

• REVIEW •

Development of Newer Approaches to Male Contraception: Prospects of Availability for Mass Application in the Near Future

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Progress in development of a simple, effective reversible male contraceptive has been difficult due to the indispensable role of testosterone in spermatogenesis and maintenance of secondary sexual characters. Efforts are in progress to develop methods, which do not interfere with testosterone production. These include blockade of FSH action by immunization against FSH or FSHR, and interfere with sperm maturation by interfering with estrogen action or by immunization against epididymal or sperm specific proteins. An evaluation of these various approaches for practical application is also presented.

Key words: male contraception; testosterone; sperm maturation; FSH; estrogen

Introduction

The need for control of population explosion in the developing countries is not necessary to over emphasize. Unfortunately due to lack of acceptable methods of fertility control for males, the entire burden of limiting the family size is borne by the female. This is mainly due to male dominated social system in some of the underdeveloped countries and the availability of choice of contraception methods for use by the female, in contrast to the limited methods available for use by the male^[1].

It is distressing to note that in spite of concerted efforts of several agencies for over 40 years, it has not been possible to develop an acceptable method of contraception for use by male other than condom and vasectomy. This has been mainly due to the requirement that any method developed for male contraception should not interfere with libido and secondary sexual characteristics which are dependent on testosterone.

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In the case of male, the important hormones needed for reproduction are follicle stimulating hormone (FSH), lutenizing hormone (LH) and testosterone (T). FSH acts on Sertoli cells and LH acts on Leydig cells to produce T which also acts on Sertoli cells. In all species so far studied, T is indispensable for normal spermatogenesis.

It is quite easy to interfere with the action of T and thus sperm production by feed back inhibition of hypothalamo-pituitary axis by administering T to males^[2]. This will be similar to the inhibition of pituitary LH which is needed for ovulation in the female by administering the female pill, which is now very widely used all over the world by women. However, in the case of male, inhibition of T production also interferes with libido which is completely unacceptable. Thus over the last 40 years efforts have been going on to develop a method of contraception for male which does not interfere with T levels. So far, 4 such methods are being considered and in the present paper an attempt is made to evaluate them and comment on the feasibility of practical application of these approaches.

Interfering with the action of FSH

The process of reproduction in all mammals is regulated by the hypothalamic gonadotropin releasing hormone (GnRH) which regulates the production of pituitary gonadotrophins FSH and LH which in turn act on testis. FSH acts on Sertoli cells and LH acts on Leydig cells to produce T; both FSH and T 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.*^[3] 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, which resulted in progressive decrease in sperm counts with the increase in antibody titer, which took about 60-90 d. 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 T levels and thus libido are unaffected during the entire course of study. Thus interfering with the action of FSH appeared to be a very promising method. Based on these results, a pilot study was undertaken to assess the responsiveness of human volunteers to immunization against ovine FSH. 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 T, a marker of Sertoli cell and seminiferous tubule function, showed marked reduction following immunization against FSH^[4].

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 titer in all the subjects through out the period if contraception has to be

achieved by blocking the action of FSH. Also there are needs for: (a) use of an alternate method of contraception until effective titer of antibody against FSH are produced in the serum; (b) more importantly requirement for periodic boosters to maintain the titer; (c) 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 alpha and beta units and together constitute the whole functional hormones.

One way to overcome the last problem was the use of receptor for FSH as the antigen instead of FSH. Accordingly, Moudgal, *et al.*^[5] used the extra cellular 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, sperm counts decreased without any effect on serum T 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 FSHR, LHR and TSHR belong to the super family of G-protein coupled receptors. In fact, it is known that in clinical situation both stimulatory and inhibitory antibodies against TSHR are encountered routinely and this is a serious problem^[6].

Once again 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 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 extra cellular domain resulted in a progressive drop in sperm count with all animals which became azoospermic by d 100. However, serum T concentrations were unaltered during the entire course of study and animals exhibited normal mating behavior^[7]. Following arrest of immunization, there was a decrease in antibody titer and all the animals exhibited normal fertility.

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 T.

Interfering with T production and supplementation with long acting androgens

The second method is to block the production of T which will inhibit spermatogenesis and administer sufficient quantity of T and maintain libido and secondary sexual characters. Over the years efforts have been made by WHO to explore the possibility of using a variety of T derivatives which act at the level of hypothalamus to inhibit the release of pituitary FSH and LH. This in turn causes inhibition of production of T which results in inhibition of sperm production although it interferes with libido; however, simultaneously slow releasing derivatives of androgen are given to maintain the libido and secondary sexual characteristics. Two long acting testosterone derivatives, testosterone enanthate^[8] and testosterone bucyclate, have been tested in human subjects^[9]. Preliminary results suggested that the contra-

ceptive efficacy was high even when spermatogenesis was not fully suppressed. Based on these observations, T was given as an implant every 4 months and progestin DMPA injected every 3 months had been tried in 55 couples as a male contraceptive and no pregnancies were reported over 12 months. This study provides the proof of the principle, which male contraception by hormonal steroids is possible^[10].

Blockade of T production can also be achieved by administration of GnRH agonists or antagonists^[11-14]. However, these compounds have to be given chronically and recent studies have clearly established that GnRH has action on non-reproductive tissues. Also, even this approach needs supplementation with androgen and use of alternate approaches until its efficacy is established. Considering the above problems associated with androgen supplementation, it is unlikely that the use of GnRH agonists or antagonists as a male contraceptive will be ever successful.

Hormonal approaches to male contraception based on the suppression of LH secretion require androgen replacement treatment to maintain sexual behavior and secondary sexual characteristics. Androgen supplementation not only involves large and frequent doses of T esters but also results in undesirable effects on the prostate gland. In an attempt to avoid such problems, a synthetic androgen, 7- α -methyl-19-nortestosterone (MENT), which is much more potent than T, has been developed. It was reported that the potency of MENT as an androgen was 4 times higher than that of T and anabolic effects of MENT on skeletal muscle were 10 times higher than those of T^[15]. In addition, MENT was also 12 times more potent than T in the suppression of serum gonadotropin. In the primate model, MENT was 10 times more potent than testosterone with regard to clinically desirable end points of gonadotropin suppression and anabolism of MENT had been tested in the adult male bonnet monkeys. In one of the studies, MENT was administered at different doses (25 μ g/d, 50 μ g/d, 100 μ g/d, 300 μ g/d and 1 000 μ g/d) either alone or in combination with estradiol 17 β (50 pg) via silastic implants to adult male bonnet monkeys (*Macaca radiata*) for a specified period. Blood and semen samples were collected at specific intervals and analysed for serum T and seminal parameters, respectively. The results of this study clearly revealed that administration of MENT at all doses tested resulted in suppression of nocturnal surge of T (by d 3), as well as a decrease in the number of spermatozoa (by d 45). Simultaneous administration of estradiol resulted in a reduction in the dose of MENT required to suppress the nocturnal surge. None of the male bonnet monkeys treated with MENT were able to impregnate females, clearly demonstrating the efficacy of MENT in blocking fertility of male bonnet monkeys^[16]. Preliminary studies revealed that administration of MENT even at high doses did not have any toxic effect assessed by hematological and biochemical parameters. Although, being more effective than T, the libido and secondary sexual characteristics were unaffected and there was no need for any further supplementation with androgen. More importantly in contrast to the large quantity of testosterone or its derivative used, MENT was effective in very low doses. Recently studies have been carried out in human volunteers who were

administered MENT subdermally by implants containing MENT acetate. Subjects, who received four implants each capable of releasing 400 µg/d became oligozoospermic to azoospermic without any side effect^[17]. The only problem is that it has to be delivered by injection or via slow release mechanism. If one can develop an orally active derivative of MENT, it can fulfill the requirements of a potential male pill, although alternative methods have to be used until the sperm counts decrease to the required level.

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 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. Though these efforts are not completely successful, they provide important clues. It is demonstrated that sperm membrane undergoes important changes during its transit through 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 fact that addition of epididymal proteins promotes the maturation of immature sperms by induction of fertilizing capacity and exposure of mature spermatozoa to antibodies that are directed towards epididymal protein impede 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. One such protein in rats called DE 37 kD is synthesized by the epithelium of the proximal epididymis originally localized in the dorsal region of sperm head and is involved in sperm egg fusion process. Active immunization against this protein in both male and female rats led to infertility^[18]. Indirect immunofluorescence assays with spermatozoa indicated that the immune serum was capable of recognizing DE on the fresh spermatozoa. This suggests that the circulating antibodies have accessed in the reproductive tract, to interfere with the function of the protein which is used as an antigen. Protein DE fulfils many of the requisites of candidate antigen for immunocontraception and research focused towards identification of the human functional analogues revealed that acidic epididymal glycoprotein related product (ARP) was its functional human counterpart. Further studies using differential cDNA screening procedure to target gene products of post-testicular origin testis as a negative control and after secondary screening with various tissues led to the cloning of 6 human epididymal proteins (HE1-HE6)^[19,20]. With the exception of HE5 (CD52), all of these represent completely novel gene products whose expression is highly restricted to epididymis. HE1 accumulates in the caudal region of epididymis, functions as cholesterol transfer protein and plays a major role in capacitation of the sperm which involves change in cholesterol/ phospholipid

ratio. HE2 is of caput in origin and plays a role in sperm-egg fusion. HE4 is secreted by distal human epithelium and is a decapacitation factor. HE5 is found to be highly concentrated in the caudal epithelium and caudal luminal fluid being a GPI anchored protein. Finally, HE6 belongs to the novel member of the 7 transmembrane domain receptor family whose ligand is not known. The large N-terminal extracellular domain of HE6 resembles that of glycoprotein hormone receptors suggesting a glycoprotein originating from testicular secretion might be a probable ligand. These novel target molecules unique to 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 used include SP-10, lactate dehydrogenase (LDH-4), PH-20 and FA-I, GP-83, GP-39, YLP-12, SAMP-32 and E-3^[21-24]. FA-1 has been tested for its contraceptive efficacy where active immunization against these proteins in monkeys and rabbits led to reduction in fertility. Studies involving 15 baboons immunized against the immunodominant epitope of the human LDH-4 conjugated to diphtheria toxin revealed a 75% reduction in fertility compared with control groups of animals^[25]. Sperm from LDH-4 immunized male baboons had diminished zona binding capacity^[26]. The other antigens tested in experimental animals include RSA-1, human SP17, Tcle-1, β H20 and SP10. Recently, it has been demonstrated that one of the isozymes in sperm which is very specifically expressed only during spermatogenesis is glyceraldehyde 3-phosphate dehydrogenase-S (Gapds). It is tightly bound to the fibrous sheath, a cytoskeletal structure that extends most of the length of the sperm flagellum. Disruption of Gapds expression by gene targeting revealed that the Gapds(-/-) males were infertile and had profound defects in sperm motility, exhibiting sluggish movement without forward progression^[27]. Although mitochondrial oxygen consumption was unchanged, sperm from Gapds(-/-) mice had ATP levels that were only 10.4% of those in sperm from wild-type mice. These results imply that most of the energy required for sperm motility is generated by glycolysis in sperm and its dependence on this sperm-specific enzyme suggest that Gapds is a potential contraceptive target, and that mutations or environmental agents that disrupt its activity could lead to male infertility.

Similarly, the fact that the proteins of vas deferens also have an important role in sperm function was revealed by knocking out P2X1 receptors. P2X1 receptors for ATP are ligand gated ion channels and presented on many excitable cells including vas deferens smooth muscle cells. A substantial component of the contractile response of the vas deferens to sympathetic nerve stimulation which propels into the ejaculate is mediated through P2X1 receptors. Male fertility is reduced by 90% in mice with a targeted deletion of the P2X receptor gene. There was neither sperm dysfunction nor effect on T levels^[28].

Another sperm bound antigen is riboflavin carrier protein (RCP). Riboflavin is a water-soluble B-complex vitamin, which is essential for the embryonic growth and is transported in the system by RCP. RCP is also involved in intratesticular vitamin transport to germ cells. *In vitro* studies demonstrated that the addition of RCP antibody had profound effects on sperm

motility. Sperms were progressively immobilized with time which was further aggravated by addition of guinea pig serum as a source of complement, with 70% decline in penetration capacity as assessed by zona-denuded hamster egg penetration test. Active immunization of male rats led to significant reduction in fertility and anti-RCP antibodies could be identified in the void spermatozoa^[29]. Bonnet monkeys actively immunized against RCP revealed that anti-RCP antibodies are indeed associated with acrosomal caps of the ejaculated spermatozoa and had impaired motility parameters. There was no change in the serum T and mating studies revealed that in repeated mating with fertile females during d 10-16 of ovulatory cycles, no pregnancies were recorded^[30].

However, none of the studies have reached a stage where it could be inferred that a particular antigen could be the candidate antigen for use as a contraceptive vaccine. Very recently, studies of employing Eppin, which is 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 titers to Eppin (epididymal protease inhibitor)^[31]. It was observed that all of these high-titer monkeys were infertile. Five out of seven (71%) anti- eppin titer males recovered fertility when immunization was stopped. This study demonstrates that effective and reversible male immunocontraception is an attainable goal.

Blockade of estrogen action

Estrogen is no longer considered as a female “only” hormone. In the adult testis, estrogen is synthesized by the Leydig cells and germ cells, and a relatively high concentration of estrogen is found in rete testis fluid. Estrogen receptors exist in the testis, efferent ductules and epididymis of most species. Disruption of the estrogen receptor alpha either in the knockout (α ERKO) or by treatment with a pure antiestrogen, results in dilution of cauda epididymal sperm, disruption of sperm morphology, inhibition of sodium transport and subsequent water reabsorption, increasing secretion of Cl⁻, and eventually decreasing fertility. In addition to this primary function of regulation of luminal fluid and ion transport, estrogen is also responsible for maintaining a differentiated epithelial morphology. This strongly suggests that estrogen or estrogen receptor alpha mediated action is an absolute necessity for fertility in the male^[32].

The observation that estrogen plays an important role in sperm maturation has suggested the possibility of interfering with estrogen action at epididymal level. Accordingly one can use estrogen receptor antagonists to block the action of estrogen. In fact, our own studies which involved chronic administration ICI 182870, a specific estrogen receptor antagonist, in adult male bonnet monkey, revealed a drastic decrease in sperm motility^[33]. ICI treatment in male rats also led to decreased fertility^[34]. Similarly, following chronic administration of TMX (tamoxifen), another E₂ receptor antagonist, the adult male bonnet monkeys were found to be infertile with severe abnormalities in sperm morphology and decreased motility^[35]. TMX treatment in male rats also affected sperm fertilizing ability^[36,37]. While

these results are very encouraging one has to remember that estrogen has several sites of action in the male including brain and unless a specific estrogen receptor blocker which is targeted only to epididymis can be developed, this approach is not going to be practical.

While the approach of interfering with the sperm maturation process is very attractive due to its non interference with T, this approach has the similar problems. Majority of the proteins which were found to be involved in sperm maturation are androgen regulated to inhibit the production of these proteins involves interference with T. Alternatively one can use the most promising protein as a candidate vaccine and to raise antibodies to interfere with action of these proteins. Also, it is accepted now that more than one antigen need to be used to produce a successful vaccine and further studies are required to delineate the type of immune response and its augmentation required to increase the contraceptive efficacy. However, as in the case of using FSH, as a contraceptive vaccine, the response to the antigen is highly variable due to genetic variability, sufficient titers which can reach the epididymal lumen, have to be maintained by periodic injections. The availability of sufficient quantity of antigen and the need for frequent injections and need to use alternative approaches during early periods of immunization are major hurdles in making this approach a reality.

Blocking passage of sperms

Blocking vas deferens by injecting styrene maleic anhydride which polymerizes *in situ* has also been proposed as a method of male fertility control^[38]. Vas occlusion involves injection of the desired compound through the skin to form plugs in vas deferens. It was reported that injection of polyurethane directly into the surgically exposed sperm duct in baboons achieved azoospermia in most of the animals by one month^[39]. The plugs were removed surgically after 9-18 months to determine the reversibility. A clinical study conducted in 10 men in China, revealed that most of the men achieved azoospermia or severe oligozoospermia, although the rate of disappearance of sperm was slow. This has led to further studies to establish whether this is related to the size of the plug or to other factors. Studies carried out in China has provided evidence that polyurethane plugs are easily removed after 1-2 years in place which sperms returned into the ejaculate and high rate of pregnancy could be achieved^[39].

Current status

It is evident from the above cited new approaches for male contraception that considerable progress has been made in developing more than one method. However, each one has its merits and demerits. Among the various approaches, immunization using either FSH or FSHR or peptides appears very attractive as the efficacy and reversibility has been clearly established. The eradication of small pox using the mass vaccination approach has been the main impetus for our effort to develop a contraceptive vaccine. A contraceptive vaccine essentially involves the use of a molecule either a protein or small ligand which has critical role in the reproductive process. Interfering with its function by using antibodies would mean

an 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 effect other than impairing fertility. Besides, it should be easy and economically viable to produce on a large scale. If we consider the above prerequisites, many of the candidate antigens identified do not fulfill the requirements. Several of them are only at the stage of establishing efficacy. Only the approaches of blocking FSH action by using FSHR peptides or the use of eppin have reached a stage of identifying the immunogenic epitopes. It is essential to identify the epitope, as it is much easier to produce them by chemical synthesis by recombinant technology. Production of large proteins by recombinant technology has inherent problems. However, once again efficacy studies with the identified epitopes have to be carried out. After establishing the efficacy of the approach using the candidate epitope(s), the appropriate adjuvants suitable for human use have to be identified and suitable for immunization schedule have to be developed to sustain a uniform immune response. It is very essential to realize that even after a candidate antigen is identified and produced in enough quantities by recombinant methods, methods are 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). In the case of sperm or epididymal antigen, enough antibodies should reach the site where these antigens are present.

With regard to hormonal contraception using androgen to block hypothalamo pituitary axis, several issues should be considered before it can be employed for mass application. These include the assessment of side effects due to the androgens and progestins used, particularly on the prostate as it is an androgen responsive tissue in addition to other anabolic effects due to the steroids; the quantity and duration need for androgen supplementation and mode of delivery as contraceptive efficacy studies by WHO revealed that there is ethnic difference between Chinese Indonesian men and Caucasians. It was found that the former were more susceptible to the contraceptive effects of steroids^[39]. Certainly a more detailed study is warranted with different populations.

Also azoospermia has been more difficult to achieve than oligospermia. Although the contraceptive efficacy of azoospermia has been convincingly established the efficacy of severe oligozoospermia is still the subject of clinical studies. The demonstration of contraceptive efficacy of oligozoospermia is considered as one of the more critical steps towards the development of male contraceptive.

The use of GnRH agonists or antagonists to interfere with T production and thus fertility also needs androgen supplementation. These compounds have to be administered chronically and recent studies have clearly established that GnRH has action on non-reproductive tissues.

With regard to the approach of interfering with sperm maturation by blocking estrogen action at the level of epididymis, an appropriate selective estrogen receptor modulator (SERM)

has to be developed which can be targeted only to epididymis without any side effect on the other estrogen responsive tissues^[40-42]. Although this is certainly feasible, it is still a long way to go before it can find practical application.

The use of agents to block the passage of sperms in vas deferens is attractive but can only be considered as a modification of vasectomy and needs surgical skills for the applicator. The reversibility also has to be assured.

Concluding remarks

The practical application of methods of male contraception based on the above findings is not going to be reality in the near future. The time frame for any of the approaches discussed above for practical use cannot be defined. Among the various approaches, the use of MENT has more promise as it is very effective at very low doses and does not need androgen supplementation. MENT or more effective derivatives can be administered by a slow release mechanism in such a way that one administration is adequate for one year. Similarly, the approach of active immunization against sperm antigen, epididymal antigens, FSH or FSHR peptides opens up the possibility of investigating the interaction between the receptor with its ligand so that a small non-toxic non-peptide organic compound which can inhibit the interaction can be developed as in the case of SERM. Even if any one of these methods are successful, one is always faced with the problem of need for an alternate approach until one can rely on the efficacy of the method, making it not a very friendly user approach. Considered this, the option left for male contraception for the time being is the age old approach of barrier method or vasectomy.

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