The Regulation of Male Fertility: An Immunological Approach*

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Methods of fertility regulation have constituted one of the most important areas of research during the past 15-20 years. Many new drugs, devices and techniques have been developed, and research is continuing to improve these and develop new ones. There is interest in and attention paid to them all over the world by scientists, politicians and the public in the more developed and the less developed countries.[1]

The World Health Organization (WHO) has accordingly convened numerous meetings of experts over the past decade to assess the state of knowledge concerning methods of fertility regulation and to make recommendations for research that should be undertaken. Population control is an issue of some complexity, and in the past, emphasis has been placed on the development and use of contraceptive methods for women.[2] The persistence of problems with the existing methods, as well as the high incidence of births which are unplanned and unwanted, and the increasing incidence of legal abortions suggest that contraceptive and sterilization technologies are still less than adequate.[3] Judging from the numbers of vasectomies performed throughout the world, it is evident that men exist who are willing to accept responsibility for contraception. Over the past decade, increasing effort has been devoted to a reexamination of the male role in contraception. Actually there are three methods of male contraception from which to choose: coitus interruptus, the use of condoms and vasectomy.[1] But none of these methods is both safe and effective. It is against this background that WHO has been attempting to stimulate research into the development of contraceptives for males. The major aim of this research is to produce a safe, effective and acceptable method of contraception that is reversible.[4]

The theoretical prospects for controlling male fertility are numerous; however, they can be considered basically under three main topics; interference with mature sperm, interference with spermatogenesis and sperm transport, and interference with the hormonal control of testicular function.[1] Immunological methods are among the means of fertility regulation that can interfere with the aforementioned reproductive process of males. These methods will be reviewed and discussed in this paper.

Antibodies against sperm and seminal plasma antigens

The development of an immunological technique for the regulation of fertility requires the identification of a biologically active antigen which will evoke a specific antibody response. Sperm antigens appear to be the most promising sources of such antigens, for they are isolated from exposure to the body's immune system.[5] It has been known since the turn of this century that antibodies can be raised against spermatozoa, both in other species and in the same species.[6] Soon after, it was found that spermatozoa are auto-antigenic as well as isoantigenic. That is, they can be used to stimulate the production of antispermatozoal antibodies in the male producing them, or in the female of the same species. It is well known that antibodies to spermatozoa might be a cause of infertility in both males and females.[7]

In the male, the sperm can be auto-antigenic because spermatogenesis occurs after puberty when sperm acquires specific antigens. By this time the body has developed immune competence to discriminate between self antigens and nonself antigens. After the embryonic life, testes and sperm are not recognizable as self antigens, and therefore can invoke an autoimmune reaction.[8] Due to the blood-testis barrier and the basement membranes of the seminiferous tubules, sperm antigens are isolated from

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the antibody-forming tissues.[9] Auto-antibody formation against sperm may be found in male patients with occlusion of the vas efferens or in those who have undergone testicular trauma or vasectomy after which extravasation of sperm may occur. Extravasation of sperm is likely to cause only humoral antisperm antibodies; it rarely induces cell-mediated immune response against testicular tissue.[10] Antisperm antibodies have been demonstrated in both the serum and seminal fluid of infertile men. Such antibodies are commonly of the IgG class as well as the IgA class.[11] These antibodies can attach themselves to motile sperm and interfere with the ability of sperm to penetrate cervical mucus. Mainly, there are three kinds of antibodies that can interfere with sperm; cause several pathological conditions such as sperm agglutination, sperm immobilization and sperm cytotoxicity [6] In recent years, a rather high incidence of antisperm antibodies in the serum of men has been reported in cases of couples that are infertile.[12] This stiuation could be due to a reduction in the migratory potential of spermatozoa and impairment of the interaction of spermatozoa with the ovum.

Human sera that react with spermatozoa were not reactive with other cells or bodily constituents; thus, it may be stated that spermatozoa possess organ-specific auto-antigens.[13] These antigens are confined to the more mature stages of spermatogenesis. In a few studies, it has been claimed that in some antibodies were directed against so-called sperm-coating antigens.[14] These are substances that spermatozoa acquire from seminal plasma during their passage through the efferent pathway. They are absent on epididymal spermatozoa. The major sperm-coating antigen of human semen has been identified as lactoferrin, which originates, as far as it concerns the genital tract, in the seminal vesicles.[14] Like somatic cells, spermatozoa also carry non-organ-specific antigens. ABO blood-group substances are present in the seminal plasma of secretors, and they coat spermatozoa.[15] Whether spermatozoa also contain intrinsic A and B antigens on the epididymal sperm is still controversial. Other nonorgan-specific antigens are HL-Antigens (HLA), and there is some evidence suggesting haploid expression.[16] Iso-antibodies against these substances could occur; however, there is no indication that these antibodies play any role in infertility. Sperm-specific enzymes, such as lactate dehydrogenase-X (LDH-X) and hyaluronidase, can also be auto-antigenic or iso-antigenic.[17] These enzymes may be essential to the process of fertilization, and thus antibodies to these enzymes may impair fertility. The probable mechanism by which this might be achieved is through a reduction in the number of viable sperm as a result of the interaction between antibodies in the reproductive tract and LDH-X in the sperm.

Immunologic aspermatogenesis and orchitis

Voisin *et al.*[18] and Freund *et al.*[19] induced testicular lesions in guinea pigs by systemic injection of testicular homogenates and Freund's complete adjuvant (FCA). This phenomenon of experimental allergic aspermatogenesis has served as a model for autoimmune disease in general, and has also been called (experimental) autoimmune aspermatogenesis or (experimental) allergic orchitis (EAO), or other combinations of these words. The tissue damage in the testes, notably orchitis and epididymitis, was the major points emphasized. The occurrence of aspermatogenesis and the formation of specific antibodies against testicular tissue and against spermatozoa were also mentioned. One can induce auto-antibodies without consequent tissue damage, or one can have both these conditions as a result. The use of incomplete adjuvant may lead only to the production of specific antibodies, but the use of complete adjuvant (with added acid-fast mycobacteria) may lead, in addition, to the development of tissue damage. The same situation is also true for sperm suspension in place of the testicular material.

Testicular damage is limited to the germinal epithelium and proceeds in stages from inhibition of maturation to complete aspermatogenesis.[20] These types of damage are limited to adult animals; this indicates that mature germ cells are auto-antigenic, whereas immature germinal tissue is not. Histological study of the testes in such immunized animals show striking changes. The earliest abnormalities as seen by electron-microscopy are changes in the formation of the acrosome of the spermatids.[21] This develops progressively and shows more extensive disease involving degeneration of spermatids, spermatocyes and finally the spermatogonia; the seminiferous tubules may look completely empty and hollow. The Sertoli cells and the Leydig cells are generally not affected. The seminal vesicle and the prostate gland remain quite normal, as can be well understood, since they belong to a different antigenic system.

The process by which testicular damage results from the immune reaction has been much debated. Some authors[22] have described early infiltration by mononuclear cells, namely lymphocytes and histiocytes; they regard this infiltration as the primary process that leads to atrophy of the germinal epithelium. However, others,[18] while observing very little or no infiltration by inflammatory cells, did see specific damage to the germinal cells; they concluded that inflammation is a secondary phenomenon. Immunized animals develop various types of humoral antibodies as well as cell-mediated immunity. It has not yet been completely elucidated how the different types of immune response are involved in testicular damage. A predominant role for cellular immunity was originally

postulated.[23] Multiple transfer of serum from actively sensitized guinea-pigs to recipients failed to induce testicular damage.[24] Transfer of lymph nodes produced different histological changes when they were injected intravenously or into a testis. More recent evidence indicates that humoral antibodies are also involved; lesions are found most frequently and in more severe forms in animals showing both humoral and cellular immune responses. It seems that the testicular defects are a result of the simultaneous presence of both humoral and cellular immunity.[25]

Antibodies against hormones that control spermatogenesis

Testes have two functions: spermatogenesis and the production of androgen, mainly testosterone. These functions are localized in different compartments in the testes, viz. seminiferous tubules and Leydig cells, respectively.[26] In mature testes, testosterone is required for the maintenance of spermatogenesis and the production of mature and fertile sperm. Spermatogenesis and testosterone secretion are controlled by the pituitary gland through the secretion of gonadotrophic hormones: follicle – stimulating hormone (FSH) and luteinizing hormone (LH), also known as interstitial cell-stimulating hormone (ICSH).[27] FSH acts on the seminiferous tubules, influencing the initiation of spermatogenesis indirectly by modifying the function of the Sertoli cells. Thus, FSH appears to be necessary for the maintenance of established spermatogenesis. LH stimulates testosterone production by the Leydig cells; by maintaining high levels of intratesticular testosterone, it indirectly stimulates spermatogenesis. On the other hand, testosterone controls the functional activity of accessory sex organs and the development of secondary sexual characteristics.

The synthesis and secretion of the two gonadotrophins are under the control of a single hypothalamic factor, namely: gonadotrophin-releasing hormone (GnRH) or luteinizing hormone-releasing hormone (LH-RH) or luteinizing hormone-follicle stimulating hormone-releasing hormone (LH-FSH-RH), etc.[28] This hormone is secreted into the portal circulation in a pulsatile manner, and it is taken into the gonadotrophic cells of the pituitary. The hormone, which is a decapeptide, is capable of releasing both LH and FSH. This relationship has been firmly established; it is known as the 'hypothalamic-pituitary-gonadal (Leydig and Sertoli cells) axis'. Testosterone exerts a negative feedback effect on LH and FSH secretion; thus the serum gonadotrophin concentrations are controlled by the androgen of the testes. Inhibin, a second testicular hormone, which is secreted by the Sertoli cells, inhibits FSH secretion by modulating pituitary responsiveness to GnRH.[29] Negative feedback may result from decreased output of GnRH or a reduction in the pituitary gland to respond to GnRH.

FSH increases the formation by Sertoli cells of an androgen-binding protein (ABP) that the Sertoli cells secrete into the extracellular fluid surrounding the cells of the germinal epithelium.[30] ABP acts as a carrier protein for androgen (testosterone) within the lumen of the seminiferous tubules and within the epididymis. The action of FSH is thus seen as providing a concentration gradient for androgen that is directed towards the tubular lumen and a transport mechanism for androgen to reach the developing germ cells and stimulate their maturation.[27] A reduction in ABP concentration might result in impaired maturation of the germinal epithelium, resulting in the histological appearance of maturation arrest.

In the wake of these findings, attempts to disrupt spermatogenesis by an immunological mechanism have been based on the concept of interfering with the secretion of hormones of the 'hypothalamic-pituitary-gonadal axis'.[37] The antibodies thus produced form stable complexes with the hormone antigen, resulting in loss of binding of the hormone to its receptors in the tissue.

Whenever immunization with hormones is considered as an approach to fertility control in males, it should be borne in mind that only spermatogenesis should be affected while androgen production, libido and sexual behaviour should remain uninfluenced. The production of antibodies against the releasing hormone (GnRH) produces infertility. However, since GnRH is responsible for the release of both FSH and LH, complete testicular atrophy affecting spermatogenesis as well as Leydig cell function is achieved. Immunization against LH will also produce testicular atrophy due to impaired Leydig cell function and thus influence libido and sexual behaviour as well. Antibodies against testosterone render the experimental animal infertile, but all other functions of the androgenic hormones are also impaired; hence, this is not considered to be a feasible approach to male fertility control. Rabbits immunized with testosterone show atrophy of the accessory glands and loss of sexual activity.

In experimental animals, such as bonnet monkeys and rats,[32] passive immunization with anti-FSH serum inhibited spermatogenesis and reduced sperm counts. Active immunization of adult rhesus monkeys with highly purified ovine FSH resulted in the production of specific FSH antibodies and suppression of spermatogenesis.[33] In these experiments, testicular volume was markedly reduced and weight decreased by 50% without any reduction in the weight of the accessory organs or the levels of serum testosterone. The Leydig cells appeared normal, but the tubule diameter and the number of germinal cells were reduced when compared with con-

trols. Furthermore, spermatids and spermatocytes seemed to be affected in number. Thus, selective suppression of FSH by antibodies may provide a means of regulating male fertility.

Treatment with either antisera against FSH as well as ABP causes a reduction in the androgen-binding protein levels in the testes, thus impairment of testicular function.[35] In contrast to the effects of antiserum to FSH in immature rats, the administration of antiserum in adult rats over a period of 14-30 days failed to induce a reduction in ABP levels.[31] This result indicates a relative insensitivity of the testes to withdrawal of FSH in adult rats, in contrast to immature rats.

IMMUNIZATION AND ITS CONSEQUENCES

Active immunization and passive use of antibodies

In designing an antifertility vaccine, two types of immunization can be adopted: active and passive immunization. Active immunity is long-lasting, exhibits immunological memory and is likely to induce an infertile state that may not be reversible. In contrast, passive immunity is short-lived requiring repetitive immunization; in addition, the resultant infertility can probably be reversed.[36] Active immunization implies the injection of the antigen (s) and the elicitation of antibodies against the same antigen (s), whereas passive immunization implies the injection of preformed antibodies.[37] The immunological mechanism generally chosen as a potential antifertility method has been the development of active immunization procedures to produce a vaccine. This approach, as opposed to passive immunization with antibodies, is based on safety consideration and the practical use of the method. Although it is possible to produce highly specific antibodies in animals capable interfering with human reproduction, their repeated administration is considered hazardous.[38] The major problem with the passive use of antibodies for fertility regulation is the danger of the formation of immune complexes in which the antigen is present in excess. On the other hand, sensitization of humans to animal immunoglobulins after the initial injection makes possible severe reactions to subsequent injections. Because the lifespan of effective antibodies would not be more than a few weeks or months, antibody administration would have to be repeated at relatively short intervals. Passive immunization would have the advantage that antibodies could be well characterized before use and the effects of treatment could be reversed simply by discontinuing therapy.[35]

To obtain an antigen for active immunization, many factors must be considered. Among these are the fact that quantities of antigen in a highly purified state must be available, the antigenic components would need to be chemically defined, the antigens are not common to normal tissue constituents, and the modification of their natural structure is possible to improve immunogenicity.[38] The following approaches to immunological contraception are necessary: (a) the devising of adequate experimental animal models, including subhuman primates, for the study of its efficacy and safety, (b) a search for evidence of naturally occurring immunity to these substances to the antigens as possible causes of infertility.[39]

The antigens and antigenicity of the substances used for producing vaccine

Immunization with sperm antigen has been shown to provoke a variety of responses. Male animals injected with homologous or autologous spermatozoa or testis homogenate form auto-antibodies against spermatozoa that are detectable in agglutination, immobilization, complement-fixation, skin sensitization, cytotoxicity, immunofluorescence, and other tests.[6] These antigens are found to be specific for spermatozoa and are confined to the more mature stages of spermatogenesis.

Agglutinating, immobilizing and cytotoxic antibodies have been detected by techniques that usually require living motile spermatozoa and recognize membrane-bound antigens. Different patterns of agglutination suggest the existence of distinct membrane antigens, specifically on the head, the tail and the tip of the sperm's tail.[7] By immunofluorescence on fixed spermatozoa, auto-antigens can also be recognized in the cytoplasm and the nucleus. Different types of staining, such as acrosomal, equatorial, postnuclear, mid-piece and tail, also show the diversity of existing auto-antigens. By means of adsorption technique, antibodies against human spermatozoa that agglutinate and immobilize the motile sperm are directed to intrinsic antigens, i.e. testicular antigens of spermatozoa. At least two auto-antigens or iso-antigens of human spermatozoa have been chemically characterized: protamine (basic nuclear protein) and the sperm specific iso-enzyme LDH-X.[40] Surface antigens involving agglutination and immobilization reactions have so far not been succesfully solubilized and isolated. Their physicochemical properties are therefore still unknown.

Antibodies in some sera were directed against the sperm-coating antigens on the spermatozoa. The major coating antigen of human semen, as mentioned before, has been identified as lactoferrin which originates, as far as it concerns the genital tract, in the seminal vesicle.[14]

Attempts were made, particularly in the guinea pig, to isolate and characterize the antigens that are able to induce autoimmune aspermatogenesis orchitis, and therefore are called aspermatogenic antigens.[7] A highly purified glycopeptide with molecular weight of 13,000 was characterized chemically.[41] Voisin *et al.*[42] have studied

further four of these antigens: namely, antigens P, S, T and Z. Antigen P is a protein that elicits complement-fixing antibodies, and reveals anaphylaxis and orchitis. Antigen S is a glycoprotein that elicits antibodies which cause passive haemagglutination and reveals orchitis as well as delayed hypersensitivity. Antigen T is a proteolipid that elicits spermatoxic, but not complement fixing, antibodies. And lastly, antigen Z is a protein that elicits cytotoxic complement-fixing antibodies which could also cause spermagglutination. It is now apparent that a careful systemic approach to the isolation of sperm antigens is required before the identification of an immunogenic substance for a vaccine can be anticipated.

FSH is essential for the initiation of the spermatogenic process and important in the adult male for the maintenance of optimum testicular function.[27] Availability of purified gonadotrophins led to the production of neutralizing antisera, which in turn provide an immunological tool. FSH is a glycoprotein hormone which shares a common alpha-subunit with TSH and LH. Thus, long-term immunization with the whole molecule could conceivably lead to formation of antibodies to this common subunit and cause partial neutralization of the TSH and LH.[43] The beta-subunit of the FSH seems more specific, thus more suitable as an antigen for active immunization. The data on the antigenicity, cross-reactivity and neutralizing ability of such fragments have been studied.[44] The advantage of this procedure is the lack of cross reaction among the subunits of FSH, TSH, LH as well as human chorionic gonadotrophin (hCG). These studies suggest that immunization with FSH-beta-subunit rather than the whole molecule of FSH be considered as an approach to contraception. Anti-FSH will subsequently inhibit the spermatogenesis process, but libido and sexual behaviour will not be affected.

Immunopathological consequences of antifertility vaccines

The greatest hazard of an immunological method of fertility regulation is auto-immunity, i.e. an immune response directed against reproductive antigen (s) cross reacting with other tissue in the form of an immunological disease.[37] According to Coombs and Gell,[45] there are four types of tissue destruction based on immunological reaction: namely, type I reaction (anaphylactic type hypersensitivity), type II reaction (cytotoxic type hypersensitivity), type III reaction (immune complex mediated type hypersensitivity) and type IV reaction (cell mediated type hypersensitivity).

Type I reactions are produced by pharmacologically active substances release from tissue cells, such as basophils and mast cells, following reaction between an antigen and a specific antibody adsorbed to the tissue cell membrane. Most of the antibodies responsible for type I reactions in humans belong to the IgE class. Allergy to seminal plasma has been described in which urticaria, pharyngeal oedema, cardiovascular symptoms and loss of consciousness occurred in women immediately after intercourse.[46] Since sperm and seminal plasma are autoantigenic, administration of these antigens could elicit a type I reaction.

Type II reactions are classically considered to be cytotoxic in character and involve the combination of IgG or IgM antibodies with antigenic determinants on a cell membrane or a free antigen may be adsorbed to a tissue components and a cross reacting antibody subsequently combines with this adsorbed antigen. Complement fixation frequently occurs in this situation and leads to cell damage. Antibodies to the pituitary hormones may be cytotoxic to hormone-producing cells in the pituitary gland.[47] In addition, antigens common to the testes and the central nervous system have been described. Rats immunized with testicular antigens developed focal immunopathology of the brain.[48]

Type III reactions are secondary to the localization of antigen-antibody complexes in tissue; inflammation is the main feature. Almost any injected foreign antigen which elicits a detectable antibody response can lead to the development of immune complex deposition. IgG and IgM are the antibody classes normally involved in the deposition of immune complex in tissues. Classical reactions of this type are the Arthus reaction and serum sickness. Antihormone antibodies may react with hormones as they are released from cells in the pituitary gland to form insoluble immune complexes and elicit an Arthus type tissue reaction. The immune complexes may circulate; they can become lodged in tissue space, such as the renal glomeruli and mediate tissue injury. Consequently, acute or chronic glomerulonephritis may develop.[49]

Type IV reactions are produced as a result of the interactions between actively sensitized lymphocytes and specific antigens. They are mediated by the release of lymphokines, direct cytotoxicity or both. They occur without the involvement of either an antibody or complement. The classical lesion of cell-mediated immune reaction is the delayed reaction which develops over a period of 24-48 hours and which has a characteristic mononuclear infiltrate. It is felt that auto-immune aspermatogenesis orchitis characterized by lymphocyte infiltration of the testes involves type IV reactions. Generally, it is difficult in such situations to decide if the cell-mediated immunity is a primary or secondary event.[25]

Some rationable behind the immunological approach to contraception

The location of target molecules and the parts of the body through which they transit are important

considerations for deciding where the immunological attack must operate. If immunization is directed against sperm antigens, it should be expressive at either the site of their production, i.e. the testes, or of their maturation and transit, i.e. the accessory reproductive organs.[38] The presence of antibodies to spermatozoa in systemic circulation is not sufficient, since these must be functional in the genital tract, particularly in the ejaculate. Unless sperm antibodies are present in the seminal plasma, agglutination of living spermatozoa does not occur.[7] These kinds of antibodies are produced locally somewhere in the genital tract or could be derived from blood plasma by means of transudation via the accessory reproductive organs, i.e. the prostate gland and seminal vesicles.[50] The latter process particularly may account for IgG antibodies, whereas secretory IgA antibodies are locally formed antibodies.

Antifertility vaccination should be reversible to permit childbearing at predictable intervals. This is one of the differences compared with conventional vaccination against infectious diseases in which termination of immunity is not usually desired. Furthermore, in the immunization against an infectious disease the targets of the immune response are nonhuman pathogens, while in an antifertility vaccine, the targets are a human substance. Thus, procedures must be developed to render antigenic components that are normally nonantigenic, such as FSH. Sperm and some components of seminal plasma are recognized as nonself antigens.[41]

Booster injection is a procedure which is usually done in conventional immunization to increase the development of antibodies.[36] On the other hand, the development of an immunological technique for the regulation of fertility requires the identification of a biologically active antigen which will evoke a specific antibody response, and one must induce an immune response that cannot be boosted by the same natural component.[37] Thus, active immunization with FSH would be inconsistent with the rationale, since this hormone is continually present in the body and therefore immunological hazards are likely.[39]

CONCLUSION

The experimental induction of immunity against antigens of the male reproductive tract has an extensive history reaching back to the turn of the century. It has been demonstrated that varying degrees of infertility can be produced in males. The responses obtained, however, vary widely in terms of immunity and infertility depending on the type of antigenic preparation, the use of adjuvants, the method and route of immunization and the immunological techniques used to evaluate results.

Substances exist which are unique to the reproductive system; both active and passive immunization of experimental animals with some of these substances have produced antifertility effects. Although these results have been encouraging, attempts to develop a contraceptive vaccine have met with several difficulties. A high degree of efficacy is necessary and for this reason, that approach has to be dropped because of poor immunogenicity. The major problems are the identification and isolation of intrinsic specific spermatozoal antigens and the characterization of specific immune responses which are casually related to infertility. The solution to these problems is a necessary prerequisite to the development of anti-sperm immunity as a safe contraceptive method.

The feasibility of immunological approaches must be evaluated from the viewpoints of specificity and safety. Potential immunological hazards of immunization using material derived from human sources necessitate the exercise of extreme caution in the development of a contraceptive vaccine. This again requires detailed knowledge of the exact chemical nature of potential antigens. In addition, exhaustive safety testing in experimental animals including subhuman primates must be carried out before proceeding to clinical trials in humans.

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