

Review

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## Sperm specific proteins-potential candidate molecules for fertility control

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### Abstract

The increase in population growth rate warrants the development of additional contraceptive methods that are widely acceptable, free from side effects and less expensive. Immunocontraception, and in particular the targeting of antibodies to gamete-specific antigens implicated in sperm egg binding and fertilization, offers an attractive approach to control fertility. The development of a contraceptive vaccine based on sperm antigen represents a promising approach to contraception. In mammals, fertilization is completed by the direct interaction of sperm and egg, a process mediated primarily by sperm surface proteins. Sperm have proteins that are unique, cell specific, immunogenic and accessible to antibodies. A few of the sperm specific proteins have been isolated and characterized. The antibodies raised against the sperm specific antigens have proved to be extremely effective at reducing sperm-egg interaction *in vitro*; fertility trials in sub-human primates would eventually prove the effectiveness of the sperm antigens in terms of contraceptive efficacy.

### Introduction

The rapidly expanding global population has turned the attention of family planning and associated reproductive health programmes and providers towards providing safe and reliable method that can be used to limit family size. The human population is projected to exceed a phenomenal 10 billion by the year 2050 AD. Further, major increase in human population will take place in developing countries as compare to developed countries. Therefore, it is necessary to develop new, safer, effective and more economical method of contraception. As a novel concept, contraceptive vaccines have been proposed as one of the possible strategy for controlling fertility. Both gametes (sperm and oocyte) have proteins on the surface that are unique, cell specific, immunogenic and accessible to antibodies. Immunological interaction with such molecules can cause block of sperm penetration and thus fer-

tilization. The present article will focus on the present status of the development of contraceptive vaccine(s) based on protein, derived from sperm cell.

Development of vaccine based on sperm antigen represents a promising approach to contraception [1]. It has been demonstrated that sperm cell has both isoantigens/ autoantigens [2] potential to generate an immunological response in both men and women, which is capable of causing infertility. Experimental evidence demonstrating reduction in fertility has been documented using preparation of autologous or isologous sperm resulting in circulating anti-sperm antibody. Up to 70% of vasectomized men form anti-sperm antibodies and 2–30% of infertility may be associated with the presence of circulating anti-sperm antibodies in the male or female subject of an infertile couple [3]. For developing sperm based vaccine, whole

sperm cell cannot be used to immunize because sperm as a cell share common protein with somatic cell [4]. Therefore only those proteins, which are, sperm specific, can be used to develop as a candidate molecule for immunocontraception.

In mammals, fertilization is completed by the direct interaction of sperm and egg, a process mediated primarily by gamete surface proteins. Fertilization is a complex process requiring the sperm to undergo a cascade of events including capacitation, acrosome reaction, binding to zona pellucida (ZP), penetration through ZP, and fusion with plasma membrane of the oocyte [5]. Therefore, an essential task in the study of sperm-egg interaction is an exploration of the capabilities of a distinct set of surface proteins, which are gamete specific. The sperm ZP binding is pivotal tissue and species-specific step of the fertilization process, and molecules involved in this event constitute attractive candidate for the contraceptive vaccine development.

## Discussion

The development of male gamete, the spermatozoa is a unique and probably one of the most complex differentiation processes in higher eukaryotes. Though the formation of a fully functional sperm involves a series of steps occurring in different parts of the testis, the main act of spermatogenesis occurs within the seminiferous tubules. The processes of spermatogenesis results in highly specialized cell structures such as the head and the flagellum. The sperm head constitutes sperm nucleus and the acrosome surrounded by moderate amount of cytoskeletal component and cytoplasm. The flagellum mainly consists of cytoskeletal structures, including the axoneme, the mitochondria sheath, the outer dense fiber and the fibrous sheath. Application of a sperm specific protein in the development of a contraceptive vaccine is contingent upon its sperm specificity, involvement in fertility. A few of the sperm specific antigens have been isolated and characterized by biochemical and immunological techniques and genes encoding for some of these antigen have been cloned and sequenced [6]. Notable among these are LDH-C<sub>4</sub> [7], PH-20 [8], SP-10 [9], SP-17 [10], FA-1 [11], FA-2 [12], NZ-1 [13], HSS now referred as SPAG9 [14], HI now referred as AKAP-4 [15], rSMP-B [16], SAMP14 [17], SAMP32 [18], TSA-1 [19], CV-1 [20].

Anti-sperm antibodies in infertile subjects have been shown to be associated with infertility [3]. In our laboratory we have screened panel of sera from infertile women. In immunoblots, one of the major and more frequently identified band around 71 kDa protein in human sperm extract was identified by serum from infertile women who had anti-sperm antibodies, who was otherwise healthy, had normal endocrine profiles and no fallopian tube con-

striction. This serum had neither autoimmune factors nor reactivity with any other human somatic tissues. Anti-71 kDa antibodies showed cross reactivity with other species of sperm, further demonstrated inhibition of sperm attachment to oocytes in an *in vitro* mouse system. In an attempt to identify a candidate protein molecule having specific function in sperm or sperm-egg interaction *per se*, exclusively expressed in testis, a testis cDNA expression library was screened using polyclonal antibodies against sperm proteins showing head to head sperm agglutination and surface localization on live human sperm [14]. A novel gene coding for human sperm surface protein SPAG9 (Sperm associated antigen 9) was cloned. Tissue specific expression studies demonstrated the presence of SPAG9 transcript only in the testis [14]. Further, studies are in progress to examine the role of the SPAG9 gene product during reproduction.

Sperm surface PH-20 antigen has been cloned and studied in a number of species including the guinea pig [21], rat [22], mouse [21], horse [23], macaque and human [24]. PH-20/2B1 antigen appears to be a bi-functional sperm plasma membrane protein. Firstly, its hyaluronidase activity allows acrosome intact sperm to penetrate the cumulus cell layer surrounding the oocyte [25] and secondly, it appears to be required for acrosome-reacted sperm to bind to the zona pellucida [26]. Antibodies generated against PH-20 and 2B1 significantly reduce sperm-zona pellucida binding *in vitro* assay [27,28]. The immunization trials in both male and female guinea pigs were reported to lead to infertility in all immunized animals [29]. However, it has subsequently been shown that autoimmune orchitis was induced in the male guinea pigs with the resultant infertility attributable to the absence of sperm in the epididymis [30]. Further, immunological response of female macaques to PH-20 sperm protein following injection of recombinant protein or synthesized peptides was studied [31]. It was reported that antigens derived from synthesized peptides and recombinant proteins representing selected regions of PH-20 molecule mounted an immune response and the circulating antibodies from immunized animals recognized macaque sperm surface PH-20 on western blot and were shown by indirect immunofluorescence to bind to the surface of macaque sperm. This data suggested that the selected regions of PH-20 molecule could be used as vaccine component.

SP-10 is a sperm-specific acrosomal protein that was first identified in the human using a monoclonal antibody [9] and subsequently cloned and sequenced in human [32], mouse [33], fox [34], baboon and macaque [35]. Immunological studies have also identified SP-10 on bovine and porcine sperm. Antibodies to SP-10 inhibit bovine *in vitro* fertilization by reducing sperm-zona secondary bind-

ing [36] and a monoclonal antibody HS 63, which was subsequently also found to recognise the mouse orthologue of SP-10, inhibits mouse *in vitro* fertilization [37]. Both of these antibodies inhibit human sperm penetration of zona-free hamster eggs. SP-10 as an immunogen have been used for immunogenicity studies employing different routes. Specific anti-SP-10 antibodies have been measured in vaginal secretions of mice following oral immunization with attenuated *Salmonella* sp. expressing human SP-10 [38] and in oviductal fluid of macaques following intramuscular immunization [39]. Antibody levels in the oviduct are of particular relevance since SP-10 is localised within the acrosomal compartment [40] and the outer acrosomal membrane complex [41] and is therefore only accessible to antibody after the acrosome reaction has been initiated. SAMP14, a novel, human acrosome membrane associated, GPI anchored member of the LY-6/uPAR receptor superfamily has been characterized and shown to have a role in sperm-egg interaction, antibodies against SAMP14 inhibit both binding and fusion of human sperm to zona free hamster eggs [17]. Panel of 2-D composite images of human sperm proteins (Encyclopaedia of human sperm proteins) have been generated, which are being characterized and are in a process of developing target molecules to interfere the sperm egg interaction with various strategies [42,43].

Testis specific lactate dehydrogenase LDH-C<sub>4</sub> has been well characterized. The sperm specific isozyme of lactate dehydrogenase LDH-C<sub>4</sub> was purified from mouse testis and was shown to suppress the fertility of female mice, rabbits, and baboon [44]. For the last two decades, LDH-C<sub>4</sub> has been reported to be an excellent antigen for use in a contraceptive vaccine. In addition, synthetic peptide of LDH-C<sub>4</sub> has been shown to induce a contraceptive effect as much as 75% following vaccination of female baboons [45,46]. Independent fertility trials with same LDH-C<sub>4</sub> peptide conjugated to a T-cell epitope from tetanus toxin (TT) in non-human primates were carried out [47]. It was demonstrated that in cynomolgus macaques, vaccination with LDH-C<sub>4</sub> did not reduce fertility.

Fertilization Antigen-1 (FA-1) is a sperm specific glycoprotein originally isolated from human and murine sperm and was subsequently also found to be expressed on sperm from rabbit, bull and macaque. The cDNA encoding murine FA-1 [48] and human FA-1 [49] has been cloned and sequenced. Antibodies to FA-1 inhibit *in vitro* fertilization in all of the above species by interfering with sperm zona interaction [50] and immunization of female rabbits and mice with FA-1 does appear to reduce fertility *in vivo* [51]. Novel human testis specific contraceptive vaccine antigen [20] was identified by subtracting single stranded cDNA of human testis with poly(A<sup>+</sup>) RNA of human peripheral blood cells. Rabbit recombinant CV

antibodies inhibited human sperm penetration of zona-free hamster oocytes, as well as human sperm binding to human oocyte zona pellucida [20,52]. Recently, testis specific antigen (TSA-1) expressed in murine sperm [53] and human sperm [19] has been cloned and characterized. In functional bioassays, recombinant TSA-1 antibodies inhibited the acrosome reaction [19] and sperm egg binding in *in vitro* assays [53]. These findings indicated that the testis/sperm specific protein has role in human sperm function and may find clinical application in the contraceptive vaccine development.

The MDC (Metalloprotease/Disintegrin/Cysteine-rich) protein are a rapidly growing family of integral membrane proteins, all of which contains a number of distinct conserved features including a metalloproteinase-like domain, a Disintegrin-like domain, a Cysteine-rich domain, a pro-domain and a transmembrane domain (also known as ADAM family) [54]. ADAM family members expressed predominantly in the testis are fertilin  $\beta$  (ADAM2), cyritestin (ADAM3; tMDC I), ADAM 5 (tMDC II), ADAM 6, ADAM 16 (xMDC 16), ADAM 18 (tMDC III), ADAM 20, ADAM 21, ADAM 24 (testase 1), ADAM 25 (testase 2), ADAM 26 (testase 3), ADAM 29 and ADAM 30. Five of these ADAMs (fertilin  $\beta$ , cyritestin, ADAM 5, ADAM 16, ADAM 18) are known to be expressed as proteins present on male germ cells and/or mature sperm of at least one species. Other ADAMs such as fertilin  $\alpha$  (ADAM 1) is expressed in other tissues but is also present on sperm [55].

Among the cell adhesion events that are mediated by ADAMs are the interactions between mammalian gamete plasma membranes during fertilization. On mouse sperm, there are at least three ADAMs that appear to participate in sperm-egg binding: fertilin  $\alpha$  (ADAM1), fertilin  $\beta$  (ADAM2), and cyritestin (ADAM3) [56]. Evidence for a specific role of fertilin  $\alpha$  in sperm-egg adhesion and/or membrane fusion is not very clear. Fertilin  $\alpha$  may have a role in adhesion, as the fertilin  $\alpha$  cysteine-rich domain appear to participate on cell adhesion, as has also been found for ADAM 12 [57]. A recombinant form of fertilin  $\alpha$  corresponding to the complete extracellular domain (*i.e.* the disintegrin-like domain, the cysteine-rich domain, and the EGF-like repeat) inhibits sperm-egg binding more effectively than does a shorter form with a truncated disintegrin-like domain [58], suggesting that the presence of disintegrin-like domain in recombinant fertilin  $\alpha$  enhances the ability of the recombinant fertilin  $\alpha$  protein to inhibit sperm-egg binding. With regards to gamete fusion, CD9 on the egg appears to be critical (59–61) although the mechanism of this protein's role in membrane fusion is unclear. On the sperm, fertilin  $\alpha$  was originally speculated to have hydrophobic fusion peptide [62] but in fact does not appear to be required for sperm-egg

fusion, as fertilin  $\beta^{-/-}$  sperm lack fertilin  $\alpha$  and are yet capable of sperm-egg fusion [63].

Fertilin  $\beta$  was one of the first "cellular disintegrins" identified and the best-characterized candidate molecule that mediates sperm-egg membrane fusion. Fertilin  $\beta$  is one subunit of the dimeric sperm antigen that cross reacts with one of the fertilization inhibiting antibodies, known as PH-30 (fertilin  $\alpha$  is the other subunit) [64]. Fertilin  $\beta$  and cyritestin have similar amino acid sequence in their disintegrin loops. Based on studies with synthetic peptides and anti-disintegrin loop antibodies, both fertilin  $\beta$  and cyritestin appear to utilize their disintegrin loop sequence to interact with the egg membrane [58,65]. Incubation of mouse eggs with recombinant fertilin  $\beta$  prior to insemination inhibits sperm-egg binding during IVF [64]. Likewise, incubation of sperm in antibodies against the disintegrin domain of mouse fertilin  $\beta$  prior to insemination also inhibits sperm-egg binding and fusion [65]. Anti-fertilin  $\beta$  antibodies also reduced the incidence of fertilization of rabbit eggs *in vitro* [64]. Finally, knockout male mice lacking either fertilin  $\beta$  [67] or cyritestin [68] are severely sub-fertile. Fertilin  $\beta^{-/-}$  sperm show greatly reduced levels of binding to the egg plasma membrane, sperm-egg fusion, migration from the uterus into the oviduct and binding to the egg-zona pellucida [67], but the few that bind are capable of sperm-egg fusion. Cyritestin $^{-/-}$  sperm show reduced ability to bind to the eggs extracellular matrix and the zona pellucida [68].

A number of glycoproteins on the surface of spermatozoa are acquired from epididymal secretions during transit through epididymis. It is quite likely that such proteins may have either a protective role or a modulation role in sperm maturation. Some have been specifically implicated in sperm-egg interactions at fertilization such as human gp 20 [69] and rat DE [70]. Protein DE (also known as acidic epididymal glycoprotein, AEG) is a 37 kDa glycoprotein that associates with the dorsal region of the sperm head during epididymal maturation. Antibody to protein DE/AEG significantly inhibits sperm penetration in zona free eggs in *in vitro* assays.

## Conclusion

In conclusion, candidate antigens have been, and are continuing to be, identified that have potential to interfere with biological process that are fundamental to the reproductive process, fertilization. It is evident from the studies that the antibodies against the sperm specific antigen have proved to be extremely effective at reducing sperm egg interactions *in vitro*. Immunizations of female subjects with sperm specific proteins lead to block of fertility in several animal models. One mechanism by which sperm antigens may cause immunoinfertility is to induce antibody formation in the female reproductive tract and in

turn block single or multiple points of sperm and egg interaction. Therefore, it may be possible, by using various formulations in delivery vehicles with suitable carriers and adjuvants and expression vectors to challenge the gut-associated lymphatic tissues (GALT) for stimulation of B-lymphocytes committed to local immunity in the reproductive tract. This, in conjunction with a systematic vaccine, could ensure antibody existence at all sites where spermatozoa could possibly transit in the female tract. Recombinant DNA technology has made possible the manipulation of the genetic material encoding sperm proteins and in bioprocess development and product purification have made available sufficient quantities of recombinant proteins for immunogenicity and fertility studies. Injections of recombinant and peptide vaccine immunogens in various animal models including sub-human primates have produced specific immune responses. Thus, the theoretical basis for the emergence of a contraceptive vaccine based upon recombinant proteins or synthetic peptides are at hand, and initial results have been promising. The technology underpinning vaccine development is constantly being developed and the introduction of DNA/RNA vaccines are certain to impact upon the immunocontraception field. DNA/RNA vaccine has several potential advantages: these are cheap and easy to produce, can be easily modified and are quite stable as compared to conventional vaccines. Current and emerging strategies such as sperm proteomics, sperm protein encyclopaedia (using 2D composite images of human sperm proteins), structural biology of sperm proteins and modelling of protein ligands interaction using X-ray and / or NMR structures will provide experimental foundation for the design of small molecule inhibitors for fertility control. Moreover gene knockout and gene silencing using RNA interference (RNAi) will provide better understanding of the molecular and biological process of sperm function and sperm-egg interaction. This understanding is required to generate clinical advances for treatment of infertility and novel contraceptive targets.

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