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Sperm-based contraceptive vaccines: current status, merits and development

Anil Suri

At the present growth rate, the world population is estimated to reach a phenomenal 8.9 billion people by the year 2050, posing a great risk of overpopulation. Therefore, new strategies of contraception are required. A novel contraceptive strategy that is receiving considerable attention is that of immunocontraception. Spermatozoa have proteins that are unique, cell specific, immunogenic and accessible to antibodies. The targeting of antibodies to gamete-specific antigens implicated in sperm function, sperm–egg binding and fertilisation could block sperm binding and thus fertilisation. The present review highlights the current status, relative merits and future directions for various sperm-based candidate antigens with regard to the development of a contraceptive vaccine.

The world's population is experiencing unprecedented demographic change. The most obvious and best-known example is the expansion in human numbers. The current world population is 6.3 billion, and this will probably rise by another 2.6 billion by 2050 (Ref. 1). This growth in human numbers has been a principal cause of the rising demand for food, water and other natural resources in the past, and this will continue to be the case for the foreseeable future. The development of novel forms of contraception is one way in which the world population crisis is being tackled.

The pursuit of the development of a safe, effective, long-acting contraceptive vaccine has involved the selection of appropriate targets within the reproductive process that are amenable to interference with antibodies. Sperm involved in reproductive function provide a variety of target antigens that have been studied with a view to controlling reproduction through vaccination (Ref. 2). Antibodies of appropriate specificity are able to block the action of sperm antigens that are obligatory for successful reproduction, and act either by steric hindrance (for example, by blocking the binding of a spermatozoon to an oocyte) or by facilitating the rapid clearance of the target antigen through the formation of immune complexes. Cases of naturally occurring sperm-agglutinating antibodies produced by infertile couples indicate that such strategies should be effective (Ref. 3) and the lack of any additional effects other than infertility due to the presence of these antibodies is reassuring.

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Under normal physiological conditions, the immune system does not respond to sperm, egg or fetus. Identification of sperm proteins capable of eliciting functionally relevant sperm antibodies is a first step towards identifying possible immunocontraceptive targets, as well as towards providing a more complete understanding of the mechanism(s) underlying immunological infertility. However, the development of contraceptive vaccines depends on various factors in addition to identifying sperm antigens, such as which adjuvant would be suitable in humans, how a high antibody titre can be maintained, how the contraceptive effect can be terminated and how side-effects can be minimised. A large number of candidate molecules for sperm-based vaccines have been identified using several approaches, including using antibodies obtained from infertile couples to screen testis cDNA expression libraries (Ref. 4) or high-resolution two-dimensional electrophoresis gels (Ref. 5). Although anti-fertility effects have been obtained in animal models with several spermatozoa antigens, sperm-based vaccines have yet to enter clinical trials. The present review highlights various ongoing studies on sperm-based antigens in the development of immunocontraceptive vaccines for men and women.

Contraception: why the need for new strategies?

Conventional methods of contraception have depended on reducing female exposure to sperm by intra-uterine devices, the contraceptive pill, hormone injections and implants, barrier methods, and device-free methods such as abstinence, withdrawal and non-vaginal intercourse. Interruption of sperm transport, either by vasectomy or percutaneous injection of liquids that form cure-in-place plugs, are attractive options for male contraception, and it is estimated that every year more than 500 000 men worldwide receive vasectomy (Ref. 6). However, this procedure can be associated with surgical complications, unwanted immunological consequences and other pathological conditions (Ref. 7). Non-occlusive devices that deviate the course of spermatozoa, so that they are either voided in urine or are destroyed, are being evaluated for their contraceptive potential (Ref. 8). Condom use has been promoted as an easy-to-use and comfortable method to prevent sperm deposition. Unfortunately, several continued hurdles have hindered the provision of condoms to those most in need. For example, physical access to condoms remains a key impediment in many places: it is estimated that only two-thirds of the world's population has ready and easy access to condoms (Ref. 9) and difficulties still exist regarding supply of condoms in developing countries, ultimately resulting in low uptake and inconsistent use in many areas.

New forms of contraception are therefore required, as many of these traditional methods are largely inappropriate, with limited acceptability and reliability. Methods such as immunocontraception provide an effective and modern contraceptive modality, a key factor in the global issue of regional population growth as well as agricultural, medical, economic and social development.

Sperm as a target for contraception

The development of sperm in the male reproductive system involves interaction between somatic cells and germinal cells, as well as complex hormonal interactions with central nervous system controls. The primary male sex organ, the testis, produces spermatozoa that travel through the epididymis and vas deferens, passing through the urethra along with secretions from the prostate, seminal vesicle, and Littre's and Cowper's glands. In the female reproductive tract, spermatozoa are actively transported from the vagina through the cervical canal and the uterine cavity to the ampulla of the oviducts, where fertilisation occurs. Proper functioning of each step, in a sequential and orchestrated manner, is necessary for successful fertilisation (Ref. 8).

The chain of events between ejaculation and fertilisation involves a sequence of molecular changes within the sperm (Ref. 10). First, during 'capacitation', sperm undergo removal of adherent seminal plasma proteins, reorganisation of their plasma membrane and development of hyperactive motility. Once the oocyte is reached, sperm bind to carbohydrates on the oocyte zona pellucida and release zona-digesting enzymes from the sperm head (known as the 'acrosomal reaction'). The spermatozoa passes through the zona pellucida and fuses with the oocyte plasma membrane, activating the egg to release cortical granules that alter the structure of the zona pellucida so as to block polyspermy.

Research on the development of sperm-based contraceptives has targeted the following five

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areas: (1) inhibition of sperm production, (2) interference with sperm function, (3) interruption of sperm transport, (4) prevention of sperm deposition, and (5) prevention of sperm–egg interaction (Ref. 8). Of these, an approach that will arrest the fertilising ability of spermatozoa, either in the testis, the epididymis or the vas deferens, is likely to find better compliance than those methods that suppress sperm production. Blocking of sperm function might be a rapid process both in onset and reversibility, and may not disrupt testicular endocrine function and thereby suppress libido. Interference with sperm functional development could be at the level of sperm motility, formation of sperm organelles or integrity of the sperm surface membrane.

Anti-sperm antibodies (ASAs)

The demonstration of an anti-fertility effect of naturally occurring anti-sperm antibodies (ASAs) has clearly fuelled the drive for controlling reproduction towards immunocontraceptive vaccines. Spermatozoa are sequestered from the immune system because of the blood–testis barrier, and therefore should be immunogenic in the male. Observations that up to 70% of vasectomised men develop ASAs and up to 30% of infertility is associated with ASAs in the male and/or the female partner of an infertile couple confirm the immunogenicity of spermatozoa. Male ASAs can be present in the serum, seminal fluid and directly on the sperm surface (Ref. 11). In females, antibodies against sperm can be found in the cervical mucus, genital tract secretions, utero-tubal fluids and follicular fluid. ASAs (IgG, IgM and IgA) can agglutinate spermatozoa and can impair sperm motility and sperm penetration through the cervical mucus, as well as interfere with several processes such as the acrosome reaction, penetration of the sperm through the zona pellucida, sperm–oocyte recognition and fusion, and pre-implantation embryonic development (Refs 12, 13, 14, 15, 16, 17, 18). ASAs on the sperm surface can also participate in complement-dependent, neutrophil-mediated sperm cytotoxicity (Refs 19, 20). Most sera positive for IgG and IgM ASAs fix homologous complement on human sperm cells by the classical pathway and contribute either to the phagocytosis of sperm cells by activated macrophages or to the complement-induced action of membrane attack complex, both of which lead to the destruction of sperm cells (Ref. 21). Therefore, the identification of various steps and factors involved in regulating the production of ASAs might open new paths in the development of a more effective immunocontraceptive.

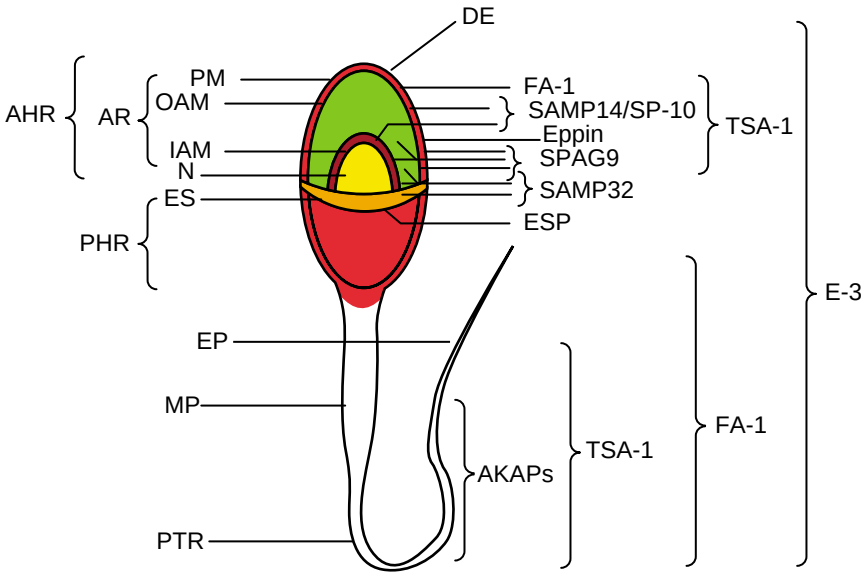
Sperm motility

The proteins playing a role in sperm motility are an enticing target for a contraceptive that could be taken either by men or women to prevent the normal progress of sperm through the female reproductive tract and therefore block the process of fertilisation. Sperm motility is regulated by the cyclic (c)AMP-dependent, protein kinase A (PKA)-mediated phosphorylation of a group of flagellar proteins. This phosphorylation is facilitated by the A-kinase anchoring proteins (AKAPs) (Fig. 1). AKAPs tether cAMP-dependent protein kinases and thereby localise phosphorylation of target proteins and initiation of signal transduction processes triggered by cAMP (Ref. 22). cAMP-dependent PKA is anchored at specific subcellular sites through the interaction of the regulatory subunit (R) with AKAPs via an amphipathic helix-binding motif. Synthetic peptides containing this amphipathic helix domain have been shown to competitively disrupt PKA binding to AKAPs, causing a loss of PKA modulation of cellular responses and thus inhibiting sperm motility (Ref. 23). In addition, phosphatidylinositol 3-kinase (PI 3-kinase) has recently been suggested to regulate sperm motility negatively by interfering with AKAP3–PKA binding (Refs 24, 25). These studies indicate that the interaction of the regulatory subunit of PKA with sperm AKAPs plays a key role in sperm motility and, moreover, that the disruption of this interaction may form the basis of a sperm-targeted contraceptive (Ref. 23).

Among the testis-specific AKAPs, AKAP4 is the most abundant protein in the sperm fibrous sheath, a cytoskeletal structure present only in the principal piece of the sperm tail. [The sperm flagellum is divided into a middle piece (MP), principal piece (PP) and end piece (EP). The fibrous sheath surrounds the axoneme and outer dense fibres in the PP, whereas the mitochondrial sheath surrounds them in the MP (Fig. 1).] Gene targeting has been used to test the hypothesis that AKAP4 is a scaffold for protein complexes involved in regulating flagellar function (Ref. 26). Although sperm numbers were not reduced in male mice lacking AKAP4, the sperm failed to show progressive motility, rendering the male mice infertile. The fibrous

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Schematic depiction of mammalian spermatozoan structure and proteins

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Figure 1. Schematic depiction of mammalian spermatozoan structure and proteins. (a) The left-hand side shows sperm structure. Abbreviations: AR, acrosomal region; AHR, anterior head region; EP, end piece; ES, equatorial segment; IAM, inner acrosomal membrane; MP, middle piece; N, nucleus; OAM, outer acrosomal membrane; PHR, posterior head region; PM plasma membrane; PTR, principal tail region. (b) The right-hand side shows the localisation of various sperm-specific proteins. Abbreviations: AKAP, A-kinase anchoring protein; DE, epididymal sperm protein; E3, epididymis-specific secretory protein; Eppin, epididymal protease inhibitor; ESP, equatorial segment protein; FA-1, fertilisation antigen 1; SAMP14, sperm acrosomal membrane protein 14; SP-10, sperm protein 10; SPAG9, sperm-associated antigen 9; TSA-1 testis-specific antigen.

sheath anlagen was 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. These findings suggest that AKAP4 is a scaffold protein required for the organisation and integrity of the fibrous sheath. Thus, sperm motility was lost in the absence of AKAP4 as a result of the failure of signal transduction and glycolytic enzymes to become associated with the fibrous sheath (Ref. 26), suggesting the possible use of AKAP4 as a target for a male contraceptive. However, despite several attempts to elucidate the role of different components of the PKA holoenzyme in the regulation of sperm motility, it is not yet clear which regulatory subunit is important for sperm motility.

Sperm capacitance

PKA plays an important role in sperm capacitation, a term that describes the molecular changes that ejaculated sperm undergo in order to fertilise the oocyte. The most significant changes experienced by sperm during capacitation are reorganisation (in composition and topology) of sperm surface antigens, changes in plasma membrane permeability, increases in intracellular second messengers (cAMP, inositol 1,4,5-trisphosphate, diacylglycerol), and increased phosphorylation of a set of proteins by different kinases (Refs 27, 28, 29, 30). The increase in cAMP activates PKA, which in turn stimulates tyrosine kinases, which finally phosphorylate a set of proteins important for capacitation (Refs 29, 31). Such signalling molecules and the processes underlying capacitation could be used as attractive candidates for the development of a contraceptive vaccine.

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Sperm-specific antigens as contraceptive targets

Although whole spermatozoa can produce an antibody response that is capable of inducing infertility in humans, they cannot be used for the development of a vaccine since there are several proteins on the sperm surface that are likely to be shared with various somatic cell plasma membranes. Thus, only those antigens that have been carefully analysed for sperm specificity should be employed for the development of an anti-sperm contraceptive vaccine (Refs 32, 33). Various sperm-specific antigens that are relevant to fertility and have potential application in immunocontraception are discussed in this section.

Sperm-associated antigen 9

A novel sperm-specific gene from human testis encoding sperm-associated antigen 9 (hSPAG9) (Ref. 4) has been evaluated as a potential candidate for the development of a contraceptive vaccine. SPAG9 is an acrosomal molecule that is not only restricted to a specific region (domain) of the acrosome (Fig. 1) but also undergoes relocation to the equatorial region in a stage-specific manner during the acrosome reaction, suggesting its potential role in sperm–egg binding (Ref. 34). SPAG9 has structural homology with c-Jun N-terminal kinase (JNK)-interacting protein 3 (Ref. 34). Recently, studies of mitogen-activated protein kinases (MAPKs) revealed that SPAG9 interacts with higher binding affinity to JNK3 and JNK2 compared with JNK1 (Ref. 34). Sperm-specific SPAG9 protein is classified as JNK-interacting protein 4 (JIP4) (Ref. 35), which is structurally related to JIP3, speculating the involvement of SPAG9-mediated signal transduction pathways in reproductive processes. Polyclonal antibodies raised against recombinant hSPAG9 recognised native protein in extracts of human sperm (Ref. 34). Furthermore, anti-SPAG9 antibodies were found to inhibit human spermatazoa from binding to human oocytes (Ref. 34). As an alternative strategy, active immunisation trials in mice using plasmid DNA encoding hSPAG9 protein resulted in an antibody response that recognised the native SPAG9 in human sperm (Ref. 36), suggesting further studies to examine its effect on fertility. hSPAG9 also shares a considerable homology with its counterpart in baboon and macaque (Refs 37, 38), suggesting that non-human primates may be useful as models for immunocontraceptive studies with this protein (A. Suri, unpublished).

PH20

A widely conserved sperm antigen (PH20) is a glycosylphosphatidylinositol (GPI)-linked protein with multiple roles in mammalian fertilisation. It has been shown to be dually expressed in testis and epididymis and is conserved in mouse, rat, fox, rabbit, macaque and human (Refs 39, 40, 41, 42, 43, 44). Immunisation trials in both male and female guinea pigs were reported to lead to 100% effective contraception in all immunised animals (Ref. 45). However, assessment of contraceptive vaccines in mice based on recombinant mouse PH20 failed to show a significant reduction in fertility, indicating that recombinant PH20 is not a useful antigen for inclusion in immunocontraceptive vaccines in mice (Ref. 46).

A possible explanation for the lack of an immunocontraceptive effect of PH20 might be the absence of epididymis-specific modifications to PH20; native PH20 proteins may have antigenic epitopes with immunocontraceptive potential not present in either of the recombinant forms used in the study. For example, native PH20 is proteolytically cleaved late in sperm maturation during passage in the epididymis in rodents such as guinea pigs (Ref. 47), rats (Ref. 48) and mice (Ref. 49). In addition, modifications to N-linked oligosaccharides on native mouse PH20 have also been reported during the passage of mouse sperm through the epididymis (Ref. 49). Neither of these modifications is likely to have occurred in the recombinant proteins. It is therefore possible that such epididymis-specific modifications to PH20 are required to provide B-cell epitopes associated with the immunocontraceptive effects seen in guinea pigs with native guinea pig PH20 isolated from sperm (Refs 45, 50).

The lack of an immunocontraceptive effect for PH20 has also been reported for bacterially produced rabbit PH20 in rabbits (Ref. 51) and, in this animal, the inability to cause sterility was directly attributed to insufficient PH20 antibodies in the male and female reproductive tracts (Ref. 51) rather than to the absence of critical epitopes in the protein. The strong immunocontraceptive effect reported for gpPH20 in guinea pigs (Refs 52, 50), but not seen for mice and rabbits, could reflect fundamental differences in the biological role of PH20 among the various species. These differences also raise questions concerning the

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creditability of employing rodents or animal species other than primates for assessing the contraceptive effect of candidate contraceptive vaccinogen. A better approach might be to test the potential of candidate sperm antigens in non-human primates before undertaking human trials.

Sperm acrosome membrane proteins

Human sperm acrosome membrane protein 32 (SAMP32) is specifically expressed in testis and is associated with the inner acrosomal membrane of principal and equatorial segments of the sperm acrosome (Ref. 53) (Fig. 1). Antibodies generated in rats in response to recombinant SAMP32 protein significantly suppressed the binding and fusion of capacitated human spermatozoa with zona-free hamster eggs (Ref. 53). These results suggest 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. 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 (Ref. 53). From the perspective of developing a contraceptive vaccine, 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 (Ref. 53).

Another acrosomal membrane protein, SAMP14, is a GPI-anchored putative receptor in the Ly-6/urokinase-type plasminogen activator receptor (uPAR) family that is exposed on the inner acrosomal membrane after the acrosome reaction (Ref. 54). SAMP14 has also been shown to be involved in sperm–egg interaction.

The definition of the molecular constituents of the acrosomal matrix and acrosomal membranes is clearly of importance to understanding the molecular mechanisms that mediate induction of the acrosome reaction and sperm–egg interactions. Therefore, molecules identified as key players in these events may be candidates for targeting by rational drug design or for inclusion in a sperm-based contraceptive vaccine.

Equatorial segment protein

The equatorial segment of the acrosome is of considerable functional importance to fertilisation. It remains intact following the acrosomal reaction, underlies the domain of the plasma membrane involved in fusion with the egg membrane, and is the site where breakdown of the sperm nuclear envelope is initiated after fertilisation (Ref. 55). Equatorial segment protein (ESP), a testis-specific protein, is unique to the equatorial segment and can serve as a marker for early specification of the equatorial segment (Ref. 56) (Fig. 1). Several research groups have identified immunoreagents that react with equatorial segment proteins similar in size to ESP (Refs 57, 58, 59). The identification of a molecule such as ESP will be useful in understanding the molecular mechanisms underlying key aspects of equatorial segment biology, including: (1) membrane trafficking during acrosome development; (2) the basis for stability and retention of the equatorial segment during the acrosome reaction; (3) molecular mediators of sperm–egg binding and fusion; and (4) the fate of the equatorial segment after fertilisation, including its role as the initiation site for breakdown of the sperm nuclear envelope (Ref. 56).

Fertilisation antige 1 and testis-specific antigen 1

Fertilisation antigen 1 (FA-1) is a sperm-specific glycoprotein localised on the post-acrosomal region of human spermatozoa (Ref. 60) (Fig. 1). Studies have reported that sera from infertile women, as opposed to fertile women, react strongly with FA-1, indicating its involvement in infertility in humans (Ref. 61). Another study demonstrated the immunoadsorption of autoantibodies by FA-1 from the surface of sperm cells of immunoinfertile men. Removal of these antibodies permitted an increased acrosome reaction and thereby the fertilisation capacity of now antibody-free sperm (Ref. 62). Furthermore, antibodies to recombinant FA-1 antigen inhibited in vitro fertilisation by interfering with the sperm zona interaction (Ref. 63) and immunisation of female rabbits and mice with FA-1 appeared to reduce fertility in vivo (Ref. 64).

Recently, testis-specific antigen 1 (TSA-1), which is expressed in murine (Ref. 65) and human sperm (Ref. 66), was cloned and characterised (Fig. 1). In functional bioassays, recombinant TSA-1 antibodies inhibited the acrosome reaction in a concentration-dependent manner (Ref. 66) and inhibited sperm–egg binding in assays in vitro (Ref. 65).

ADAM family proteins

The successful union of sperm and oocytes requires specialised cell–cell interactions that

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include cell recognition, adhesion, signalling and fusion of gametes. 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 comprising α and β subunits (Ref. 67). Fertilin α and β are the first identified members of a family of membrane proteins that have the following domains: pro-, metalloprotease, disintegrin, cysteine-rich, epidermal growth factor (EGF)-like, transmembrane and cytoplasmic. This protein family has been named ADAM because all members contain a disintegrin and metalloprotease domain. Fertilin localises to the whole head of testicular spermatozoa, undergoes site-specific cleavage in the epididymis and, as a result, relocates to the post-acrosomal domain on cauda epididymal spermatozoa (Ref. 47). Fertilin has a putative role in sperm–oolemma binding and fusion via integrin/disintegrin-like interactions (Ref. 68). However, in vivo trials have revealed that the induction of very high serum IgG antibody titres to sperm fertilin is insufficient to cause infertility in the rabbit (Ref. 69); this situation is similar to that observed for PH20, which also failed to cause infertility in female rabbits (Ref. 51). Further experiments are needed to measure the relationship between the levels of fertilin in sperm, and antibody titres and the nature of the immune response in the various regions of the reproductive tract (Ref. 69).

Epididymal proteins

Spermatazoa are produced in the testis and gain the ability to fertilise an egg as they transit the epididymis. During spermatogenesis, many germ cell proteins are produced that are necessary for the function of mature sperm (Refs 70, 71, 72). Likewise, as sperm transit the epididymis, they acquire proteins necessary for normal function from the epididymal fluid, which is a secretory product of the epididymal epithelium (Refs 70, 71, 72). These epididymal proteins might act directly in the process of gamete recognition and interaction, or might play a vital role in sperm–egg fusion. Additionally, there is evidence to suggest an indirect participation of epididymal proteins in fertilisation (e.g. through alteration of the sperm surface by glycosylation, hydrolysis, lipid removal, etc) (Refs 73, 74).

Eppin

Epididymal protease inhibitor (Eppin; Fig. 1) represents the first member of a family of protease inhibitors on human chromosome 20 that were characterised by being cysteine rich and carrying two types of protease inhibitor consensus sequences (Ref. 75). Three splice variants of Eppin are expressed differently: Eppin-1 is expressed in the testis and epididymis, Eppin-2 in the epididymis only, and Eppin-3 in the testis only. Eppin on the surface of spermatozoa and in semen is bound to semenogelin-I (Ref. 76), which is the principle protein component of the coagulum of ejaculated semen. The Eppin–semenogelin complex appears to aid the survival of spermatazoa in the female reproductive tract. Semenogelin-I is bound to the sperm surface and its N-terminal end (amino acids 45–136) has been demonstrated to be a sperm motility inhibitory factor (Refs 77, 78). Liquefaction, which is a physiological process occurring in ejaculated semen, removes semenogelin-I from the sperm surface, allowing spermatozoa to migrate along the female reproductive tract. By contrast, failure to remove semenogelin from the sperm surface results in the inhibition of capacitation (Ref. 79).

Recently, a study demonstrated that effective and reversible male immunocontraception could be achieved by immunisation of primates with Eppin. Seven out of nine males (78%) developed sustained high titres (>1:1000) to Eppin, although boosters (100 μg) were required approximately every 3 weeks. All these high-titre monkeys were infertile. Furthermore, five out of seven (71%) males with high anti-Eppin titres recovered fertility when immunisation was stopped (Ref. 80). This may be explained by the fact that the antibodies to Eppin interfere in the interaction of normal Eppin with the sperm surface and with semenogelin. Although this method has shown promise in male monkeys, its immunocontraceptive effect in humans needs to be evaluated.

Epididymal protein DE

The epididymal sperm protein DE (Fig. 1), originally identified in rat (Ref. 81), is localised on the dorsal region of the acrosome and migrates to the equatorial segment, the region through which the sperm fuses with the egg, concomitantly with the occurrence of the acrosome reaction (Ref. 82). Polyclonal anti-DE antibodies significantly inhibit the percentage of

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penetrated zona-free rat eggs. These findings support a role for this protein in sperm–egg fusion (Ref. 83). Furthermore, immunisation of rats with protein DE produced a significant and reversible reduction in male and female fertility by a specific inhibition of sperm fertilising ability (Refs 84, 85). Given the potential use of DE for fertility regulation, the availability of the recombinant DE protein will allow further 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.

Epididymis-specific secretory protein E-3

Epididymis-specific secretory protein (E-3) is a novel and epididymis-specific secretory protein that is incorporated into the rat sperm flagellum (Fig. 1). E-3 is an isoantigen containing defensin- and lectin-like motifs, and is similar to β-defensins, having an α-helical structure followed by three β-sheets. E-3 is a secreted epididymal protein that becomes associated with the sperm as it transits through the corpus and cauda. Although the nature of the association between E-3 and the maturing spermatozoa is not known, the presence of these defensin-like proteins in the male and female reproductive tracts in humans and rodents (Refs 86, 87) might 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. β-defensins also play multifunctional roles apart from antibacterial activity (Ref. 88), including interaction with plasma membranes of *Xenopus* oocytes and acting as chemoattractants to macrophages (Ref. 89), indicating a possible role in sperm–egg interaction 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 have yet to be defined, its molecular characteristics and pattern of expression suggest that it might act in sperm maturation, in sperm–egg binding, as a decapacitation factor, or as a defensin to protect sperm from bacterial infection.

Sperm-specific isozyme of lactate dehydrogenase

Sperm-specific lactate dehydrogenase LDH-C_4 has been well characterised. LDH-C_4 was purified from mouse testis and was shown to suppress the fertility of female mice, rabbits and baboons (Ref. 90). For the past two decades, LDH-C_4 has been reported to be an excellent antigen for use as a contraceptive vaccine. A synthetic peptide of LDH-C_4 has been shown to induce a contraceptive effect as great as 75% following vaccination of female baboons (Refs 91, 92). However, an independent fertility trial with the same LDH-C_4 peptide conjugated to a T-cell epitope from tetanus toxin in another non-human primate (*Cynomolgus macaque*) revealed no contraceptive effect (Ref. 93), questioning the efficacy of LDH-C_4 as a contraceptive candidate.

Current and future research directions

Inducing cell-mediated immunity

In mammals, fertilisation is completed by the direct interaction of sperm and egg, a process mediated primarily by antigens present in the gametes. Induction of an immune response against gamete-specific antigens represents one of the approaches to develop an anti-fertility vaccine. To this end, several immunocontraception trials using animal model systems have been reported in recent years. However, the results of these studies have been largely disappointing. The fundamental flaws that might account for the poor success of most of these preliminary immunocontraceptive trials include the assumption that the generation of high antibody titres will be sufficient to cause a significant reduction in fertility. However, studies suggest that such a premise may not be well founded for a number of reasons. The fertility testing of LDH-C_4 in baboons showed animals with low titres not becoming pregnant and, perhaps more significantly, animals with high titres becoming pregnant (Ref. 91). A similar lack of correlation between serum antibody titres and vaccine efficacy has been reported for vaccines against various human diseases such as malaria (Ref. 94), schistosomiasis (Ref. 95) and leishmaniasis (Ref. 96). In these systems, in vivo protection seems to be related to T-cell activation and to soluble factors such as interferon γ and tumour necrosis factor (Refs 97, 98). The possibility exists, therefore, that a similar mechanism of action is involved in the contraceptive effect of LDH. Several other investigators (Refs 99, 100) have examined the role of cell-mediated immunity (CMI) in immunological infertility and have provided evidence that soluble factors produced by activated lymphocytes and macrophages

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in reproductive tissues could be significant mediators of natural or induced infertility. Therefore, the ability of various gamete-specific antigens to stimulate appropriate CMI responses might be a critical factor in the development of successful contraceptive vaccines for human use (Ref. 91).

Polyepitope vaccines

The problems associated with the designing of anti-fertility vaccines are similar to the development of anti-cancer vaccines: it is required that both types of vaccine should not affect normal tissues or their function, should be able to overcome host downregulation of autoreactive immune cells, and should be efficacious in as many individuals as possible to reduce the development of genetic resistance (Ref. 101). To address these problems, some anti-cancer vaccines under development have used vaccines composed of multiple different tumour-antigen-derived peptides (Ref. 102) or DNA-encoded polyepitopes (Ref. 103) administered simultaneously, or multiple antigen peptides derived from several antigens conjoined into a single artificial construct (Ref. 104). Since individual peptides are often ineffective at inducing immunity at the level sufficient for blocking fertilisation, the use of multiple peptide antigens and immune enhancers would be an appropriate approach to develop anti-fertility vaccines. One possibility is that monospecific antibodies to sperm antigens may be combined for immunocontraceptive purposes in the form of intravaginal sperm-specific spermicides. Indeed, a recombinant mini-antibody has been engineered to the tissue-specific carbohydrate epitope located on the sperm glycoform of the CD52 antigen (Ref. 105). Its efficacy in agglutinating human sperm in a tangled pattern might make it a useful candidate for such applications.

Potentially, polyepitope vaccines can more closely mimic cases of documented natural autoimmunity where the physiological effect appears to require simultaneous immune responses against a number of different antigenic molecules (Ref. 106). From sequence comparisons with other species, mouse-specific immunocontraceptive peptides with key roles in reproduction have been identified in mouse proteins, 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 (Ref. 107). 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. The study concluded that infertility induced in mice with a range of peptide-based vaccines is dependent on antigen formulation. Similarily, immunisation with peptides from sperm-specific LDH-C_4 (Ref. 91), the sperm peptide P10G (PGGGTLPPSG; Ref. 108), and multiple antigen peptides from the sperm antigen YWK-II (YAL-198, YAL-201 and YAL-212, corresponding to the extracellular domains of a human sperm protein; Ref. 109) have all achieved varying degrees of infertility.

B-cell epitopes

One approach to develop an immunocontraceptive vaccine might be to use defined, synthetic, B-cell and T-cell epitopes. Specific B-cell epitopes on native antigens are mainly involved in affecting fertility. Short synthetic peptides representing B-cell epitopes are, however, incapable of inducing B cells to differentiate and produce antibody unless associated with epitopes that are recognised by helper T cells. Traditionally, carrier proteins, to which the synthetic epitope is coupled, are used to induce the necessary T-cell activation. In the case of gamete-derived epitopes, such conjugates reduce fertility rates in primates and mice, demonstrating the viability of this approach (Refs 45, 110). However, conjugation procedures have disadvantages: for example, antigen specificity may be altered (Refs 111, 112), batch-to-batch variation in the conjugation reaction makes quality control difficult, and multiple injections with the same carrier protein could induce either hypersensitivity or immune suppression (Refs 113, 114). A synthetic chimeric peptide containing a linear B-cell epitope (from the sperm protein Sp17) and a promiscuous T-cell epitope demonstrated a strain-specific reversible reduction in the fertility rate of BALB/c mice, eliminating the need for conjugation to a carrier protein (Ref. 115).

Antigen delivery

It is important to note an important flaw in the area of contraceptive research relating to the

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universal use of systemic immunisation as the sole route of antigen delivery. Whereas systemic immunisation regimes may lead to high serum IgG levels, these levels do not correlate with specific antibody levels in the reproductive tract or with contraceptive efficacy. Hence, an alternative antigen delivery approach is required that will induce an effective local immune response in the reproductive tract. An important example is the sperm-specific antigen SP-10, whose gene has been cloned into avirulent *Salmonella typhimurium* for use in oral administration. Since *S. typhimurium* naturally invades and persists in gut-associated lymphoid tissue (GALT), oral immunisation with attenuated *Salmonella* expressing foreign antigens was shown to stimulate antigen-specific secretory, humoral and cellular immune responses in serum and vaginal secretions (Ref. 116).

Moreover, the finding that naked DNA can be taken up and efficiently expressed by cells when injected into mammalian tissues has opened up an exciting new approach for developing anti-fertility vaccines. DNA vaccines have been shown to elicit both humoral and CMI responses (Refs 117, 118) that are quantitatively similar to live attenuated vaccines but without the inherent safety hazards of infectious agents. A recent study involving pcDNA–hSPAG9 plasmid DNA encoding hSPAG9 demonstrated the feasibility of generating an immune response to sperm-specific proteins by DNA vaccination in mice (Ref. 36). Antibodies generated in this study recognised native SPAG9 in human sperm and inhibited sperm adherence to, or penetration in, zona-free hamster eggs (Ref. 36). Although the studies are preliminary, DNA vaccines suggest a potentially important method for controlling fertility in consideration of its cost effectiveness, safety and ease of production.

Novel targets

Post-meiotic stages of male germ cell maturation also represent an interesting target system for the development of novel male contraceptive agents. In the human, these stages represent a period of only about 16 days differentiation; thus, targeting these cells would represent a contraceptive approach with a relatively rapid onset and equivalent recovery. Results from the Human Genome Project suggest that these cells also express a high number of very specific transcripts, although whether all of these are functional and/or essential for sperm differentiation and function requires more research. Until recently, however, these haploid stages were relatively inaccessible to molecular research because of the lack of appropriate model systems and methods (Ref. 6). This situation has recently improved, with several new techniques involving manipulation of primary cells and seminiferous tubules, germ cell transplantation and the development of new immortalised cell lines. New biochemical approaches are also yielding more information about haploid-specific transcription factors, such as germ cell nuclear factor. It is therefore to be expected that several new targets for a potential post-meiotic male contraceptive will soon become available for pharmaceutical development.

Conclusion

Various studies have recently generated experimental data in animals (including non-human primates) that firmly establishes the basis for contraceptive vaccine development using sperm-based recombinant proteins or synthetic peptides. Moreover, new strategies and different 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 nuclear magnetic resonance analysis, to name a few, are expected to provide the experimental foundation for the design of small molecule inhibitors having anti-fertility effects. In addition, gene-knockout and RNA interference strategies will no doubt contribute towards a better understanding of the molecular biology of sperm function and sperm–egg interactions. Such data would not only aid in the identification of novel contraceptive targets, but would also be crucial in any future clinical advances for the treatment of infertility.

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Figure
Figure 1. Schematic depiction of mammalian spermatozoan structure and proteins.

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