29]. However, a modified version of the CTP vaccine which includes additional hCG β-chain specific epitopes in the form of an analogue of the aa38-57 loop peptide (the analogue having reduced LH cross-reactivity compared to the native sequence) has subsequently been developed [28] and is awaiting possible WHO approval for use in future clinical trials.

**Follicle-stimulating hormone (FSH)**

Following successful pre-clinical trials in both rats and bonnet monkeys, FSH has undergone phase I clinical trials for use as a male contraceptive [30,31]. Two types of vaccine were used, employing sheep FSH β-chain alone or the intact αβ-heterodimer of the sheep hormone. In both cases the glycoprotein was purified from sheep pituitary and formulated with alum as the adjuvant. Whilst the vaccine was reported as being well tolerated [30], and although all the men receiving the vaccine responded by producing FSH-specific antibodies, the antibody levels were too low to cause a reduction in sperm count. Further trials have been proposed using recombinant glycoproteins and different doses to those used previously [31].

5. **Pre-clinical studies**

**Riboflavin carrier protein (RCP)**

This vitamin carrier is present on ovulated oocytes and on spermatozoa. Immunisation of female rodents and primates with chicken RCP stimulates the production of antibodies capable of blocking pregnancy at around the time of implantation, or perhaps earlier during fertilisation [32]. Vaccination of male rats and bonnet monkeys [32] with RCP has been shown to decrease fertility, and several B-cell epitopes have been identified that could potentially be used in an RCP-based vaccine [33].

**Gonadotropin-releasing hormone (GnRH)**

GnRH (also referred to as luteinising hormone releasing hormone, LHRH) stimulates the pituitary to release FSH and LH (Figure 2). Thus, vaccines utilising GnRH would suppress both sperm and testosterone production in males. This complicates their use as contraceptive vaccines because androgen supplementation is necessary
to maintain secondary sex characteristics and libido [34]. Although GnRH-based vaccines have been produced for use as contraceptives in animals [35], their development so far for use in humans has been aimed primarily at treating sex hormone-dependent diseases including cancer [24,36]. It has also become apparent that GnRH exists in several different forms and that these isoforms can bind with differing selectivities to different GnRH receptors [37,38]. These findings clearly have implications for the specificity and safety of GnRH-based vaccines. Studies are underway to develop vaccines which will have the desired effect of eliminating or reducing fertility but without causing pathology [39].

**hCG**

The main drawback with the hCG-based HSD vaccine was that it was only effective in 80% of the immunised women. A major effort is therefore being made to develop synthetic carrier sequences that provide T-cell epitopes which, following processing, are able to bind to a much broader spectrum of MHC class II molecules and therefore promiscuously recruit the necessary T-cell help [40]. Work is also progressing on the development and use of novel adjuvants containing chitosan (a polymer of D-glucosamine and N-acetyl-D-glucosamine) for the provocation of strong antibody responses to hCG [41]. The potential for using biodegradable agents to encapsulate the vaccine is also being investigated [42]. In order to produce a financially viable vaccine in large quantities it is highly likely that the hormone will need to be produced as a recombinant molecule. Correctly folded recombinant hCG β-chain has been produced both in prokaryotic (*E. coli*) [43] and eukaryotic (monkey CVI cells) [44] expression systems. For a CTP-based vaccine, a fusion protein consisting of the CTP together with the B subunit of *E. coli* heat-labile toxin has recently been shown to be immunogenic in mice without the need for an additional adjuvant [45].

Vaccination with the full length hCG β-chain, in comparison to vaccination with the unique CTP component, results in antibodies being produced which partially cross-react with LH [24,28]. This is not surprising given that there is an 85% amino acid sequence homology between amino acids 1-110 of the β-chains of hCG and LH. In the phase II clinical trial of the HSD, although anti-LH antibodies were induced, no unwanted side effects were observed [17]. However, for a vaccine which could be administered over a substantial part of a female’s reproductive life it might be desirable to have a highly specific immunogen capable only of inducing anti-hCG antibodies. Indeed, this was the rationale for the use of the CTP in the WHO supported vaccine [28]. Another approach is to take the full length β-chain and selectively mutate amino acids involved in cross-reactive epitopes whilst maintaining those involved in the hCG-unique epitopes [46]. One such mutant, hCGβ(R68E) containing an arginine → glutamic acid replacement at position 68 in the protein sequence, has a
substantially reduced ability to induce antibodies which cross-react with LH. This diminished cross-reactivity was seen both when the vaccine was administered intramuscularly to rabbits [47] or intranasally to mice [48].

An alternative strategy to the use of a vaccine is to employ passive immunisation, with administration of preformed antibody against the target antigen. This would overcome many of the safety concerns inherent in the use of active immunisation because there is no requirement to activate the host’s own immune response. Passive administration of antibody also has the advantage that the effect is immediate, rather than having to wait for the immune response to build up before a contraceptive effect is obtained as is the case following conventional immunisation. On the negative side, passive immunisation is likely to be more expensive and requires repeated administration. Some means of allowing continual release of the antibody would probably need to be employed in order to optimise this approach. A humanised antibody capable of neutralising hCG has recently been produced for use in passive immunisation [24].

**Zona pellucida**

The principal oocyte antigens proposed for use in contraceptive vaccines are those associated with the zona pellucida glycoprotein coat of the egg [49,50]. The three major molecules are ZPA (=ZP2), ZPB (=ZP1) and ZPC (=ZP3) [51]. Zona pellucida based vaccines afford some degree of protection against pregnancy in non-human primates [52-56], although immunisation with the intact glycoproteins often has unacceptable side effects including disturbances in the menstrual cycle, hormonal profiles and folliculogenesis, and the provocation of ovarian pathology. However, immunisation of cynomolgus monkeys with rabbit recombinant ZPB, conjugated to protein A as a carrier and with muramyl dipeptide as an adjuvant, led to the production of antibodies able to inhibit sperm binding to ZP in vitro without causing pathology in vivo [52]. Unfortunately, the efficacy of this particular version of the vaccine as an antifertility agent in these monkeys was not investigated. More recently, intramuscular injection of female baboons with recombinant bonnet monkey ZPB conjugated to a diphtheria toxoid carrier, with squalene and Arlacel A as adjuvant, prevented pregnancy in all of the four animals in the study without inducing ovarian disruption [55]. Contemporaneously with this report, a study from the U.S. company Zonagen Inc. showed that immunisation of both cynomolgus monkeys and of baboons with recombinant human ZPB rendered the animals infertile for a period of ≥9 months (up to 35 months in the case of the cynomolgus monkeys) [57]. Although some of the animals in the Zonagen study exhibited disruption of the menstrual cycle, this was a temporary side effect. However, further studies in baboons did not achieve contraceptive levels of antibody in all the animals and therefore Zonagen have recently
decided to suspend their research into these vaccines in order to concentrate their efforts on other products [http://www.zonagen.com].

Another interesting study [51] showed that immunisation of outbred mice with a fusion protein containing the 200 amino acid N-terminal region of ZPA together with the sperm antigen Sp17 reduced fertility by 78%. Although in this study the Sp17 acted primarily as a carrier, these authors propose that the use of other sperm antigens together with the N-terminal region of ZPA may produce an even more effective contraceptive vaccine.

Most of the current effort on oocyte vaccines involves isolating those epitopes which are capable of activating B lymphocytes in the absence of a pathogenic T lymphocyte response [56, 58-68]. An immunodominant B cell epitope, the amino acids GPLTLELQI, is shared between pig, bonnet monkey and human ZPB. This sequence is recognised by sera from both rabbits and baboons which have been immunised using a crude preparation of pig ZP antigens [58]. Other epitope mapping studies have delineated the sequence DAPTDWCDSDLP from bonnet monkey ZPB [66] and the sequence LDPEKLTL present in human and rabbit ZPA [68] as potential candidates for a zona pellucida-based vaccine. Marmoset vaccination studies suggest, however, that the use of more than one ZP epitope may be necessary in order to achieve protection, but so far it has not proved possible to identify a combination of ZP epitopes which are able to prevent pregnancy in the absence of pathological changes in the ovaries [67].

**Spermatozoa**

Most types of contraceptive vaccines are deliberately aimed at breaking immunological tolerance to self antigens. An exception would be the immunisation of women with a vaccine based upon one or more sperm antigens. Antibodies in cervical mucus could cause agglutination of the spermatozoa and thereby prevent their access to the upper reproductive tract, whereas antibodies in the oviductal fluid could inhibit sperm-egg binding. As already mentioned, cases of naturally occurring sperm-immobilising antibodies produced by the female partner of infertile couples would seem to indicate that such strategies stand a good chance of success [10-12]. One route to the identification of antigens for use in an anti-sperm vaccine is to employ antibodies obtained from infertile couples to screen cDNA expression libraries derived from spermatozoa [69]. A large number of sperm-specific and sperm-associated antigens have been identified (Table 1) using this and other
approaches. Some of these are only present in the acrosome, a structure which becomes exposed on the sperm surface during the process of capacitation occurring within the female reproductive tract. Thus, antibodies to acrosome-restricted antigens would need to be present at sufficient concentration in the oviductal fluid to bind when the antigens are revealed. The majority of sperm antigens are, however, accessible to antibodies prior to capacitation and vaccines based on these antigens would potentially be applicable to both males and females. However, approximately $10^5$-$10^9$ sperm are present in the male and all of these would need to be neutralised. This contrasts dramatically with the situation in the upper reproductive tract of the female where only tens or hundreds of sperm would need to be inhibited. Nonetheless, optimal immunisation strategies to elicit antibody in the male reproductive tract are beginning to be explored [90], so a sperm vaccine designed for use in males may eventually prove feasible if high levels of antibody production can be achieved.

Some encouraging results have been obtained with a number of sperm antigens, but generally a sufficiently high efficacy remains elusive. Thus, a chimaeric peptide consisting of an immunodominant epitope from rabbit sperm protein 17 (SP17) and a promiscuous T cell epitope (aa94-104) from bovine RNase reversibly reduced fertility in female Balb/c strain mice from 72% in controls to 29% in the vaccinated animals [75]. Fertility in female B6 strain mice was reduced by up to 70% following immunisation with another candidate antigen, recombinant murine FA-1 [82]. Using a peptide representing amino acids 1-17 of the sperm-coating seminal plasma protein inhibin conjugated to bovine serum albumin, fertility was abolished in 75% of immunised male rats [83]. Studies in non-human primates have also usually failed to prevent pregnancy in at least some of the vaccinated animals. For instance, a chimaeric peptide consisting of aa5-19 of the sperm-specific isozyme of lactate dehydrogenase (LDH-C₄) and a promiscuous T cell epitope (aa580-599) from tetanus toxin reversibly reduced fertility in female baboons [72] from 77% in controls to 29% in vaccines. This vaccine has also been used to immunise male baboons, in whom antibodies developed which were capable of reducing sperm-egg binding [91]. However, in female cynomolgus macaques a slightly different version of the vaccine with the same peptide sequence conjugated to a T-cell epitope from tetanus toxin failed to reduce fertility [92]. In the latter study high antibody titres were present in the serum but not in vaginal fluids.

The Canadian company Immucon (www.immucon.com) is developing a vaccine intended for use by either females or males and based upon the sperm-associated antigen P26h. For use in males the aim is to produce a vaccine that will be effective for a period of 12 months, with fertility being regained 4-6 weeks following the contraceptive period. For use in females the vaccine is aimed to provide protection from pregnancy for 18 months. The company intends to commercialise this vaccine between the years 2005 and 2007. However, a
recent publication [93] describes the immunisation of male hamsters with a recombinant fusion protein comprising P26h fused to maltose-binding protein (MBP). Only a 20-25% decrease in fertilisation rates were observed when the immunised hamsters were mated with superovulated females, indicating that antibodies to the recombinant P26h show lower inhibitory properties than antibodies to native P26h.

As mentioned above with regard to hCG, an alternative approach to active immunisation with sperm antigens is to use passive immunisation with anti-sperm antibodies. This strategy has been proposed for antibodies against sperm antigens identified using a phage display library [94], and for a hybridoma-derived monoclonal antibody against a sperm glycoform of CD52; the sperm agglutination antigen-1 (SAGA-1) [88].

6. Expert Opinion

There are a number of potential advantages to using vaccination as a means of contraception (Figure 5) and the feasibility of using this approach to control fertility is now established [17]. However, there are certain issues that still need addressing (Figure 6). Predominantly these relate to safety and efficacy.

All of the contraceptive vaccines that have so far entered phase I and phase II clinical trials depend upon inducing an immune response to self antigens, in direct contrast to conventional vaccines which are aimed at inducing an immune response to a foreign pathogen. Would the antibodies required to neutralise the reproductive process lead to tissue damage? For some contraceptive vaccines, clinical trials in human volunteers have confirmed previous primate studies demonstrating a lack of adverse side-effects [17,27,31]. Additionally, those women who took part in the phase II clinical trial of the hCG HSD vaccine and who subsequently became pregnant had uneventful pregnancies and their children developed normally in comparison with their siblings [24,95]. Although there was a rise in natural autoantibodies in individuals vaccinated with the WHO hCG CTP vaccine and, in about one third of the subjects, in autoantibodies reacting with the somatostatin-producing δ-cells in the Islets of Langerhans in the pancreas, these autoantibodies were not associated with any detectable autoimmune-mediated damage [96]. Similar increases in autoantibodies are probably associated with vaccines against infectious diseases, and are caused by a combination of polyclonal B-cell activation by the adjuvant and molecular mimicry by the immunogen [96]. However, only very limited clinical trials of contraceptive vaccines have been carried out to date. Whilst the lack of major side effects seen in these trials would suggest that further clinical trials are warranted using vaccines designed to have improved efficacy, it will be necessary to very carefully monitor the volunteers for any adverse side effects.
With respect to contraceptive vaccines based on gamete antigens, pathological sequelae have occurred in a number of animal studies using sperm- or egg-based antigens. The problems of ovarian pathology which have often been seen following vaccination with intact ZP antigens have been mentioned above, but there are indications that these might eventually be overcome by using highly purified antigens or a judicious combination of peptides. Similar problems have sometimes also been noted with vaccines based upon sperm antigens. For example, although complete abolition of fertility was seen in female (i.e. immunised with a foreign antigen) and male (i.e. immunised with a self antigen) guinea pigs using affinity purified PH-20, in males this was not always reversible and led to autoimmune orchitis [79].

Regular booster injections of the contraceptive vaccines are needed to maintain effective levels of antibody [17,24]. In the absence of revaccination, protection from pregnancy is only afforded over a period of weeks or months rather than years. Thus, initial concerns that such vaccines might result in a permanent and undesired infertility have proven unfounded. Nevertheless, some concerns have been raised that these vaccines might be open to abuse. It would, therefore, be highly desirable if contraceptive vaccines could be user-administered. Recent advances in understanding mucosal and cutaneous immunity open up the possibility of self-administration by nasal spray or skin patch. Assurance that protective levels of antibody are present might be possible by using a home ELISA test to monitor antibody levels in the vagina or perhaps, if shown to be representative of reproductive tract immunity to a particular antigen, in saliva. Approaches towards generating longer term immune responses with protection assured for a minimum specified period of time are highly desirable and include the use of slow- or pulsatile-release vehicles [42]. Ideally these vaccines should guarantee a contraceptive effect for a stated period of time, say six or twelve months, and then a booster could be administered at the end of that time period without the cumbersome necessity of monitoring antibody levels. Return to normal fertility within a reasonable time frame in the absence of a booster should also be a required feature. An alternative to vaccination that has already been mentioned is to use passive immunisation, i.e. administration of preformed antibody, which would provide short term protection with a return to full fertility once the antibody has been catabolised [24]. For longer term contraception, in order to avoid repeated administration a slow release formulation will almost certainly be needed in the case of passive immunisation.

Although it is possible to screen individuals for antibody responsiveness in order to exclude the minority of people in whom a contraceptive vaccine would not prove to be effective, this would be a far from ideal solution. Ideally, the responder rate must be improved such that the overall efficacy at the population level is comparable
to other commonly used family planning methods. The most likely way of achieving this is to couple the antigen to peptides which represent helper T-cell epitopes capable of being presented by the MHC molecules of genetically diverse individuals [24,28,40,72]. Specifically regarding the hCG-based vaccine, another issue that may need to be addressed is the previously mentioned extensive sequence homology between the $\beta$-chains of hCG and of LH. In the phase II clinical trials with the HSD cross-reactivity with human LH ranged from 10-75%, but there was no cross-reactivity with FSH or TSH. Regulatory authorities might desire the removal of LH cross-reactive epitopes from future generations of the vaccine.

Overall, it would seem likely that contraceptive vaccines will eventually be produced that are generally acceptable in terms of both safety and efficacy. To ensure a high level of efficacy at the population level it may be necessary, however, to incorporate a number of different antigens into the final vaccine formulation.
Figure legends

Figure 1
World population growth

Figure 2
Gonadotropin-releasing hormone (GnRH) produced by the hypothalamus stimulates the production of follicle-stimulating hormone (FSH) and luteinising hormone (LH) from the pituitary. These gonadotropins act upon the gonads and thereby stimulate the production of the gametes. Human chorionic gonadotropin (hCG) is produced by the early embryo and appears to have a direct role in facilitating implantation as well as stimulating the ovary to maintain the corpus luteum. This ensures the continued production of progesterone by the ovary, preventing menstruation and thus maintaining the pregnancy. Each of these stages is amenable to immunologically-mediated disruption.

Figure 3
The X-ray crystallographic structure of hCG [24]. The α-chain is in blue and the β-chain in green. The position of the N-linked oligosaccharides is illustrated in a stick representation. The C-terminus (CTP) of the β-chain, which contains four O-linked oligosaccharides, was not visible in the crystal structure due to the lack of an ordered conformation in this part of the molecule. In the HSD (see text) the human α-chain shown here was replaced by the similar α-chain from sheep.

Figure 4
The anti-hCG response in four individuals (one per panel) vaccinated with the HSD. The arrows denote the day of the first and subsequent injections with the vaccine. The dots at the top of each graph represent onset of menstruation. The solid horizontal line near the top of the graphs represent cycles in which the women were exposed to the risk of pregnancy, and the dotted line near the bottom of the graph represents the 50ng/ml level of neutralising antibody required to protect against pregnancy. Reproduced with permission from TALWAR GP, SINGH O, PAL R et al.: A vaccine that prevents pregnancy in women. Proc. Natl. Acad. Sci. USA (1994) 91: 8532-8536. Copyright 1994 National Academy of Sciences, U.S.A.

Figure 5
The potential advantages of a vaccine approach to contraception.
Figure 6

Some issues relating to the use of contraceptive vaccines.

Table 1

Some examples of spermatozoa antigens that have been proposed as candidates for use in contraceptive vaccines.
References


** An extensive web-based source of information on all matters relating to population.


* Useful review


* A good overview of some important studies investigating the use of contraceptive vaccines for the control of animal populations.


** The successful phase II trial of the hCG-based HSD vaccine.


*An excellent overview of broad interest.


*Provides evidence that human chorionic gonadotropin may be a tumour-associated antigen in a range of different cancers.


*Excellent review of the current state of knowledge regarding implantation of the foetus.


*Excellent review of the current state of knowledge regarding implantation of the foetus.


*The X-ray crystallographic structure of hCG.


*Extensive overview of hCG-based active and passive immunisation.


*Provides important evidence that endogenous hormones probably will not act as boosters for contraceptive vaccines.


**The WHO-sponsored trial using the C-terminal peptide (CTP) from hCG.


**Well worth digging out this authoritative review.


* Summarises the results of the phase I trial of an FSH-based vaccine, and includes supporting data from studies in rats and bonnet monkeys.


*Further important evidence that both GnRH and GnRH receptors comprise several different isoforms, showing a hitherto unappreciated complexity.*


*Another paper indicating the potential complexity of different GnRH isoforms and receptors.*


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* This study utilized a fusion protein between sperm and egg antigens; similar approaches may eventually lead to a highly efficacious vaccine.


*A small but important study providing optimism for the use of ZP antigens in contraceptive vaccines.*


*Review from one of the leading groups investigating sperm antigens as components of contraceptive vaccines.*


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**Acknowledgments**

Research in the author’s laboratory has been supported in part by grants from The Wellcome Trust, Sir Jules Thorn Charitable Trust, and The Cleveland General and Immunological Trust.