Expert Opinion

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Vaccines & Antibodies

Contraceptive vaccines targeting sperm

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Overpopulation is a global problem of significant magnitude, with grave implications for the future. Development of new contraceptives is necessary, as existing forms of birth control are unavailable, impractical and/or too expensive for many individuals due to sociological, financial or educational limitations. Immunocontraception and, in particular, the targeting of antibodies to sperm-specific antigens implicated in sperm–egg binding and fertilisation offers an attractive approach to control fertility. Sperm-specific antibodies may impair fertility by inhibiting sperm motility, by reducing penetration of the cervical mucus by sperm, or by interfering in sperm capacitation or the acrosome reaction; alternatively, antisperm antibodies may invoke the complement cascade, resulting in sperm lysis. The antibodies raised against sperm-specific antigens have proved to be extremely effective at reducing sperm–egg interactions *in vitro*; fertility trials in subhuman primates will eventually be needed to prove the effectiveness of the sperm antigens in terms of contraceptive efficacy before trials in humans can be justified. In addition, existing and emerging strategies (such as sperm proteomics, the determination of molecular and structural details of sperm proteins, and the modelling of protein–ligand interactions using X-ray and/or NMR structures to name a few) are expected to provide the experimental foundation for the design of small molecule inhibitors with antifertility effects. The technology underpinning vaccine development is constantly being developed and the introduction of DNA/RNA vaccines is certain to impact upon the field of immunocontraception.

Keywords: acrosomal protein, contraceptive vaccine, epididymal protein, sperm antigens

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1. Introduction

The world's population has doubled in size over the past 40 years, reaching 6.4 billion in 2004. Another 3 billion individuals may be added to the global population over the next 50 years, including a 4% increase in the population of developed countries to > 1.2 billion and a 55% increase in developing nations' populations to > 8 billion [101]. Modern methods of contraception and birth control in use at present are impractical and/or too expensive for widespread use in different parts of the world. Therefore, it is necessary to develop new, safer, effective and more economical methods of contraception. Contraceptive vaccines have been proposed as one of the possible strategies for controlling fertility [1]. Immunocontraception as a means to control fertility has gathered a growing number of advocates in recent decades. This method aims to modify how specific proteins involved in the reproductive process are recognised by the immune system. If the immune system is tricked into thinking that these proteins are foreign material – antigens (proteins) – it will intervene and mount an antibody response that attacks the protein and interferes with its role in reproduction. This strategy has the potential to be highly specific to

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both species and to the reproductive process, and can involve antigens that will not affect reproductive behaviour. Thus, immunocontraception may provide the most easily administered and inexpensive means of creating a block to fertility.

Development of vaccines based on sperm antigens represents a promising approach to contraception [2]. The application of a sperm antigen in the development of a contraceptive vaccine is contingent on its sperm specificity, its involvement in fertility, and on raising a high antibody titre locally in the genital tract that is capable of intercepting the fertilisation process [3]. It has been demonstrated that a sperm cell has both isoantigens and autoantigens [4], with potential to generate an immunological response in both men and women, which is capable of causing infertility. The correlation of antisperm antibodies (ASAs) in some individuals with unexplained infertility suggests a role for these antibodies in blocking fertilisation [5]. The incidence of immunity to sperm in infertile couples is estimated to be 9 – 36% [6]. ASAs are thought to impair fertility by inhibiting sperm motility [7], sperm penetration of the cervical mucus, capacitation [8] or the acrosome reaction [9]; or they may invoke the complement cascade, resulting in sperm lysis [10]. Thus, ASAs and their cognate antigens may provide the basis for immunological control of fertility in the form of a birth control vaccine [11]. However, strictly speaking, any immunoglobulin that binds to sperm should not be called an ASA unless it is directed against a sperm antigen that plays a role in fertilisation and fertility [12]. This article will focus on the present status of the development of contraceptive vaccine(s) based on proteins derived from sperm cells.

2. Candidate sperm vaccinogens

For developing a sperm-based vaccine, whole sperm cells cannot be used for immunisation as they share common proteins with somatic cells. Therefore, only those proteins that are sperm-specific (**Figure 1**) can be used to develop candidate molecules for immunocontraception. Panels of 2D composite images of human sperm proteins (Encyclopaedia of Human Sperm Proteins) have been generated, which are being characterised and developed as target molecules able to interfere with the sperm–egg interaction employing various strategies [13]. A number of sperm proteins have been cloned and sequenced, and the recombinant proteins or their peptides have been evaluated as contraceptive targets in laboratory animals [14], but so far sperm-specific lactate dehydrogenase (LDH-C_4) is the only antigen that has been reported to reduce fertility in baboons [15]. However, an independent fertility trial of LDH-C_4 in another non-human primate (*Cynomologus macaque*) revealed no contraceptive effect [16]. Therefore, the search for new sperm antigen candidates and evaluation of their immunocontraceptive effect in non-human primates has become important for developing effective human contraceptive vaccines.

2.1 Acrosomal antigens

After sperm entry into the perivitelline space, the final stages of sperm–egg interaction include the binding and fusion of the sperm and egg plasma membranes, and entry of the sperm into the egg. Sperm binding to the egg surface occurs on the lateral face of the head [17], with the firm point of attachment between the sperm and egg plasma membranes occurring at the equatorial segment. Sperm must undergo the acrosome reaction before they can penetrate the zona pellucida and fuse with the egg plasma membrane. Modification of the sperm plasma membrane occurs during capacitation and the acrosome reaction to expose oocyte interaction proteins on the sperm surface. These modifications include unmasking sites and movement of sites to the correct location on the sperm head in preparation for fusion with the oocyte plasma membrane [18]. Migration of individual sperm proteins, including potential sperm ligands for the oocyte, between sperm surface domains to the equatorial and postacrosomal regions has been demonstrated in some rodent species [19,20]. This relocalisation possibly causes changes in protein density and level of expression, and thus serves to change the membrane environment.

2.1.1 Sperm-associated antigen 9 (SPAG9)

Recently, a novel human sperm-specific gene has been cloned and characterised, referred to as sperm-associated antigen 9 (*SPAG9* – human version denoted as *hSPAG9*) [21,22]. SPAG9 is an acrosomal molecule, which is not only restricted to a specific region (domain) of the acrosome, but also undergoes relocation in a stage-specific manner during the acrosome reaction [22]. Therefore, SPAG9 is a potential candidate for development of a contraceptive vaccine attributed to its sperm specificity and role in sperm binding to the zona pellucida. An antibody generated against recombinant human SPAG9 showed crossreactivity with sperm from other animal species, further resulting in inhibition of sperm binding to oocytes in *in vitro* rodent and human systems [21-23]. As an alternative strategy, the feasibility of generating an immune response to sperm-specific hSPAG9 protein using a DNA vaccine was evaluated. It was observed that plasmid DNA encoding hSPAG9 protein successfully elicited anti-hSPAG9 antibodies in mice. The antibodies thus generated recognised native SPAG9 protein in human sperm and inhibited sperm binding with zona-free hamster egg, suggesting that further active immunisation studies employing SPAG9 plasmid DNA would be useful to examine its effect on fertility [24].

SPAG9 gene encoding SPAG9 protein has also been cloned and sequenced from non-human primates (baboon and macaque), which has shown that SPAG9 protein exhibited conserved amino acid sequences. The presence of a high level of amino acid homology and recognition of macaque and baboon SPAG9 by hSPAG9 antibody implies their common function and origin in the biological past [25,26]. As *hSPAG9* was cloned from human testis and its contraceptive effects cannot be examined in humans at the present time, a suitable animal model is needed to

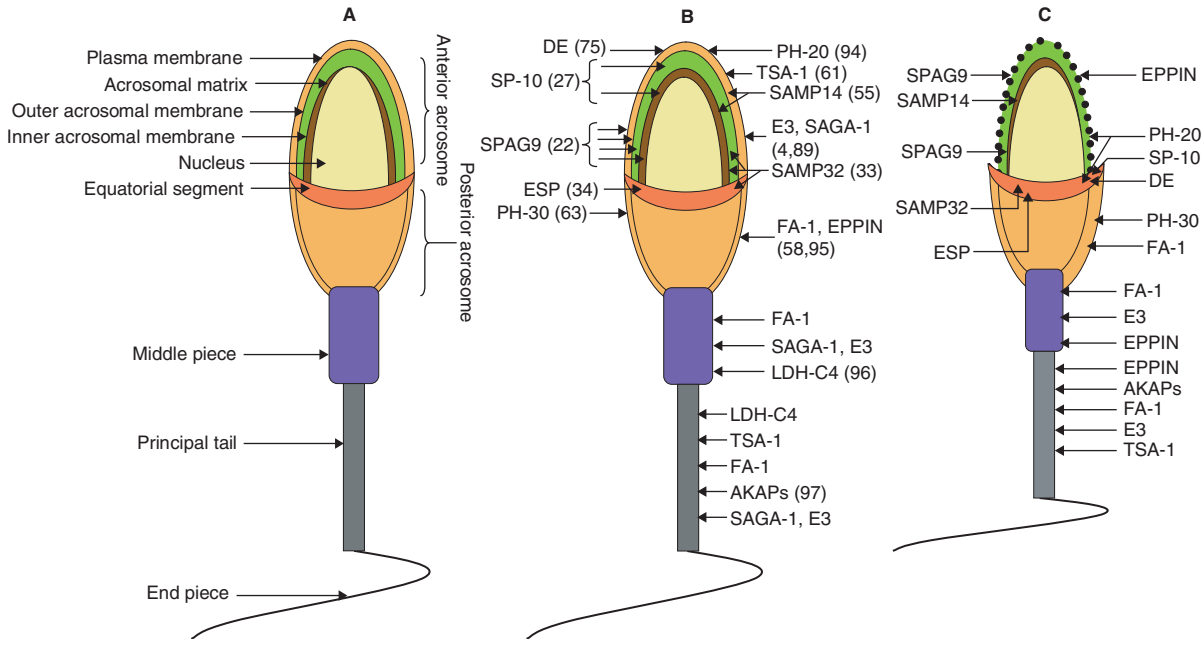


Figure 1. Schematic model of mammalian spermatozoa depicting sperm structure (A), localisation of various sperm-specific proteins in intact (B) and acrosome-reacted spermatozoa (C).

AKAP: A-kinase anchoring protein; DE: Epididymal sperm protein; ESP: Equatorial segment protein; FA: Fertilisation antigen; LDH-C4: Sperm-specific lactate dehydrogenase; SAGA: Sperm agglutination antigen; SP: Sperm protein; SPAG: Sperm-associated antigen; TSA: Testis-specific antigen.

investigate its immunocontraceptive effect. The regions of highest homology in SPAG9 protein from human and non-human primates provide important information for the rational design of recombinant hSPAG9 contraceptive vaccine formulations and suggest the appropriateness of testing fertility trials on these primates using hSPAG9 as a contraceptive vaccinogen. At present, the author's group are in the process of investigating the immunocontraceptive potential of SPAG9 protein in a macaque model.

2.1.2 Sperm protein-10 (SP-10)

Sperm protein-10 (SP-10) is a sperm-specific acrosomal protein that was first identified in humans using a monoclonal antibody [27], and was subsequently cloned and sequenced from humans [28], baboons and macaques [29]. Active immunisation trials have been performed in female baboons using the human recombinant SP-10. These baboons developed antibodies that were reactive with the cognate antigen. However, in spite of the presence of high titres of anti-SP-10 antibodies, there was only a partial reduction in fertility in a few animals [30]. Further studies demonstrated that recombinant sperm-specific antigen SP-10 induces IgG and IgA antibodies in primate oviductal fluids after systemic immunisation, which recognise the endogenous SP-10 molecule on both human and macaque sperm [31]. An important application of the human intra-acrosomal protein SP-10 has been to insert the SP-10 gene into avirulent *Salmonella typhimurium* for use in oral administration. As *S. typhimurium* naturally invades and persists in gut-associated lymphoid tissue (GALT), oral immunisation with attenuated *Salmonella* expressing foreign antigens stimulates antigen-specific secretory, humoral and cellular immune responses. A cDNA sequence encoding human SP-10 was cloned and expressed to a high level in an avirulent strain of *S. typhimurium.* Oral immunisation of female BALB/c mice with this recombinant *Salmonella* elicited high-titre anti-SP-10 IgG antibodies in serum and IgA antibodies in vaginal secretions. 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. These results are the first indication that a gene encoding a human sperm antigen can be delivered in an oral immunogen vector and induce a secretory immune response against sperm-specific antibodies in the reproductive tract. This discovery could lead to the development of a simple, safe, efficient and easy to use immunocontraceptive, and opens the way for the development of additional vectors that induce secretory immunity in the female reproductive tract [32].

2.1.3 Sperm acrosome membrane protein 32 (SAMP32)

Human sperm acrosome membrane protein (SAMP)32, specifically expressed in testis, was shown to be associated with the equatorial segment of the sperm acrosome [33]. Rat antibodies to the recombinant SAMP32 protein significantly suppressed the binding and the fusion of capacitated human spermatozoa with zona-free hamster eggs in comparison to preimmune serum. Serum from an ASA-positive infertile man strongly reacted with the recombinant SAMP32 antigen,

suggesting that it might be one antigen related to immune infertility. From a contraceptive vaccine development perspective, the fact that antibodies from ASA-positive humans reacted with recombinant SAMP32 indicated that at least some of the immunogenic epitopes recognised by the humoral response are present in the expressed and purified recombinant antigen. This indicated that recombinant SAMP32 is suitable for vaccine testing in primates. These results suggested that SAMP32 might have a role in one or more events of primary and secondary binding and in fusion of sperm with the oolemma or in sperm internalisation. Experiments are under way to clarify the role of SAMP32 in the fertilisation process [33].

2.1.4 *Equatorial segment protein (ESP)*

The equatorial segment of the acrosome, which underlies the domain of the sperm that fuses with the egg membrane, plays an indispensable role during fertilisation. Equatorial segment protein (ESP), a testis-specific protein, was cloned, characterised and was shown to be first seen in the developing acrosomal vesicles of round spermatids. ESP is unique to the equatorial segment and can serve as a marker for early specification of the equatorial segment [34]. Several research groups have previously identified immunoreagents that react with equatorial segment proteins similar in size to ESP [35-37]. The identification of a molecule such as ESP will be useful in understanding the molecular mechanisms underlying key aspects of equatorial segment biology, including:

- membrane trafficking during acrosome development
- the basis for stability and retention of the equatorial segment during the acrosome reaction
- molecular mediators of sperm–egg binding and fusion
- the fate of the equatorial segment after fertilisation, including its role as the initiation site for breakdown of the sperm nuclear envelope [34]

2.1.5 *Sperm adhesion molecule 1 (SPAM1/PH-20)*

A widely conserved sperm antigen, the sperm adhesion molecule 1 (SPAM1 or PH-20) is a glycosylphosphatidyl inositol (GPI)-linked protein with multiple roles in mammalian fertilisation. It has been shown to be dually expressed in testis and epididymis, and this is conserved in mouse, rat, fox, rabbit, macaque and human [38-43]. Antibodies generated against PH-20 significantly reduce sperm–zona pellucida binding in *in vitro* assays [40]. PH-20 is the only sperm protein that has been demonstrated to give 100% effective contraception when used to immunise an animal model. The immunisation trials in both male and female guinea-pigs were reported to lead to infertility in all immunised animals [43]. However, assessment of contraceptive vaccines in mice based on recombinant mouse sperm protein PH-20 failed to show significant reduction in fertility, indicating that recombinant PH-20 is not a useful antigen for inclusion in immunocontraceptive vaccines in mice [44]. The possible explanation may be the absence of epididymis-specific modifications to PH-20, required to provide B cell epitope associated with the immunocontraceptive effects seen in guinea-pigs with native PH-20 [45-47]. A lack of immunocontraceptive effect for PH-20 has also been reported for bacterially produced rabbit PH-20 in rabbits [42], and in this animal the inability to cause sterility was directly attributed to insufficient PH-20 antibodies in the male and female reproductive tracts [48] rather than to absence of critical epitopes in the protein. The strong immunocontraceptive effect reported for gpPH-20 in guinea-pigs [45-47], but not seen for mice and rabbits, could reflect fundamental differences in the biological role of PH-20 among the various species and its unsuitability as contraceptive vaccine when delivered as a recombinant protein in mice [44].

PH-20 appears to be a multifunctional protein, as it shows hyaluronidase activity (i.e., it is involved in the digestion of the extracellular matrix to disperse the cumulus cells). SPAM1 (PH-20) is also known to play a role in secondary zona binding after the acrosome reaction [49] and in the Ca^{2+} signalling associated with acrosomal exocytosis. Human SPAM1 was shown to be essential for the hyaluronic acid (HA)-induced [Ca^{2+}] increase in sperm after progesterone stimulation of the acrosome reaction [50]. Acrosome reactions occur *in vivo* under conditions where the important interacting molecules are at their highest levels. It is known that sperm are exposed *in vivo* in the oviduct to increasing levels of progesterone, which is produced by the cumulus cells during ovulation [51]. In addition, prior to ovulation, the cumulus cell–oocyte complex synthesises an extensive extracellular matrix that is enriched in HA. The recent finding that SPAM1 is expressed throughout the murine female reproductive tract and uterus [52] suggests that, following the arrival of sperm in the oviduct, all of the important molecular components are in place to provide the interactions that will enhance the rate of acrosome reactions. It is unknown whether or not all of these functions of SPAM1 occur in all mammals, particularly the mouse (a widely used model), where *Spam1*-null mutants are fertile despite a delay in removal of the cumulus cells [53]. Fertility of *Spam1*-null mice might be explained by the presence of two other non-somatic hyaluronidases, *Hyalp1* and *Hyal5*, which is 60% homologous to *Spam1* [53]. However, as the human orthologues of these genes are non-functional or absent, an essential role for *SPAM1* in human fertilisation remains unchallenged. Thus, it is important to fully investigate all of the functions of SPAM1 as well as the activation of the transcript in the mammalian testis during spermiogenesis. Recently, *in situ* hybridisation of testis sections indicated that transcription of SPAM1 mRNA starts in round spermatids. The transcriptional activity of spermatids stops at the time of nuclear condensation. As the transcript was observed in low levels in elongated spermatids, it may be inferred that it has a lifespan of 48 h. Further studies are required to understand the role of SPAM1 and its eventual use as a candidate molecule for contraception [54].

2.1.6 *Sperm acrosome membrane protein 14 (SAMP14)*

SAMP14 represents a GPI-anchored putative receptor in the Ly-6/urokinase plasminogen activator receptor (uPAR) family that is exposed on the inner acrosomal membrane after the acrosome reaction. The SAMP14 sequence predicted a GPI-anchored protein with a signal peptide, a transmembrane domain near the carboxyl terminus, and a putative transamidase cleavage site in the protein. Attachment of SAMP14 to the membrane by a lipid anchor was confirmed by its sensitivity to phosphatidylinositol phospholipase C. SAMP14 has a single functional domain similar to the Ly-6 and uPAR superfamily of proteins and shown to have a role in sperm–egg interaction. Antibodies against recombinant SAMP14 inhibited both binding and fusion of human sperm to zona-free hamster eggs [55]. By analogy to uPAR, SAMP14 may play a role in spatial restriction of proteolytic processes at the surface of the inner acrosomal membrane. As a Ly-6/uPAR family member, SAMP14 merits further evaluation as a GPI-linked receptor that is retained on the inner acrosomal membrane after acrosomal exocytosis. Several other GPI-anchored antigens found on the cells of the immune system, namely, CD62, CD55 and CD73, are also found anchored to the sperm surface [56]. The functions of these GPI-anchored molecules in sperm are largely unknown and require further investigations.

2.1.7 *Fertilisation antigen (FA)-1 and testis-specific antigen (TSA)-1*

Fertilisation antigen (FA)-1 is a sperm-specific glycoprotein localised on the postacrosomal region of bovine and human spermatozoa [57], and the cDNA encoding human FA-1 [58] has been cloned and sequenced. Antibodies to FA-1 inhibited *in vitro* fertilisation by interfering with the sperm–zona interaction [59], and immunisation of female rabbits and mice with FA-1 does appear to reduce fertility *in vivo* [60]. The cloned FA-1 cDNA will also help to gain further insights into the transcriptional control of testis-specific genes during spermatogenesis, as well as for studying the species specificity and molecular mechanisms involved in sperm activation and sperm–zona interactions [58]. Recently, testis-specific antigen (TSA)-1, expressed in murine sperm [61] and human sperm [62], has been cloned and characterised. In functional bioassays, recombinant TSA-1 antibodies inhibited the acrosome reaction [62] and sperm–egg binding in *in vitro* assays [61]. These findings indicated that the testis/sperm-specific protein has a role in human sperm function and may find clinical application in contraceptive vaccine development.

2.2 Proteins from a disintegrin and metalloprotease domain (ADAM) family

A group of sperm-specific molecules involved in cell–cell adhesion mainly belong to the family of a disintegrin and metalloprotease domain (ADAM) proteins. Sperm–egg plasma membrane fusion is preceded by sperm adhesion to the egg plasma membrane. Cell–cell adhesion frequently involves multiple adhesion molecules on the adhering cells. One such sperm surface protein with a role in sperm–egg plasma membrane adhesion is fertilin, a transmembrane heterodimer (alpha and beta subunits). Fertilin alpha and beta are the first identified members of a new family of membrane proteins that each has the following domains: pro-, metalloprotease, disintegrin, cysteine-rich, EGF-like, transmembrane and cytoplasmic domains. This protein family has been named ADAM because all members contain a disintegrin and metalloprotease domain. Intriguingly, many of the antigens that are sequestered into specific surface domains in the testis and subsequently become targets for post-testicular processing are those found to be important for gamete interactions during fertilisation. Particularly instructive in this respect is the behaviour of PH-20 and PH-30 antigens on guinea-pig spermatozoa. PH-30 antigen, renamed 'fertilin', also localises to the whole head of testicular spermatozoa, undergoes site-specific cleavage in the epididymis and, as a result, relocates to the postacrosomal domain on cauda spermatozoa [45,63]. Fertilin has a putative role in sperm–oolema binding and fusion via integrin/disintegrin-like interactions [64]. However, *in vivo* trials revealed that the induction of very high serum IgG antibody titres to the sperm autoantigen fertilin is insufficient to cause infertility in the rabbit [65], like PH-20, which also failed to cause infertility in female rabbits [42]. Further experiments are needed to measure the relationship between the levels of fertilin in sperm, antibody titres and the nature of the immune response in the various regions of the reproductive tract [65] for the eventual development of candidate molecules for contraception.

2.3 A-kinase anchoring protein (AKAP)

Infertility due to sperm antibodies may be attributed to a number of mechanisms, which essentially fall into two categories. The first is antibody disrupting pivotal events of the fertilisation process, such as capacitation, acrosome reaction or sperm–egg fusion [66]. The second is impairment of sperm motility, preventing their normal progress through the female reproductive tract. Thus, proteins that play a role in sperm motility could be an enticing target for a contraceptive that could be taken by either men or women to block the process of fertilisation. The mammalian sperm tail, which is mainly responsible for sperm motility, is characterised by its complex cytoskeletal structure [67]. The nine outer dense fibres that surround the microtubular axonemes are encompassed by a mitochondrial sheath in the midpiece and the fibrous sheath in the principal piece. The fibrous sheath is believed to influence the degree of flexibility, plane of flagellar motion and the shape of the flagellar beat. Various proteins associated with the fibrous sheath identified in recent studies indicate that it also has an active role in sperm motility [67-73].

Sperm motility is regulated by the cAMP-dependent protein kinase (PKA)-mediated phosphorylation of a group of flagellar proteins. This phosphorylation is facilitated by a group of proteins known as A-kinase anchoring proteins (AKAPs). AKAPs tether cAMP-dependent protein kinases

and thereby localise phosphorylation of target proteins and initiation of signal transduction processes triggered by cAMP. AKAPs can also be scaffolds for kinases and phosphatases and form macromolecular complexes with other proteins involved in signal transduction [68].

Nearly half of the proteins in fibrous sheaths isolated from mouse sperm are AKAP4. AKAP4 is transcribed only in the postmeiotic phase of spermatogenesis and encodes the most abundant protein in the fibrous sheath. Similarly, a human testis-specific gene (EMBL nomenclature AKAP4), cloned from a testis cDNA expression library, encodes a protein having regional homologies to the domain of AKAP and may act as a regulatory protein in the flagellum for sperm motility [21]. Gene targeting was used to test the hypothesis that AKAP4 is a scaffold for protein complexes involved in regulating flagellar function [69]. Sperm numbers were not reduced in male mice lacking AKAP4, but sperm failed to show progressive motility and male mice were infertile. The fibrous sheath anlagen formed, but the definitive fibrous sheath did not develop, the flagellum was shortened and proteins usually associated with the fibrous sheath were absent or substantially reduced in amount. However, the other cytoskeletal components of the flagellum were present and appeared fully developed, concluding that AKAP4 is a scaffold protein required for the organisation and integrity of the fibrous sheath. The effective sperm motility is lost in the absence of AKAP4 due to failure of signal transduction and glycolytic enzymes to become associated with the fibrous sheath [69], suggesting the possible use of AKAP4 as a target for a male contraceptive. Despite several attempts to elucidate the role of different components of the PKA holoenzyme in regulation of sperm motility, it is not yet clear which regulatory subunit is important for sperm motility. PKA is anchored at specific subcellular sites through the interaction of the regulatory subunit (R) with PKA-anchoring proteins (AKAP3) via an amphipathic helix-binding motif. Synthetic peptides containing this amphipathic helix domain competitively disrupt PKA binding to AKAPs, cause a loss of PKA modulation of cellular responses and thus inhibit sperm motility [72]. Phosphatidylinositol 3-kinase has been recently suggested to negatively regulate sperm motility by interfering with AKAP3–PKA binding [70,71]. Studies infer that interaction of the regulatory subunit of PKA with sperm AKAP3 is a key regulator of sperm motility and that disruption of this interaction using cell-permeable anchoring inhibitor peptides may form the basis of a sperm-targeted contraceptive [72,73].

2.4 Epididymal proteins

The epididymal microenvironment is important in the development of sperm, which undergo a series of morphological, physiological and biochemical changes that are tightly regulated by androgens. These include, for instance, changes in plasma membrane lipids, proteins and glycosylation, alterations in the outer acrosomal membrane, gross morphological changes in the acrosome in some species, and crosslinking of nuclear protamines and proteins of the outer dense fibre and fibrous sheath. It is well-documented that androgen-regulated glycoproteins are added to, deleted from, or masked in the sperm membrane during passage through the epididymis. A number of glycoproteins on the surface of spermatozoa are acquired from epididymal secretions during transit through the epididymis. It is quite likely that such proteins may have either a protective or a modulatory role in sperm maturation/function. Although numerous secreted epididymal proteins that interact with spermatozoa have been identified and characterised, our understanding of sperm maturation is far from complete.

2.4.1 Epididymal protein (DE)

The epididymal sperm protein DE is a candidate molecule to mediate gamete membrane fusion in rats [74]. Originally localised on the dorsal region of the acrosome, DE migrates to the equatorial segment concomitantly with the occurrence of the acrosome reaction [75]. Relocation of DE to the equatorial segment, the region through which the sperm fuses with the egg [76], together with the results of experiments showing that the polyclonal anti-DE antibody significantly inhibited the percentage of penetrated zona-free rat eggs, supported a role for this protein in sperm–egg fusion [77]. Immunisation of rats with DE protein produced a significant and reversible reduction in male and female fertility by a specific inhibition of sperm fertilising ability [78,79]. Given the potential use of DE for fertility regulation, the availability of the recombinant DE protein will further allow research on the structure–function relationship of DE and will also provide an important tool to continue exploring the use of this protein for contraceptive development.

2.4.2 Epididymis-specific secretory protein (E-3)

Epididymis-specific secretory protein (E-3) is a novel and epididymis-specific secretory protein localised to the rat sperm flagellum. It is an isoantigen resembling β-defensins, a lectin, or both. The results suggested that the E-3 gene is predominantly expressed in the corpus and cauda of the epididymis, and that the secreted E-3 protein is associated with spermatozoa. However, the nature of the association between E-3 and the maturing spermatozoa is unknown. Based on its predicted secondary structure, E-3 is similar to β-defensins, having an α-helical structure followed by three β-sheets. The presence of these defensin-like proteins in the male and female reproductive tracts in humans and rodents [80,81] may protect sperm and the epididymis from bacterial infections. Confirmation of this possibility for E-3 awaits tests of antibacterial activity against a variety of organisms. It has recently been shown that β-defensins also play multifunctional roles apart from antibacterial activity [82], including interactions with plasma membranes of *Xenopus* oocytes and as chemoattractants to macrophages [83], indicating a possible role in sperm–egg interactions and fertilisation. Thus, it is possible

that E-3 (and perhaps other similar family members) may have assumed different roles in spermatozoa. Although the functional properties of E-3 are yet to be defined, its molecular characteristics and pattern of expression suggest that it might act in sperm maturation, sperm–egg binding, as a decapacitation factor, or as a defensin to protect sperm from bacterial infection [3].

2.4.3 *Epididymal protease inhibitor (Eppin)*

The successful contraception of male non-human primates (*Macaca radiata*) was achieved recently by immunisation with Eppin, a testis/epididymis-specific protein [84]. This study demonstrated that effective and reversible male immunocontraception in primates is an attainable goal. Seven out of nine males (78%) developed high titres to Eppin, and all of these high-titre monkeys were infertile. Five out of seven (71%) high-anti-Eppin titre males recovered fertility when immunisation was stopped. The study revealed that a high serum titre, sustained over several months, achieves an effective level of contraception. Eppin on the surface of spermatozoa and in semen is bound to semenogelin [85], which is involved in coagulum formation in the ejaculate. Antibodies to Eppin interfere with the normal Eppin interaction with the sperm surface and with semenogelin, hinting at a possible mechanism to explain the infertility due to Eppin.

2.4.4 *Sperm agglutination antigen (SAGA)-1*

Sperm agglutination antigen (SAGA)-1 is a polymorphic (~ 15 – 25 kDa) glycoprotein secreted in the epididymis that is localised over the entire plasma membrane of human sperm [86]. SAGA-1 was identified as the target of an anti-carbohydrate monoclonal antibody (H6-3C4) immortalised from an infertile woman [87], linking this antigen to the cause of immunological infertility [88]. The murine antihuman IgG_1 sperm monoclonal antibody (S19) that displays multiple sperm-inhibitory activities [87,89] recognised a unique *N*-linked glycan of SAGA-1 [88]. The peptide core of SAGA-1 is identical to CD52, a GPI-anchored glycoprotein found on the surface of human lymphocytes and spermatozoa. The identification of unique sperm surface epitopes that are not expressed or exposed in the female reproductive tract is a key element in the development of antibody-based contraceptives. S19 immunoreactivity was identified only in the epididymis and on spermatozoa, indicating that the S19 carbohydrate moiety was a male reproductive tract-specific epitope [88], supporting the use of the S19 epitope as a contraceptive immunogen and the suitability of the S19 monoclonal antibody as an intravaginal contraceptive. Furthermore, a recombinant antibody was engineered to a tissue-specific carbohydrate epitope located on human SAGA-1 [90]. Studies have shown that recombinant ASA (RASA) alone was able to agglutinate human spermatozoa. RASA represents an example of an active recombinant antibody generated to the entire human sperm surface. RASA itself, or in conjunction with additional SAGAs generated against sperm surface proteins, may serve as a topical agglutinin. In addition, generation of an active mini-antibody to the sperm surface opens the path to engineering chimeric fusion proteins [91] or complement-recruiting diabodies [92] with specialised cytotoxic properties using RASA as a targeting domain. Adequate large-scale expression, purification and delivery strategies must be developed and feasibility trials performed as a prerequisite to availability.

3. Expert opinion, conclusion and future prospects

Most antifertility vaccines involve the deliberate elicitation of an autoimmune response. Given that autoimmunity *per se* does not equate with autoimmune disease, this may not necessarily be a cause for worry; vaccines generating spermatozoa-specific antibodies in the female genital tract should further negate these concerns. Sperm-specific antibodies may impair fertility by inhibiting sperm motility, by reducing penetration of the cervical mucus by sperm, or by interfering in sperm capacitation or the acrosome reaction; alternatively, antisperm antibodies may invoke the complement cascade, resulting in sperm lysis. Documented cases of naturally occurring infertility, where the presence of ASAs in the female partner is the only attributable cause, indicate the potential feasibility of the approach. The lack of additional physiological consequences of the presence of such antibodies is reassuring. Although an antisperm vaccination strategy should work in males as well, a few hurdles can be envisaged. The sheer number of spermatozoa that would have to be neutralised (~ 10^8 – 10^9, compared with the tens or hundreds present in the female upper reproductive tract) obviously presents a more formidable challenge. Neutralising antibodies would be required to enter the lumenal compartment of the male reproductive tract, and this might be best achieved by inducing secretory IgA responses in the prostate. As the immunology of the male reproductive tract remains poorly understood at present, this remains a difficult objective.

Several candidate sperm antigens have been tested for immunogenicity and antifertility effects, and many have shown promise in experimental animals. However, no single antigen has yet shown the levels of efficacy demanded of an antifertility vaccine. Therefore, an effective immunocontraceptive vaccine would probably consist of several sperm-specific antigenic epitopes in a single formulation. Mouse-specific immunocontraceptive peptides have been identified in mouse proteins with key roles in reproduction from sequence comparisons to other species, and have been tested for efficacy as immunocontraceptive antigens. Peptides were derived from granulocyte-macrophage colony-stimulating factor, the placental 27-kDa heat-shock protein, leukaemia inhibitory factor receptor, oviduct glycoprotein, proliferin, prolactin, sperm protein SP56, and mouse zona pellucida subunits 1 and 3 (ZP1, ZP3). Fertility of female BALB/c mice was reduced after immunisation with several peptides either conjugated to a carrier protein or in the form of recombinant

polyepitopes [93]. The study concludes that infertility induced in mice with a range of peptide-based vaccines is dependent on antigen formulation and genetic factors, but does not necessarily correlate with peptide-specific antibody levels.

Preformed monospecific antibodies to sperm antigens may also be combined in intravaginal sperm-specific spermicides for immunocontraceptive purposes. A recombinant mini-antibody has been engineered to the tissue-specific carbohydrate epitope located on the sperm glycoform of the CD52 antigen; its efficacy in agglutinating human sperm in a tangled pattern might make it a useful candidate for such applications. By a judicious use of delivery vehicles, carriers and adjuvants, it may become possible to challenge the GALT for the elicitation of local immunity in the reproductive tract. In conjunction with a systemic vaccine, this strategy could ensure the persistence of antibodies at all sites of spermatozoa transit.

Recombinant DNA and bioprocess technologies have made available recombinant proteins for immunogenicity and fertility studies. There now exist experimental data in animals (including subhuman primates) that firmly establish the basis for contraceptive vaccine development based on recombinant proteins or synthetic peptides. The technology underpinning vaccine development is constantly being developed and the introduction of DNA/RNA vaccines is certain to impact upon the field of immunocontraception. Existing and emerging strategies and methodologies (such as sperm proteomics, the determination of molecular and structural details of sperm proteins, and the modelling of protein–ligand interactions using X-ray and/or NMR structures, to name a few) are expected to provide the experimental foundation for the design of small molecule inhibitors with antifertility effects. Moreover, gene knockout and RNA interference strategies will no doubt contribute towards a better understanding of the molecular biology of sperm function and the sperm–egg interaction. Such data would not only aid in the identification of novel contraceptive targets, but would also be critical in any future clinical advances for the treatment of infertility.

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