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Review

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 fertilization. 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 autologus or isologus 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 under go 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_4 [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 constriction. 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 with in 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 SAPM14 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_4 has been well characterized. The sperm specific isozyme of lactate dehydrogenase LDH-C_4 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_4 has been reported to be an excellent antigen for use in a contraceptive vaccine. In addition, synthetic peptide of LDH-C_4 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_4 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_4 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^+) 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 feature 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 member expressed predominantly in the testis are fertilin β (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 β, 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 α (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 α (ADAM1), fertilin β (ADAM2), and cyritestin (ADAM3) [56]. Evidence for a specific role of fertilin α in sperm-egg adhesion and/or membrane fusion is not very clear. Fertilin α may have a role in adhesion, as the fertilin α cysteine-rich domain appear to participate on cell adhesion, as has also been found for ADAM 12 [57]. A recombinant form of fertilin α 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 α enhances the ability of the recombinant fertilin α 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 α was originally speculated to have hydrophobic fusion peptide [62] but in fact does not appear be required for sperm-egg

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

Fertilin β was one of the first "cellular disintegrins" identified and the best-characterized candidate molecule that mediates sperm-egg membrane fusion. Fertilin β is one subunit of the dimeric sperm antigen that cross reacts with one of the fertilization inhibiting antibodies, known as PH-30 (fertilin α is the other subunit) [64]. Fertilin β and cyritestin have similar amino acid sequence in their disintegrin loops. Based on studies with synthetic peptides and anti-disintegrin loop antibodies, both fertilin β and cyritestin appear to utilize their disintegrin loop sequence to interact with the egg membrane [58,65]. Incubation of mouse eggs with recombinant fertilin β prior to insemination inhibits sperm-egg binding during IVF [64]. Likewise, incubation of sperm in antibodies against the disintegrin domain of mouse fertilin β prior to insemination also inhibits sperm-egg binding and fusion [65]. Anti-fertilin β antibodies also reduced the incidence of fertilization of rabbit eggs *in vitro* [64]. Finally, knockout male mice lacking either fertilin β [67] or cyritestin [68] are severely subfertile. 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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References

1. Naz RK, Sacco A, Singh O, Pal R, Talwar GP: **Development of contraceptive vaccines for humans using antigens derived from gametes (spermatozoa and zona pellucida) and hormones (human chorionic gonadotrophin).** *Current Status Hum Reprod Update* 1995:1-18.
2. Rao J, Herr JC, Reddi PP, Wolkowicz MJ, Bush JA, Sherman NE, Black M, Flickinger CJ: **Cloning and characterization of a novel sperm-associated isoantigen (E-3) with defensin- and lectin-like motifs expressed in rat epididymis.** *Biol Reprod* 2003, **68:**290-301.

3. Suri A, Chhabra S, Upadhyay S: **Identification of human sperm antigen recognized by serum of an immunoinfertile woman: a candidate for immunocontraception.** *Am J Reprod Immunol* 1996, **36:**317-326.
4. Naz RK, Menge AC: **Antisperm antibodies: origin, regulation, and sperm reactivity in human infertility.** *Fertil Steril* 1994, **61:**1001-1013.
5. Primakoff P, Myles DG: **Penetration, adhesion, and fusion in mammalian sperm-egg interaction.** *Science* 2002, **296:**2183-2185.
6. Chiu WW, Chamley LW: **Use of antisperm antibodies in differential display western blotting to identify sperm proteins important in fertility.** *Hum Reprod* 2002, **17:**984-989.
7. Goldberg E, Herr JC: **LDH-C_4 as a contraceptive vaccine.** In: *Reproductive Immunology* Edited by: *Gupta SK. New Delhi: Narosa Publishing House*; 1999:309-315.
8. Primakoff P, Lathrop W, Woolman L, Cowan A, Myles D: **Fully effective contraception in male and female guinea pigs immunized with the sperm protein PH-20.** *Nature* 1988, **335:**543-547.
9. Herr JC, Flickinger CJ, Homyk M, Klotz K, John E: **Biochemical and morphological characterization of intra-acrosomal antigen SP-10 from human sperm.** *Biol Reprod* 1990, **42:**181-189.
10. Lea IA, Richardson RT, Widgren EE, O'Rand MG: **Cloning and sequencing of cDNAs encoding the human sperm protein, SP-17.** *Biochim Biophys Acta* 1996, **1307:**263-266.
11. Naz RK, Alexander NJ, Isahakia M, Hamilton MD: **Monoclonal antibody to a human sperm membrane glycoprotein that inhibits fertilization.** *Science* 1984, **225:**342-344.
12. Naz RK, Morte C, Garcia-Framis V, Kaplan P, Martinez P: **Characterization of a sperm-specific monoclonal antibody and isolation of a 95-kilodalton fertilization antigen-2 from human sperm.** *Biol Reprod* 1993, **49:**1236-1244.
13. Naz RK, Zhu X: **Molecular cloning and sequencing of cDNA encoding for a novel testis-specific antigen.** *Mol Reprod Dev* 1997, **48:**449-457.
14. Shankar S, Mohapatra B, Suri A: **Cloning of a novel human testis mRNA specifically expressed in testicular haploid germ cells, having unique palindromic sequences and encoding a leucine zipper dimerization motif.** *Biochem Biophys Res Commun* 1998, **243:**561-565.
15. Mohapatra B, Verma S, Shankar S, Suri A: **Molecular cloning of human testis mRNA specifically expressed in haploid germ cells, having structural homology with the A-kinase anchoring proteins.** *Biochem Biophys Res Commun* 1998, **244:**540-545.
16. Takikawa M, Kamada M, Maegawa M, Yamano S, Irahara M, Aono T, Futaki S, Ohmoto Y, Koide SS: **Evaluation of two sperm antigens, rSMP-B and YWK-II, as targets for immunocontraception.** *Zygote* 2001, **9:**145-151.
17. Shetty J, Wolkowicz MJ, Digilio LC, Klotz KL, Jayes FL, Diekman AB, Westbrook VA, Farris EM, Hao Z, Coonrod SA, Flickinger CJ, Herr JC: **SAMP14, a novel, acrosomal membrane-associated, glycosylphosphatidylinositol-anchored member of the Ly-6/urokinase-type plasminogen activator receptor superfamily with a role in sperm-egg interaction.** *J Biol Chem* 2003, **278:**30506-30515.
18. Hao Z, Wolkowicz MJ, Shetty J, Klotz K, Bolling L, Sen B, Westbrook VA, Coonrod S, Flickinger CJ, Herr JC: **SAMP32, a testis-specific, isoantigenic sperm acrosomal membrane-associated protein.** *Biol Reprod* 2002, **66:**735-744.
19. Santhanam R, Naz RK: **Novel human testis-specific cDNA: molecular cloning, expression and immunological effects of the recombinant protein.** *Mol Reprod Dev* 2001, **60:**1-12.
20. Naz RJ, Zhu X, Kadam AL: **Cloning and sequencing of cDNA encoding for a novel human testis specific contraceptive vaccinogen: role in immunocontraception.** *Mol Reprod Dev* 2001, **60:**116-127.
21. Lathrop WF, Carmichael EP, Myles DG, Primakoff P: **cDNA cloning reveals the molecular structure of a sperm surface protein, PH-20, involved in sperm-egg adhesion and the wide distribution of its gene among mammals.** *J Cell Biol* 1990, **111:**2939-2949.
22. Hou S-T, Ma A, Jones R, Hall L: **Molecular cloning and characterization of rat sperm surface antigen 2B1, a glycoprotein implicated in sperm-zona binding.** *Mol Reprod Devel* 1996, **45:**193-203.
23. Meyers SA, Rosenberger AE: **A plasma membrane-associated hyaluronidase is localized to the posterior acrosomal region of stallion sperm and is associated with spermatozoal function.** *Biol Reprod* 1999, **61:**444-451.
24. Lin Y, Kimmel LH, Myles DG, Primakoff P: **Molecular cloning of the human and monkey sperm surface protein PH-20.** *Proc Natl Acad Sci* 1993, **90:**10071-10075.
25. Lin Y, Mahan K, Lathrop WF, Myles DG, Primakoff P: **A hyaluronidase activity of the sperm plasma membrane protein PH-20 enables sperm to penetrate the cumulus cell layer surrounding the egg.** *J Cell Biol* 1994, **125:**1157-1163.
26. Hunnicutt GR, Mahan K, Lathrop WF, Ramarao CS, Myles DG, Primakoff P: **Structural relationship of sperm soluble hyaluronidase to the sperm membrane proteinPH-20.** *Biol Reprod* 1996, **54:**1343-1349.
27. Primakoff P, Hyatt H, Myles DG: **A role for the migrating sperm surface antigen PH-20 in guinea pig sperm binding to the egg zona pellucida.** *J Cell Biol* 1985, **101:**2239-2244.
28. Shalgi R, Matityahu A, Gaunt SJ, Jones R: **Antigens on rat spermatozoa with a potential role in fertilization.** *Mol Reprod Dev* 1990, **25:**286-96.
29. Primakoff P, Lathrop W, Woolman L, Cowan A, Myles D: **Fully effective contraception in male and female guinea pig immunized with the sperm protein PH-20.** *Nature* 1988, **335:**543-546.
30. Tung KS, Primakoff P, Woolman-Gamer L, Myles DG: **Mechanism of infertility in male guinea pigs immunized with sperm PH-20.** *Biol Reprod* 1997, **56:**1133-1141.
31. Deng X, Meyers SA, Tollner TL, Yudin AI, Primakoff PD, He DN, Overstreet JW: **Immunological response of female macaques to the PH-20 sperm protein following injection of recombinant proteins or synthesized peptides.** *J Reprod Immunol* 2002, **54:**93-115.
32. Wright RM, Suri AK, Kornreich B, Flickinger CJ, Herr JC: **Cloning and characterization of the gene coding for the human acrosomal protein SP-10.** *Biol Reprod* 1993, **49:**316-325.
33. Reddi PP, Naaby-Hansen S, Aguolnik I, Tsai JY, Silver LM, Flickinger CJ, Herr JC: **Complementary deoxyribonucleic acid cloning and characterization of mSP-10: the mouse homologue of human acrosomal protein SP-10.** *Biol Reprod* 1995, **53:**873-881.
34. Beaton S, ten Have J, Cleary A, Bradley MP: **Cloning and partial characterization of the cDNA encoding the fox sperm protein FSA-Acr.1 with similarities to the SP-10 antigen.** *Mol Reprod Dev* 1995, **40:**242-252.
35. Freemerman AJ, Wright RM, Flickinger CJ, Herr JC: **Cloning and sequencing of baboon and cynomolgus monkey intra-acrosomal protein SP-10: homology with human SP-10 and a mouse sperm antigen (MSA-63).** *Mol Reprod Dev* 1993, **34:**140-148.
36. Coonrod SA, Herr JC, Westhusin ME: **Inhibition of bovine fertilization in vitro by antibodies to SP-10.** *J Reprod Fertil* 1996, **107:**287-297.
37. Liu MS, Yang Y, Pan J, Liu HW, Menge AC, Lee CY: **Purification of an acrosomal antigen recognized by a monoclonal antibody and antifertility effects of isoimmune serum.** *Int J Androl* 1989, **12:**451-463.
38. Srinivasan J, Tinge S, Wright R, Herr JC, Curtiss R 3rd: **Oral immunization with attenuated *Salmonella* expressing human sperm antigen induces antibodies in serum and the reproductive tract.** *Biol Reprod* 1995, **53:**462-471.
39. Kurth BE, Weston C, Reddi PP, Bryant D, Bhattacharya R, Flickinger CJ, Herr JC: **Oviductal antibody response to a defined recombinant sperm antigen in macaques.** *Biol Reprod* 1997, **57:**981-989.
40. Foster JA, Klotz KL, Flickinger CJ, Thomas TS, Wright RM, Castillo JR, Herr JC: **Human SP-10: acrosomal distribution, processing, and fate after the acrosome reaction.** *Biol Reprod* 1994, **51:**1222-1231.
41. Olson GE, Winfrey VP, Neff JC, Lukas TJ, Nag Das SK: **An antigenically related polypeptide family is a major structural constituent of a stable acrosomal matrix assembly in bovine spermatozoa.** *Biol Reprod* 1997, **57:**325-334.
42. Mandal A, Klotz KL, Shetty J, Jayes FL, Wolkowicz MJ, Bolling LC, Coonrod SA, Black MB, Diekman AB, Haystead TA, Flickinger CJ, Herr JC: **SLLP1, A unique, intra-acrosomal, non-bacteriolytic, c lysozyme-like protein of human spermatozoa.** *Biol Reprod* 2003, **68:**1523-1537.

43. Wolkowicz MJ, Shetty J, Westbrook A, Klotz K, Jayes F, Mandal A, Flickinger CJ, Herr JC: **Equatorial segment protein defines a discrete acrosomal subcompartment persisting throughout acrosomal biogenesis.** *Biol Reprod* 2003, **69:**735-745.
44. Goldberg E, Shelton JA: **Immunologic properties of LDH-C_4 for contraceptive vaccine development.** In: *Male contraception advances and future prospects* Edited by: *Zatuchni GI, Goldsmith A, Sciarra JJ, Spieler J. Philadelphia: Harper and Row*; 1986:435-446.
45. O'Hern PA, Bambra CS, Isahakia M, Goldberg E: **Reversible contraception in female baboons immunized with a synthetic epitope of sperm-specific lactate dehydrogenase.** *Biol Reprod* 1995, **52:**331-339.
46. O'Hern PA, Liang ZG, Bambra CS, Goldberg E: **Colinear synthesis of an antigen-specific B-cell epitope with a 'promiscuous' tetanus toxin T-cell epitope: a synthetic peptide immunocontraceptive.** *Vaccine* 1997, **15:**1761-1766.
47. Tollner TL, Overstreet JW, Branciforte D, Primakoff PD: **Immunization of female Cynomolgus macaques with a synthetic epitope of sperm-specific lactate dehydrogenase results in high antibody titres but does not reduce fertility.** *Mol Reprod Dev* 2002, **62:**257-264.
48. Naz RK: **Application of sperm antigens in immunocontraception.** *Front Biosci* 1996, **1:**87-95.
49. Naz RK, Zhu X: **Molecular cloning and sequencing of cDNA encoding for human FA-1 antigen.** *Mol Reprod Dev* 2002, **63:**256-68.
50. Naz RK, Brazil C, Overstreet JW: **Effects of antibodies to sperm surface fertilization antigen-1 on human sperm-zona pellucida interaction.** *Fertil Steril* 1992, **57:**1304-1310.
51. Naz R, Zhu X: **Recombinant fertilization antigen-1 causes a contraceptive effect in actively immunized Mice.** *Biol Reprod* 1998, **59:**1095-1100.
52. Chauhan AC, Naz RK: **Effect of antibodies to sperm-specific recombinant contraceptive vaccinogen (rCV) on murine fertilization: search for an animal model to examine its contraceptive potential.** *Mol Reprod Dev* 2001, **60:**425-432.
53. Trivedi RN, Naz RK: **Testis-specific antigen (TSA-1) is expressed in murine sperm and its antibodies inhibit fertilization.** *Am J Reprod Immunol* 2002, **47:**38-45.
54. Kuno K, Kanada N, Nakashima E, Fujiki F, Ichimura F, Matsushima K: **Molecular cloning of a gene encoding a new type of metalloproteinase-disintegrin family protein with thrombospondin motifs as an inflammation associated gene.** *J Biol Chem* 1997, **272:**556-562.
55. Evans JP: **Fertilin beta and other ADAMs as integrin ligands: insights into cell adhesion and fertilization.** *Bioessays* 2001, **23:**628-639.
56. Evans JP: **Sperm disintegrins, egg integrins, and other cell adhesion molecules of mammalian gamete plasma membrane interactions.** *Front Biosci* 1999, **4:**D114-31.
57. Blobel CP, Wolfsberg TG, Turck CW, Myles DG, Primakoff P, White JM: **A potential fusion peptide and an integrin ligand domain in a protein active in sperm-egg fusion.** *Nature* 1992, **356:**248-252.
58. Evans JP, Schultz RM, Kopf GS: **Roles of the disintegrin domains of mouse fertilins alpha and beta in fertilization.** *Biol Reprod* 1998, **59:**145-152.
59. Le Naour F, Rubinstein E, Jasmin C, Prenant M, Boucheix C: **Severely reduced female fertility in CD9 deficient mice.** *Science* 2000, **287:**319-321.
60. Miyado K, Yamada G, Yamada S, Hasuwa H, Nakamura Y, Ryu F, Suzuki K, Kosai K, Inoue K, Ogura A, Ogura A, Okabe M, Mekada E: **Requirement of CD9 on the egg plasma membrane for fertilization.** *Science* 2000, **287:**321-324.
61. Kaji K, Oda S, Shikano T, Ohnuki T, Uematsu Y, Sakagami J, Tada N, Miyazaki S, Kudo A: **The gamete fusion is defective in eggs of CD9-deficient mice.** *Nat Genet* 2000, **24:**279-282.
62. Rochwerger L, Cohen DJ, Cuasnicu PS: **Mammalian sperm-egg fusion: The rat egg has complementary sites for a sperm protein that mediates gamete fusion.** *Dev Biol* 1992, **153:**83-90.
63. Cho C, Ge H, Branciforte D, Primakoff P, Myles DG: **Analysis of mouse fertilin in wild-type and fertilin$\beta^{-/-}$ sperm: Evidence for c-terminal modification, α/β dimerization, and lack of essential role of fertilin in sperm-egg fusion.** *Dev Biol* 2000, **222:**289-295.
64. Evans JP, Kopf GS, Schultz RM: **Characterization of the binding of recombinant mouse sperm fertilin beta subunit to mouse eggs: Evidence for adhesive activity via an egg beta-1 integrin-mediated interaction.** *Dev Biol* 1997, **187:**79-93.
65. Yuan R, Primakoff P, Myles DG: **A role for the disintegrin domain of cyritestin, a sperm surface protein belonging to the ADAM family, in mouse sperm-egg plasma membrane adhesion and fusion.** *J Cell Biol* 1997, **137:**105-112.
66. Hardy CM, Clarke HG, Nixon B, Grigg JA, Hinds LA, Holl MK: **Examination of the immunocontraceptive potential of recombinant rabbit fertilin subunits in rabbit.** *Biol Reprod* 1997, **57:**879-886.
67. Cho C, Bunch DO, Faure JE, Goulding EH, Eddy EM, Primakoff P, Myles DG: **Fertilization defects in sperm from mice lacking fertilin beta.** *Science* 1998, **281:**1857-1859.
68. Shamsadin R, Adham IM, Nayernia K, Heinlein UA, Oberwinkler H, Engel W: **Male mice deficient for germ-cell cyritestin are infertile.** *Biol Reprod* 1999, **61:**1445-51.
69. Focarelli R, Giuffrida A, Capparelli S, Scibona M, Fabris FM, Francavilla F, Francavilla S, Giovampaola CD, Rosati F: **Specific localization in the equatorial region of gp20, a 20 kDa sialylglycoprotein of the capacitated human spermatozoon acquired during epididymal transit which is necessary to penetrate zona-free hamster eggs.** *Mol Hum Reprod* 1998, **4:**119-125.
70. Cuasnicu PS, Conesa D, Rochwerger L: **Potential contraceptive use of an epididymal protein that participates in fertilization.** In: *Gamete interaction: prospects for immunocontraception* Edited by: *Alexander NJ, Spieler JM, Waites GMH. New York: Wiley-Liss Inc*; 1990:143-153.