Contraceptive vaccines: current status and problems in mass application

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Progress in the development of contraceptive vaccines for males and females is reviewed. Based on the criteria which need to be met with, none of the proposed candidate antigens meets the requirements for use as a contraceptive vaccine for human application. One of the major problems is the need for periodic injections to maintain required titre and use of an alternate method until effective titres are obtained. Some of the problems associated with active immunization approach can be overcome by the use of preformed, highly specific, potent antibodies. Some progress has been achieved in this direction by the use of humanized single chain monoclonal antibodies to human chorionic gonadotropin.

Keywords: Contraceptive vaccines, fertility control, hormones, reproduction.

REPRODUCTION is central to the propagation of species for every living organism. Over the last few decades there has been an explosive growth in the world population and it is expected to reach more than 6 billion, if corrective measures are not taken soon. This rapid increase in population in developing countries is causing serious stress on the meagre resources as the per capita availability of food is declining. In spite of the green revolution and several technological advances and their applications which have been instrumental in increasing food production and availability, the ever-increasing population in the developing countries has negated all these achievements; added to this although not undesirable, is the decline in the death rate and a corresponding increase in longevity[1]. In this review the current status in the development of male and female contraceptive vaccines for use in humans is briefly reviewed, followed by a discussion on the problems associated with the use of these approaches in mass application. Only results of studies which have potential for human use have been discussed, although several approaches are still in the experimental stages regarding their efficacy using laboratory animal models.

India ranks first among the countries which adopted an official family-planning programme as early as 1950. Over the years considerable efforts and resources were spent in making the population adopt one of the several methods of fertility control available, including the pill, barrier method, intra-uterine (IUD) device and tubectomy for females, and vasectomy and barrier method for males.

Although some progress has been made using the above methods in bringing down the birth rate in urban areas, it has not made any impact on the decrease in the overall population. This has been mainly due to the inherent problems associated with each of these methods. Although the use of oral pill as a fertility control method is quite prevalent in most of the developed countries, it is not effective in developing countries due to large-scale illiteracy and other associated problems in poorly nourished women. The barrier method has problems of one of the partners objecting to its use citing a variety of reasons. Tubectomy is the terminal method not acceptable to many. Other approaches such as female condom, emergency contraception or use of RU486 are not popular due to high cost and requirement of clinical supervision to a certain extent. Thus, none of the currently available methods are ideal. Vassal or tubal ligations are essentially terminal procedures, and recanalization surgery is expensive and return to fertility is not assured in every case. Although IUDs are quite popular in rural settings, the rate of noncompliance is quite high due to the fact that many women experience pelvic pain. For men, the only methods available are the barrier method and vasectomy. The barrier method needs the involvement of the male partner, which is lacking to a large extent. Although vasectomy is a simple surgical procedure, it is not popular due to misconceptions and the problem of fear of irreversibility. Considering these problems there is an urgent need to develop a method of contraception which is simple, easy, effective, relatively cheap, reversible and more importantly, effective over a long period for use at will, both by males and females.

It is in this context that the approach of immunocontraception has attracted the attention of investigators, considering its simplicity and effectiveness compared to other methods. The eradication of small pox using the mass vaccination approach has been the main impetus for efforts world over to develop a contraceptive vaccine. In fact, long before the idea of developing vaccines for prevention of malaria, tuberculosis or other infectious diseases was considered, efforts were in progress for the development of a contraceptive vaccine for controlling population growth, particularly in the developing countries. The success of the use of a vaccine for small pox,

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the ease of operation, cost-effectiveness, and long-term protection were some of the factors which were the driving force in the efforts to develop contraceptive vaccines. However, almost a quarter century after the idea was first considered, investment of considerable amount of resources and efforts by several competent teams, we are nowhere near the actual use of the proposed vaccines. The concept of contraceptive vaccines is radically different from the idea of conventional vaccines for infectious diseases. Unlike vaccines for infectious diseases, wherein no physiological process in the immunized subject is interfered with, the use of contraceptive vaccine is intended for interfering with a physiological process associated with reproduction in normal, healthy subjects. In addition, the antigens used are the isologous antigens from egg, sperm or certain reproductive hormones. Since these isologous antigens may not be as immunogenic as foreign antigens, as in the case of those from organisms that are responsible for infectious diseases, maintenance of the antibody titre in the immunized subjects over a period is a problem. A contraceptive vaccine essentially involves the use of a molecule, be it a protein or any other small ligand, which has a critical role in the reproductive process, and interference with its function by use of antibodies will result in impairment in reproduction. The primary requirement for a molecule to be a candidate vaccine is that it should have an indispensable specific role in the reproductive process and its absence should not have any adverse effects other than impairment in fertility. Besides, it should be easy and economically viable to produce the molecule on a large scale. The rationale of the birth-control vaccines is to induce humoral and/or cell-mediated immune response against a key reproductive hormone or a gamete antigen, or an important process involved in reproduction, so that it is interfered with in a reversible manner, and fertility is regained on decline of the immune response.

Historically, many more efforts have been directed towards development of immunocontraceptives for use in females because in a traditionally male-dominated society such as in India and some developing countries in South East Asia, family planning has primarily been considered as the woman's responsibility. In addition, our knowledge about female reproductive biology has preceded our understanding about the male reproductive system. Also, the sites of interception in the female reproductive system are more compared to those in the males. Furthermore, in the case of the female, one has to deal with only one egg, whereas in the male the proposed method should be capable of interfering with the fertilizing ability of millions of sperms.

Fertility control methods

Mammalian reproduction is exquisitely regulated by a cascade of hormones. In the case of females, follicle stimulating hormone (FSH) is necessary for follicular maturation and luteinizing hormone (LH) for induction of ovulation. Following fertilization, the blastocyst produces chorionic gonadotropin (CG), which is indispensable for maintenance of early pregnancy. Interference with action of any of these hormones by specific antibodies will interrupt fertility. In the case of females, the most successful approach has been the use of contraceptive pill, which interferes with the production of FSH and LH, and thus with the process of ovulation[2].

Female contraceptive vaccine

The concept of immunocontraception for use in females was first demonstrated by McLaren[3], and Edwards[4]. They showed that immunization of female mice against spermatozoa can significantly decrease their fertility. Subsequently, Moudgal *et al.*[5] reported the possibility of employing antibodies to LH/human chorionic gonadotropin (hCG) for the purpose of fertility control. Based on the results obtained by using specific antibodies to LH to inhibit ovulation implantation and terminate pregnancy in rodents[6–8], it was suggested that antibodies to LH which exhibit cross-reaction with hCG can be used to inhibit CG action and terminate gestation in monkeys[9] and women. Subsequently, pioneering studies by Talwar and his group[10–12] demonstrated the efficacy of antibodies to CG in terminating pregnancy in baboons. The baboon CG cross-reacts with hCG, and antibodies generated in monkeys against the vaccine β-hCG-tetanus toxiod (TT) were given to baboons in which pregnancy was ascertained by (i) appearance in circulation of CG bioactivity and (ii) persistence and raising of progesterone, which normally diminishes towards the end of the cycle. Following administration of the antibodies, the baboon CG activity in circulation declined to undetectable levels with a simultaneous fall in serum progesterone levels, resulting in termination of pregnancy which occurred about 48 h later; the characteristic sex swelling started in the next cycle. The reversibility of the effects of antibodies was established by the fact that when sufficient quantity of gamma globulins was administered to a pregnant baboon, the pregnancy continued without any adverse effects. The progeny born to this baboon and others similarly treated had normal development and on becoming adults, they were competent to reproduce.

These encouraging results from the baboon study prompted preclinical toxicology studies with hetero species dimer (HSD) vaccine, which was able to induce antibodies to hCG in all women, and the response was reversible as indicated by lack of any effect on ovulation and menstrual regularity. After this, phase-II clinical trials were carried out using HSD of the beta-subunit of hCG associated with the α-subunit of ovine LH and conjugated to TT and diphtheria toxoids (DT) as carriers. Women of

proven fertility (mothers of two children, the second child of 1 year) who were sexually active were recruited in the study after obtaining written consent. They were from amongst those who regularly attended the family-planning clinics. The eligibility criteria required subjects to be having ovulatory and regular cycles. The subjects received three injections of the vaccine intramuscularly at monthly intervals and were asked to report to the clinic twice a month for clinical and laboratory examination; one soon after the menstrual period, and the second during the third week of the cycle for a blood sampling for luteal progesterone. A test for pregnancy was mandatory in case the menstrual period was delayed. Where the titres showed a tendency to decline below 50 ng/ml, the subject had to take a booster injection to continue in the trial, or she had the option to withdraw from the trial. Following primary immunization, till such time as the antibody titres reached 50 ng/ml, the subjects had an IUD inserted by the clinic, which was removed after the titres had passed the putative threshold. It was ensured that during the trial period, no other contraceptive was used. Women were asked to maintain a menstrual diary and a record of sexual intercourse.

The vaccine was found to be quite effective in curtailing fertility when the antibody titre was above 50 ng/ml (hCG bioneutralization capacity), when the antibody titres fell to less than 35 ng/ml, fertility was regained[13,14]. This was the first proof of the principle for immunocontraception for humans in which the HSD–hCG vaccine adsorbed on alum was tested on women. The first dose of the vaccine had 1 mg of sodium phthalylated lipopolysaccharide and the ovine source of α-subunit is 'foreign' to humans. The use of this antigen resulted in antibodies totally free of cross-reaction with human FSH and human thyroid stimulating hormone (TSH) in the subjects, and the partial cross-reaction with human luteinizing hormone (hLH) did not impair ovulation.

All women generated antibodies to hCG and out of 162 subjects, 148 completed the schedule of three primary injections; however, only 119 (80%) produced titres above 50 ng/ml. A total of 1224 cycles which had a titre at and above 50 ng/ml were recorded. Only one pregnancy occurred in a woman having an antibody titre above the putative threshold, which indicated that the rest of the subjects remained protected from becoming pregnant. That the antibodies were indeed protective was established by the fact that the postcoital tests conducted in eight women who had volunteered for it in their mid-cycle, revealed that their cervical mucus score was high, and the sperm number and motility were such that in each case they should have led to conception, but for the anti-hCG antibodies in circulation. Thus vaccination was highly effective at and above the 50 ng/ml titre[15]. An important outcome of this clinical trial was that protection against pregnancy was achieved without derangement of menstrual regularity.

However, the results of this clinical trial revealed that there is a latent period of about 2–3 months for the antibodies to build up to protective levels following primary immunization, although this problem does not arise at the secondary booster stage, where a single injection raises the antibody titres within days. It is to be noted that this problem of generating antibodies of the required titre is inherent in all vaccines. The only way to provide instant coverage and protection is passive immunotherapy with antibodies of required titre. In this connection it should be noted that a microsphere formulation and novel immunization regime reduced the latent period to 15 days in rats for a gonadotropin releasing hormone (GnRH) vaccine[16].

One of the most important problems with the active immunization approach is the high variability in response of the subjects to the vaccine. The study conducted using HSD vaccine generated above protective threshold titres in only 60–80% of subjects. It is obvious that for the approach to be 100% effective, all the subjects should respond by way of producing the minimum required titres. Over the years several improvements in vaccine preparation have been reported and these include replacing the DT/TT carriers by a set of promiscuous T non-B-cell peptides. Studies using mice revealed that conjugation of β-hCG to a cocktail of peptides not only enhanced the quantum of immune response, but also was effective in generating antibody response in mice of different genetic backgrounds, which suggests the possibility of obtaining a uniform response in all subjects using this approach[17,18].

Passive immunological approaches

Some of the inherent problems associated with the hCG and other birth control vaccines that are dependent on the generation of uniform response in all the subjects can be overcome by passive immunization with preformed antibodies. For use of hCG as a vaccine, phase-II clinical trials with the HSD–hCG vaccine provided the much needed information on the titre of antibodies required for preventing pregnancy. With the availability of the basic information, it was easy to calculate the amount of antibody to be administered for efficacy. The main advantage with this approach is that the uncertainty of adequate antibody response to the vaccine in any individual in the active immunization approach is not encountered and one can be sure of the efficacy of the treatment in every subject. More importantly, the efficacy would be immediate, with no lag period for action. Another advantage would be the enhanced safety, as the characteristics of the antibody in terms of its specificity would be predetermined, and is not dependent on an individual's response to the vaccine and the type of antibody produced. The duration of the effect would be a function of the biological

half-life of the antibodies and the effectiveness will be for a limited period, and thus reversibility would be certain.

The avidity of antibodies generated in the subjects in phase-II efficacy trials revealed that an antibody having a titre of $10^{10}\ M^{-1}$ was adequate to interfere with pregnancy. Further analysis revealed that interestingly, the response in all women immunized with the vaccine and protected from becoming pregnant was to a dominant epitope characterized by monoclonal antibody 206 (MoAb206), and the proportion of antibodies in women's sera competing with MoAb206 varied from 40% to 90%. The MoAb206 cross-reacts fully with MoAb P3W80/PIPP, a mouse hybrid clone developed earlier[17,18]. Interestingly, it was found that the epitope for both these mouse monoclonals is in part overlapping and resides in a conformation in the core part of β-hCG common to and β-hCG and hCG. The MoAb P3W80 PIPP has a Ka of $3 \times 10^{10}\ M^{-1}$, and high specificity for binding with hCG; cross-reaction with hLH was < 5%. The antibodies were totally devoid of reactivity with hFSH, and other pituitary hormones (growth hormone/prolactin). The antibody was capable of neutralizing the hCG bioactivity in both *in vitro* (Leydig cell assay) and *in vivo* (immature mouse testosterone response to hCG) systems. More importantly, it is to be noted that these properties were similar to those of antibodies present in vaccinated women's sera[12]. This monoclonal was converted into a chimeric recombinant antibody (cPIPP) in which the variable part of the monoclonal was fixed with human IgG and human k chain. The MoAb P3W80 PIPP had all the desired properties for immunological intervention of pregnancy. The chimeric antibody was expressed in plants at a yield of 20 mg/kg of fresh leaves[19,20]. The antibody retained the affinity and high specificity of mouse monoclonal and was found to be bioeffective in that it prevented hCG-induced increase in uterine weight in mice. If this can be produced in bulk and administered during the required period, for example, around implantation, it could be effective in terminating pregnancy in 100% of the subjects. A further wishful development for this passive immunization approach would be to make the preformed antibodies orally effective, which will eliminate the problem of administering the antibody by injection.

GnRH as a vaccine

GnRH is a 'self' hormone like hCG and is a low molecular weight peptide. It requires conjugation with a carrier, for which both TT and DT have been employed to render it immunogenic. The native GnRH has no free amino or carboxyl group for chemical conjugation. Nonetheless, in synthesizing the peptide, either the amino or carboxyl end can be kept free for linkage. One can also introduce an additional amino acid, cysteine, at the N-terminal to facilitate conjugation. Conjugates have been made employing all these possibilities, and they are all immunogenic. The preferred mode arising from these studies was to retain the native pyroglutamate and glycinamide at the N- and C-terminals, and substitute glycine by D-lysine at position 6. There were three advantages in adopting this strategy. The catabolism of GnRH in the body occurs by enzymatic cleavage at the position-6 glycine. Its substitution by D-lysine slows down the metabolic degradation of GnRH and prolongs its biological half-life. Lysine creates the functional $\dot{\varepsilon}$ amino group for conjugation. Last, but not the least important, is the freezing of the conformation of thus synthesized GnRH analogue to the native GnRH conformation. Knowledge-based computer graphic studies indicate the bending of the GnRH chain around position 6 in such a manner that the carboxy- and amino-terminal amino acids are proximal[21].

Fertility control of both male and female animals by active and passive immunization against GnRH

One big advantage with GnRH is that it is present in males and females and its structure is identical. In both sexes regulation of gonadotropin secretion by the pituitary is by GnRH, and a vaccine against GnRH essentially can be used both in males and females. Antibodies to GnRH have been tested extensively in rodents and non-human primates for fertility control. A single injection of a monoclonal antibody neutralizing the GnRH bioactivity caused suppression of oestrus of normally cycling mice. The effect lasted for several cycles, after which normal cycling and fertility were regained[22]. In male rats immunized against the GnRH vaccine, a marked decline in testosterone and in the size of the testes with a block in spermatogenesis was observed. Primates immunized against GnRH vaccine and boosted at intervals had a long-lasting effect on curtailment of fertility. However, the undesirable effect was that their cyclicity was also affected.

The GnRH monoclonal antibody given to female dogs at the beginning of the signs of heat arrested the progression of oestrus. This was reflected in behaviour, by olfactory attraction to male dogs, vaginal cytology and sex hormone profiles. Administration of the same quantity of an irrelevant MoAb did not produce the same effect. This property of the antibody can be exploited for reversible control of fertility of companion animals[23].

In short, immunization against GnRH blocks the fertility of both male and female animals. The same vaccine is effective in rodents and primates. Both active and passive immunization can be employed to regulate the fertility in a reversible manner. However, the main problem with this approach in the male is blockade of testosterone production which is totally undesirable and in female

interruption of the cyclicity. Although it is claimed that the problem of inhibition of testosterone in the male and consequent effects can be taken care of by supplementing with testosterone, several issues such as the dose and frequency of administration and the adverse effect of testosterone on prostate need to be addressed[24].

Although it may not be possible to include hCG and GnRH-based contraceptive vaccines in the list of fertility control methods for immediate use in humans, they certainly have a potential for application in clinical situations. hCG is elevated in trophoblastic tumours, and antibodies to hCG can be employed to monitor the progress of cancer[25–27]. The expression of α and β or both subunits of hCG in a variety of cancers has been reported[28–30]. It has been suggested that cancer cells can be considered as dedifferentiated cells and thus assume embryonic character resulting in the production of oncofoetal protein, namely hCG. It is pertinent to note that the glycoprotein hormones, which include hCG, TSH, LH and FSH, all belong to the group of cysteine knot growth factors[31] and stimulate growth of certain cell types. It was reported that chago cancer lung cells make α-hCG, which acts as an growth provider to these cells. The growth of these cells was found to be inhibited by antibodies to α-hCG, which also inhibited the growth of tumour in nude mice in a dose-dependent manner when given along with tumour cells[32].

The use of anti-GnRH vaccine in controlling prostate cancer has also been reported and clinical studies with subjects generating antibodies to GnRH given as alhydrogel resulted in beneficial effect, as evaluated by decrease in the level of testosterone and prostate-specific antigen and regression of prostatic mass[33].

Use of gamete-based antigens as vaccines

Both sperm and egg carry antigens, an immune response against which would interfere with fertility. These are attractive targets, and if an effective vaccine against gamete antigens could be made, interference at prefertilization stage can be achieved. Considering this, it is no wonder that a number of laboratories are engaged in developing such vaccines. Twenty-two vaccines (11 each for sperms and oocytes) are under development. However, at present none of these has progressed to the stage of phase-I clinical trials, reflecting the difficulties in making these vaccines. The vaccine against lactate dehydrogenase isoenzyme C_4, the first antigen identified as unique to the sperm and on which extensive, elegant work has been carried out by Goldberg and colleagues over the last 28 years, has at best given 63–70% protection in primates, in spite of the use of potent adjuvants[34].

Zona pellucida (ZP) glycoprotein plays an important role during the interaction of oocyte with sperm. ZP proteins have been isolated and characterized from rodents, pig and monkeys[35]. Some of these have also been cloned and expressed. Comparison of the deduced amino acid sequence of the ZP glycoproteins from various species revealed a variable degree of sequence identity. The observation that antibodies generated against ZP glycoproteins of a given species recognize ZP glycoproteins of an other species has made heterologous immunization a feasible approach. It has been observed that antibodies against porcine ZP glycoproteins show a high degree of immunological cross-reactivity with the human ZP[36], and several studies have been carried out on porcine ZP protein. Using heat-solubilized porcine ZP, active immunization has been carried out in female rabbits, dogs and non-human primates. These studies revealed the efficacy of zona antibodies to curtail fertility[37–39].

Further studies were carried out with highly purified zona proteins as well as zona proteins expressed by recombinant technology as vaccines. The results indicated that antibodies to these zona proteins were effective in interfering with fertility in female dogs and non-human primates[40]. However, the results also revealed the presence of atretic follicles with degenerate oocytes, suggesting that the ovarian function is also affected. It has been suggested that in all the promising oocyte ZP antigens tried as vaccines, the epitopes for B- and T-cells co-exist, and activation of T-cells rapidly causes autoimmune oophritis and destruction of follicles, and thereby permanent sterility. The antigen has therefore to be carefully selected to induce only the B-cell (and not T-cell) response. With this objective in mind, a good deal of progress has taken place in obtaining the gamete antigens by DNA recombinant technology, and in the delineation of the structure of epitopes recognized by appropriate monoclonal antibodies. The ligand on the sperm, binding with the receptor on the oocyte ZP has also been identified, and this should offer a promising site for blocking the penetration of the egg by the sperm[18]. Recently, the structural aspects and use of zona antigens as contraceptive vaccines and their current status has been reviewed in detail by Gupta *et al.*[35]. Although the putative role of individual ZP proteins has been elucidated using a mouse model, it has been concluded that this may not hold good for the ZP proteins from other species. Considering this lacuna in our understanding of the precise role of various ZP proteins and the serious problem of damage to the ovary following immunization against zona antigens, the use of zona antigens as contraceptive vaccines does not appear to be promising in the near future. However, immunocontraceptive vaccine based on zona proteins have great potential for controlling wildlife population. This approach has been tried not only in controlling the street-dog population, but also to reduce the burden of rabies using recombinant dZP3 RV-G. However, in all these cases, the major hurdle is the delivery of vaccine which will provide long-lasting antibody titres[40].

Contraceptive vaccines for males

In the case of males, the important hormones needed for reproduction are FSH, LH and testosterone. In all species studied so far, testosterone is indispensable for normal spermatogenesis.

It is easy to interfere with the action of testosterone and thus sperm production by feedback inhibition of hypothalamo-pituitary axis by administering testosterone to males. This will be similar to the inhibition of pituitary LH that is needed for ovulation in the females by administering the female pill, which is now widely used all over the world by women. However, in the case of males, inhibition of testosterone production also interferes with libido, which is completely unacceptable. Thus over the last 40 years, efforts are being made to develop a method of contraception for males which does not interfere with testosterone levels.

Interfering with the action of FSH

As indicated earlier, the process of reproduction in all mammals is regulated by the hypothalamic GnRH, which regulates the production of pituitary gonadotropins, FSH and LH, that in turn act on the testis. FSH acts on Sertoli cells and LH acts on Leydig cells to produce testosterone; both FSH and testosterone together act on Sertoli cells to produce a variety of factors. These factors together act on germ cells to regulate the process of spermatogenesis, which culminates in the production of spermatozoa.

Pioneering studies by Moudgal *et al.*[41], have established that FSH is obligatory for initiation and maintenance of quantitative spermatogenesis in primates. Using adult male bonnet monkeys (*Macaca radiata*) as a model, studies were carried out by active immunization of monkeys against sheep FSH, that resulted in progressive decrease in sperm counts with the increase in antibody titre, which took about 60–90 days. All the monkeys were found to be infertile when breeding studies were carried out with proven fertile females. This was reversible when the immunization was interrupted. The most important feature of this approach is that the testosterone levels and thus libido are unaffected during the entire course of study. Thus interfering with the action of FSH appeared to be a promising method. Based on these results, a pilot study was undertaken to assess the responsiveness of human volunteers to immunization against ovine FSH[42]. All the subjects responded to immunization by producing specific antibodies to FSH, with no changes in other glycoprotein hormones and more importantly, testosterone. Seminal plasma transferrin, a marker of Sertoli cells and seminiferous tubule function, showed marked reduction following immunization against FSH.

However, this approach has some practical problems which include the availability of sufficient quantity of recombinant FSH for large-scale usage and variability in the immune response of the subjects. It is known that the response of the subjects to immunization against different antigens is highly variable and it is necessary to maintain the minimum effective antibody titre in all the subjects throughout the period, if contraception has to be achieved by blocking the action of FSH. Also, there is a need for: (i) use of an alternate method of contraception until effective titre of antibody against FSH are produced in the serum; (ii) requirement for periodic boosters to maintain the titre and more importantly; (iii) the possibility of production of antibodies which may cross-react with other hormones such as LH and TSH with which FSH shares structural homology in both α and β units that together constitute the whole functional hormone.

One way to overcome the last problem is the use of receptor for FSH as the antigen instead of FSH. Accordingly, studies were carried out by Moudgal *et al.*[43], to use the extracellular domain of the FSH receptor, which is involved in ligand binding, as an antigen in the adult male bonnet monkeys. Results revealed that in these monkeys, there was decrease in sperm count without any effect on serum testosterone levels, and all the immunized monkeys were infertile. Though this approach eliminates the problem of production of antibodies to other related hormones, it still suffers from the disadvantage of production of antibodies to other receptors for related glycoprotein hormones, since receptors of FSH, LH and TSH belong to the super family of G-protein-coupled receptors. In fact, it is known that in the clinical situation both stimulatory and inhibitory antibodies against the TSH receptor are encountered routinely, and this is a serious problem[44].

An effort was made to overcome this problem also, by choosing the highly specific regions of FSH receptor which bind to FSH, but do not share any homology with other related receptors. Three such specific peptides were expressed on filamentous phages and these phages were used as antigen in adult male bonnet monkeys. Active immunization of proven fertile adult male bonnet monkeys against phage-expressed FSH receptor-specific peptides from the extracellular domain resulted in a progressive drop in sperm count, with all animals becoming azoospermic by day 100. However, serum testosterone concentrations were unaltered during the entire course of study and animals exhibited normal mating behaviour[45]. Following the arrest of immunization, there was a decrease in antibody titre and all the animals exhibited normal fertility. However, this approach also suffers from the problem of need for periodic injections to maintain the required titre and use of alternate contraceptive methods until the required titres are attained in the serum of subjects.

An important outcome of these studies is the demonstration of inhibition of spermatogenesis and induction of infertility by neutralization of endogenous FSH without interfering with testosterone.

Interfering with sperm maturation

Active immunization against sperm antigens

It is well established that testicular sperms do not have forward motility and are incapable of fertilizing the egg. Testicular sperms have to pass through the rete tubules and epididymis to undergo the process of maturation, which involves acquisition of forward motility and fertilizing capacity. In view of this, intensive studies are in progress all over the world to understand the process of sperm maturation. These efforts, though are not completely successful, provide important clues. It has been demonstrated that the sperm membrane undergoes important changes during its transit through the epididymis. Epididymal secretory proteins are associated with the sperm surface during maturation and participate in the development of fertilizing capacity of the sperms. Their role as mediators of sperm maturation is supported by the observation that addition of epididymal proteins promotes the maturation of immature sperms by induction of fertilizing capacity and forward motility. Furthermore, exposure of mature spermatozoa to antibodies that are directed towards epididymal protein impedes the fertilizing capacity of the spermatozoa. Thus, induction of antibodies that interfere with the association of the protein to the sperm surface or mask these proteins on the sperm surface in the male tract and reduce the functional capacity of sperm could serve as potential contraceptives[24]. One such protein identified in rats is called DE (37 kDa) and is synthesized by the epithelium of the proximal epididymis. It is involved in the sperm–egg fusion process, and active immunization against this protein in both male and female rats led to infertility[46]. Indirect immunofluorescence assays with spermatozoa indicated that the immune serum was capable of recognizing DE on the fresh spermatozoa, suggesting that the circulating antibodies have access in the reproductive tract, to interfere with the function of the protein which is used as an antigen. A careful analysis of the available data revealed that protein DE fulfils many of the requisites of a candidate antigen for immunocontraception. In view of this, further studies were directed towards identification of the human functional analogues. These revealed that acidic epididymal glycoprotein-related product (ARP) was its functional human counterpart. Further studies using differential cDNA screening procedure led to the cloning of six human epididymal proteins (HE1–HE6)[47,48] and with the exception of HE5 (CD52), all of these represent completely novel gene products whose expression is highly restricted to the epididymis. These novel target molecules unique to the epididymis may serve as a target for contraception, either by developing specific drugs which interfere with its function or by developing neutralizing antibodies by active immunization. Other sperm-specific proteins that have been identified include SP-10, lactate dehyrogenase (LDH-4), PH-20 and FA-I, GP-83, GP-39, YLP-12 and SAMP-32 (ref. 49).

However, none of the studies has reached a stage where it could be inferred that a particular antigen could be the candidate for use as a contraceptive vaccine. Recently, studies employing Eppin, a testis/epididymis specific protein, as a candidate vaccine have reported some success in adult male bonnet monkeys. Seven out of nine males (78%) employed in the study developed high titres against Eppin[50]. It was observed that all of these high-titre monkeys were infertile. Five out of seven (71%) anti-Eppin titre males recovered fertility when immunization was stopped. Based on the results of this study it was concluded that effective and reversible male immunocontraception is an attainable goal.

Summary

Among the several antigens which include hCG, GnRH, and zona proteins, the only promising one for use is in females as a contraceptive vaccine is hCG. Antibodies to hCG induced in females were highly effective in preventing conception without affecting the cyclicity. However, one major problem was the variation in the titre in different subjects. This can be overcome by use of passive immunization approach which consists of administering highly specific and effective humanized antibody to hCG around the fourth or fifth day after ovulation, which will neutralize hCG needed for implantation. An extension of this approach will be the development of an orally active antibody to hCG, which can be taken like a pill on the required days. With regard to several other antigens believed to be important in female reproduction, most of them are still at the laboratory stage. As far as use of GnRH based vaccine is concerned, it suffers from a major drawback in that it suppresses secondary sexual characters and behaviour. Moreover, recent studies have shown that GnRH is produced in several non-hypothalamic sites and the exact target for this GnRH and its role is still not fully understood. Thus, GnRH as a vaccine fails to meet the important requirements to be specific, safe and effective. As far as zona antigens are concerned, it has been concluded that the 'long term active immunization studies in non-human primates with identified B-cell epitope based antigens must be undertaken to establish their safety in an unambiguous manner before these can be considered for human application'[18].

With regard to immunocontraceptive approach for males the only promising candidate is FSH. Its efficacy and reversibility without interfering with libido has been established. It has been concluded that among the possible hormones that have undergone trials, the FSH/FSHR-based formulation is the only one that does not require supplementation with exogenous androgens[51].

Although preliminary results with Eppin, which is a protein involved in sperm maturation are promising,

considerable work needs to be done in terms of its safety and the ability of the antibodies to reach in sufficient quantity at the target, i.e. the epididymis.

Although the approach of interfering with the sperm maturation process is attractive due to its non-interference with testosterone, this approach has several problems. Majority of the proteins which were found to be involved in sperm maturation are androgen-regulated and to inhibit the production of these proteins involves interference with testosterone. Alternatively, one can use the most promising protein as a candidate vaccine and to raise antibodies to interfere with the action of these proteins. Also, it is accepted now that more than one antigen needs to be used to produce a successful vaccine, and further studies are needed to delineate the type of immune response and its augmentation required to increase the contraceptive efficacy.

Conclusion

Considering the several requirements mentioned in the early part of this review, which must be met with for a molecule to be considered for use as a contraceptive vaccine, many of the candidate antigens identified so far do not fulfil the requirements. Several of them are only at the stage of establishing efficacy.

It is essential to identify the epitope in the most promising vaccine which needs all the requirements, as it is much easier to produce smaller peptides by chemical synthesis or improve their immunogenicity by recombinant technology. Production of large proteins by recombinant technology has inherent problems. However, once again efficacy studies with the identified peptide have to be carried out. After establishing the efficacy of the approach using the candidate peptide, the appropriate adjuvants suitable for human use have to be identified and suitable immunization schedules have to be developed to sustain a uniform immune response. It is essential to realize that even after a candidate antigen (peptide) is identified and produced in enough quantities by recombinant methods, procedures need to be developed to sustain an effective uniform response in 100% of the subjects (which is a daunting task). The main drawback in the application of this approach is the need for periodic injections (which will be a major problem in developed countries with high illiteracy and rural population). An alternate approach of contraception must be employed until sufficient titres are reached. In the case of sperm or epididymal antigen, enough antibodies should reach the site where these antigens are present.

Considering all the facts mentioned above, one may conclude that immunocontraception has no place in fertility control. In fact, to quote from a report by the committee on contraceptive research and development, 'There has been much discussion of the desirability of curtailing investment in immunocontraception, primarily in response to concerns expressed by some women's groups and by individual analysts, that immuno-contraception will not prove to be a biomedically appropriate contraceptive option for women in general and that research and development investment in the area would be better placed elsewhere'[1]. However, one should realize that immunocontraception is an important tool in identifying the potential target and candidate antigens. The approach has the power to provide insights into fundamental immunological and physiological structure and mechanism studies that could enrich development of the applications[52]. Following the identification of the candidate molecule using immunological approach, it is the modern approach of understanding the molecular basis of interaction of the hormone with its receptor, which will provide the basis for developing non-protein/non-peptide-based, orally active mimetics to interfere with the action of the hormone. Thus, the knowledge gained out of the immunological approach will be indispensable in developing future contraceptives, although immunocontraception itself may not have a place of its own in fertility control programmes at present.

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