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Male Infertility and the Genetics of Spermatogenesis

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Infertility affects an estimated 10% of couples, and in roughly half of these cases the defect can be traced to the man. Several groups have expressed the concern that male-infertility rates may be on the rise in humans (Carlsen et al. 1992), as they are in many other species, possibly as a result of environmental toxins such as analogues of sex hormones. Male infertility has also attracted a great deal of recent attention from geneticists and molecular and cell biologists, who have created targeted disruptions of numerous testis-specific genes in the mouse. Knockout technology has made that organism a favored experimental model for studies of gametogenesis (see Greenhouse et al. 1998 [this issue]); lessons from the mouse seem, in many instances, to be applicable to our species, and they may help to elucidate both intrinsic and acquired male infertility. Physiological studies in the mouse indicate that spermatogenesis and the later steps in sperm maturation and activation are subject to complex regulation, and they implicate these regulatory events as possible causes of infertility in men.

The human Y chromosome has been the focus of much of the genetic analysis of male infertility. The smallest of the 24 human chromosomes, the Y contains only 2% of the haploid genome. Long before the cloning of genes was possible, cytological studies had implicated the Y chromosome in normal and abnormal male development. More recently, 12 novel genes or families, 10 with full-length cDNA sequences available, were identified by a systematic search of the nonrecombining region (NRY) of this chromosome (Lahn and Page 1997). Among the genes in the NRY, the sex-determining gene on the Y chromosome (*SRY*) is well known to be crucial for testis formation. Microdeletions within the Y chromosome have been observed in 6.4% of azoospermic or oligospermic men (Gromoll et al. 1998). Analysis of these deletions demonstrates that at least three loci other than *SRY* (the azoospermic-factor loci AZFa, AZFb, and AZFc) are required for normal sperm development. Not all of the relevant Y-chromosomal genes have been identified, but some candidate genes exist: *RBM,* for AZFb, and *DAZ* (deleted in azoospermia) and *SPGY,* for AZFc. Remarkably, all of these candidate genes, as well as the homologous autosomal genes *DAZLA* and *SPGYLA,* encode putative RNA-binding proteins that are expressed solely in the male gonad. A conserved role for *DAZ* and *DAZLA* in spermatogenesis is suggested by their homology with a *Drosophila* gene, *boule,* which is required for male fertility in the fly (Chai et al. 1997). Moreover, mice of either sex that have a homozygous *Dazla* deletion are incapable of producing gametes, demonstrating that *Dazla* is essential for the differentiation of germ cells (Ruggiu et al. 1997). Many other genes that are required in spermatogenesis are shown in table 1. An outstanding challenge remains—to understand the relationship of these diverse genes to the developmental events that begin with the establishment of the male germ line and that end with the fusion of sperm to the oocyte

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Stages of Sperm Development and Maturation

The long journey of the sperm begins when the embryonic primordial germ cells (PGCs), responding to soluble stem-cell factor, migrate into the undifferentiated gonad (Loveland and Schlatt 1997). PGCs then differentiate into prospermatogonia and reside in a quiescent state inside the testicular seminiferous tubules. Gonadotropic stimulation at the onset of puberty induces spermatogenesis (the meiotic divisions giving rise to the sperm) followed by spermiogenesis (the differentiation of the sperm cell, from haploid round spermatid to flagellated sperm). Methylation of imprinted genes (see Bestor 1998 [in this issue]) occurs between the spermatogonial and the spermatocyte stages. During the last stage of spermiogenesis, the nucleus flattens and condenses, as nonhistone basic proteins such as protamines displace the typical histones that associate with nuclear DNA, transcriptional activity in the spermatid is silenced, and nucleosomal structure is lost (Eddy et al. 1993). At the

same time, the remaining cytoplasm is jettisoned as a "cytoplasmic droplet." The resulting sperm then enter the lumen of the tubule. Finally, sperm cells are exported to the epididymis, where they are stored until they are ejaculated from the male reproductive tract (Yanagimachi 1994). By the last stages of spermiogenesis, when sperm are released into the lumen of the tubule, their ribosomes are nearly absent, and their endoplasmic reticulum (ER) has been lost from the cytoplasm (Clermont and Rambourg 1978). Because they have no machinery to produce proteins, all of the factors that sperm will require for ascending the female reproductive tract either must be synthesized in advance and stored or must be provided from the outside—for example, by the cells of the cauda epididymis.

Sperm morphogenesis is accomplished inside the testis, but testicular sperm remain physiologically "immature." During transit, mammalian sperm undergo epididymal maturation, but they remain unable to fertilize oocytes. The final preparatory step, capacitation, occurs only when sperm have resided in the female genital tract for some time. This ill-defined process appears to be necessary for sperm to undergo a further morphological and physiological transition, the acrosome reaction, once they encounter the zona pellucida (ZP) of the egg. The acrosome, a caplike structure covering the anterior portion of the sperm nucleus, contains multiple hydrolytic enzymes that are released by exocytosis prior to fertilization. Simultaneously, extensive changes occur in all sperm compartments (head and flagellum, membrane, cytosol, and cytoskeleton). Factors originating from epididymal fluid and seminal plasma are lost or redistributed, membrane lipids and proteins are reorganized, and complex signal-transduction mechanisms are initiated (Yanagimachi 1994).

With the advent of intracytoplasmic sperm injection (ICSI) in the treatment of infertility, it has become clear that the various postmeiotic steps in spermatogenesis—which are clearly required for sperm to reach the oocyte and to cross the oolemma in vivo—are irrelevant to human zygote formation per se. With ICSI, primary spermatocytes, "immature" testicular sperm, and even intentionally damaged samples, containing only physiologically dead sperm, all appear to be "fertile" in mice (Wakayama et al. 1998).

Functional Equivalence among Sperm

Fundamental to Mendelian genetic analysis is the assumption that gametes have an equal chance of contributing their haploid genomes to a zygote, whatever alleles they carry. Sperm, of course, are far from being genetically homogeneous, so their functional equivalence is not obvious. Indeed, transmission distortion has been clearly documented in the mouse, particularly in studies of the t-complex on the proximal third of mouse chromosome 17. Certain t-haplotypes contribute to defective sperm function in fertilization. Males carrying two t-haplotypes (tx/ty mice) are sterile; their sperm have very poor motility and are unable to fertilize eggs. However, males carrying one t-haplotype (t/+) are fertile, and the sperm carrying the t-haplotype are preferentially transmitted to the pups (Johnson et al. 1995). Transmission distortion has also been suggested in a variety of human traits, but the published evidence in most such cases is not conclusive (however, for an example of human transmission distortion carried through the female, see Naumova et al. 1998 [in this issue]). It appears that the assumption of functional equivalence, though not universally valid, is nevertheless generally correct in both mice and humans.

Two developmental mechanisms have come to light that may preserve this equivalence. First, proteins and mRNA are synthesized in premeiotic germ cells and are distributed among developing spermatids. This suggests that much of the regulation of later events in sperm maturation must act at the posttranscriptional and posttranslational levels, as is indeed the case (see below). Second, haploid spermatids share mRNA and proteins through cytoplasmic bridges that persist after meiosis until the late stages of spermiogenesis. Transgenic animals that synthesize the human growth hormone exclusively in postmeiotic germ cells distribute this gene product to all of their sperm, even when the transgene is present only at a single locus in the diploid genome (Braun et al. 1989). Moreover, the product of the X-linked *Akap82* gene, which is expressed postmeiotically and which is required for the organization of the sperm tail, is found in all spermatids, again indicating sharing of cytoplasmic contents (Moss et al. 1997). Thus, there is no doubt that, in their differentiation, sperm make use of the products of both sets of parental chromosomes. Still uncertain is the relative importance, in human spermiogenesis, of premeiotic synthesis and of postmeiotic sharing of cytoplasmic contents.

Regulation of Gene Expression in the Testis

Many proteins are required uniquely in spermatogenesis, and, as in most systems, much of the tissue-specific regulation in the testis is accomplished at the transcriptional level. Testis-specific promoters have been described for a number of such genes, including those for protamine-1 and -2, transition protein-1 and -2 (Tp1 and Tp2), mitochondrial-capsule selenoprotein, acrosin, and calmegin (Watanabe et al. 1995). Certain genes that are expressed in the soma as well as in the gonad are transcribed from promoters that are inactive outside the testis. Thus, a form of the angiotensin-converting enzyme (ACE) that is expressed only in postmeiotic sper-

Table 1

Targeted Disruption of Mouse Genes Related to Male Infertility

Gene Name	Wild-Type Expression Pattern	Phenotype in Spermatogenesis	Other Effects	Reference(s)
A-myb (myb family of nuclear sequence–specific DNA-binding proteins)	Predominantly in male germ cells and in female breast ductal epithelium	Blocked at pachytene stage	Impaired mammary-gland development	Toscani et al. (1997)
Ace	Somatic ACE in vascular endothelial cells and several epithelia, testicular ACE in spermatids	Inefficient sperm ascendancy of oviduct, impaired binding of ZP-binding ability	Low blood pressure	Krege et al. (1995)
Atm (mutated in ataxia-telangiectasia)	Nearly ubiquitous, high in spleen, thymus, testis	Blocked at zygotene/pachytene stage	Growth retardation, immune defects, lack of primary oocytes in female	Xu et al. (1996)
Acrosin (sperm acrosomal enzyme)	Spermatid, low levels at pachytene stage	Fertile	Normal	Baba et al. (1994)
Bax (apoptotic regulator [partner of BCL2])	Transcribed in wide variety of cells from early embryogenesis	Blocked at round-spermatid stage, accumulation of atypical premeiotic germ cells	Atretic follicles with excess granulosa cells, hyperplasia of thymocytes and B cells	Knudson et al. (1995)
Bmp-8B (bone morphogenetic protein 8B)	Testis, prominent in stage 6–8 round spermatids	Small testes, decreased spermatogonia, increased apoptosis in germ cells	Normal	Zhao et al. (1996)
Crem	Premeiotic germ cells, CREM-τ in pachytene spermatocytes	Blocked at round-spermatid stage	Normal	Blendy et al. (1996); Nantel et al. (1996)
Calmegin	Spermatocyte to round spermatid stages	Normal-looking but infertile sperm, impaired binding of ZP	Normal	Ikawa et al. (1997)
Dazla (cytosolic RNA-binding protein)	Expressed exclusively in gametogenic cells	No germ cells beyond spermatogonial stage	No eggs in follicles in female	Ruggiu et al. (1997)
Estrogen receptor	Hypothalamus, anterior pituitary gland, reproductive organs	Dilated seminiferous tubules, disrupted spermatogenesis	Decreased sexual receptivity in female	Eddy et al. (1996)
GalTase (galactosyl transferase)	Sperm surface, also highly expressed in mammary, low expression in neurons	Fertile	Normal	Asano et al. (1997)
Hr6b (ubiquitin-conjugating DNA-repair enzyme)	Ubiquitous	Blocked at elongating-spermatid stage	Normal	Roest et al. (1996)
Hsp70.2 (heat-shock protein)	Spermatocyte	Blocked at pachytene stage	Normal	Dix et al. (1997)
Igf1 (insulin-like growth factor-1)	Mainly in liver, testicular-expression variation (depending on species)	Small testes, sterile in vivo but fertile in vivo	Limited growth (~30% of normal size); in female, no ovulation, hypoplastic myometrium	Baker et al. (1996)
Mlh1 (DNA mismatch–repair enzyme)	Virtually ubiquitous	Blocked in pachytene stage	Prone to tumors, female infertility with reduced numbers of oocytes	Edelmann et al. (1996)
Nhlh2 (basic helix-loop-helix transcription factor)	Specific regions of developing CNS and peripheral nervous system	Few or no spermatids	Fourfold reduction of FSH level, obesity, female infertility with reduced numbers of oocytes	Good et al. (1997)

(*continued*)

Table 1 (continued)

Gene Name	Wild-Type Expression Pattern	Phenotype in Spermatogenesis	Other Effects	Reference(s)
Pc4	Spermatocytes and round spermatids	Normal-looking but infertile sperm, low fertility in vivo	Normal	Mbikay et al. (1997)
Pms2 (postmeiotic segregation)	Ubiquitous	Infertile with morphologically abnormal sperm	Prone to sarcomas and lymphomas	Baker et al. (1995)
Prolactin receptor	Virtually ubiquitous	Infertility in 20% of males	Impaired mammary-gland development in female, reduced ovulation, implantation failure	Chappell et al. (1997)
RARα (retinoic acid receptor-α)	Ubiquitous	No spermatogenic cells in most tubules	Normal	Lufkin et al. (1993)
RARγ (retinoic acid receptor-γ)	Presomitic caudal region and fronto-nasal mesenchyme at 8.0 dpc, also in adult skin	Sterile squamous metaplasia of seminal vesicles and prostate	Growth deficiency, increased mortality	Lohnes et al. (1993)
c-Ros	Various epithelial-cell types	Infertile in vivo, fertile in vitro	Normal	Sonnenberg-Riethmacher et al. (1996)
RXRβ (retinoic acid receptor-β)	Transcribed in many tissues, Sertoli cells in testis	Faulty lipid metabolism in Sertoli cells, oligoasthenoteratozoospermia	Embryonic and perinatal death of fraction of −/− mice	Kastner et al. (1996)
Telomerase	Active in germ cells and in highly proliferative stem cells	Absence of spermatogenic cells in 6th generation of −/− mice	Compromised hematopoietic proliferation, female sterility in 6th generation of −/− mice	Lee et al. (1998)
Zfx (zinc-finger protein)	Virtually ubiquitous	50% reduction in sperm counts	Small size, loss of viability, low female fertility, few oocytes	Luoh et al. (1997)

matogenic cells and sperm is transcribed from a promoter that is located within intron 12 of the somatic *ACE* gene. The proper cell- and stage-specific expression of this testis-specific ACE requires only a small portion of the immediate upstream, including a cyclic AMP-responsive element (CRE [Howard et al. 1993]). In Sertoli cells, a unique testicular form of the hematopoietic transcription factor GATA-1 is expressed from a promoter located 5′ to the first erythroid exon, and the remaining exons are used in common by both testis and erythroid transcripts. This promoter allows GATA-1 to act as a developmental stage– and spermatogenic cycle–specific transcriptional regulator in Sertoli cells (Yomogida et al. 1994).

Alternative splicing may also take place in the testis, to generate unique male germ-line–specific isoforms of common proteins. Human CD46, a transmembrane protein that acts in somatic tissues to inactivate complement, is alternatively spliced in the testis. The spermatid isoform of CD46 localizes not to the plasma membrane but to the acrosomal membrane. CD46 is exposed to the sperm's extracellular environment only after the acrosome reaction, and it may participate in sperm/egg recognition (Anderson et al. 1993; Seya et al. 1993). Likewise, the *Crem* (CRE modulator) gene generates a large family of transcripts by alternative splicing and alternative promoter usage. These transcripts encode a variety of activating and inhibitory transcriptional regulators. CREM-τ is expressed in premeiotic germ cells in low amounts and in the antagonist form, but, from the pachytene-spermatocyte stage onward, the splicing pattern of this transcript changes, and the CREM-τ activator accumulates in extremely high amounts (Foulkes et al. 1992). Various target genes that are expressed in postmeiotic germ cells are reported to be transcriptionally activated by CREM-τ. Germ cells from CREM-deficient mice arrest postmeiotically at the first step of spermiogenesis, and they undergo apoptosis at a significantly increased rate; these animals lack any late spermatids, and their expression of mRNAs for protamine-1 and -2, Tp-1 and -2, and other sperm proteins is dramatically reduced (Blendy et al. 1996; Nantel et al. 1996).

Protamine-1 is also an example of a large class of genes that undergo translational regulation in the male germ line. The protamine-1 mRNA is first transcribed postmeiotically in round spermatids, but it is stored in an untranslatable form as a ribonucleoprotein particle for as long as 1 wk before it is translated. It is proposed that sequence-specific RNA-binding proteins interact with the protamine 3′ UTRs and mediate the temporal control of protamine expression (Fajardo et al. 1994). The use of RNA-binding proteins may be a common feature of the developmental regulation of long-lived mRNA (Wu et al. 1997).

Posttranslational Control of Spermatid-Membrane Proteins

Various proteins have been proposed as candidate adhesion molecules on sperm. These include sp56, PH-20, zonadhesin, p95, and sp17 (implicated in the sperm/ZP interaction) and fertilin, cyritestin, and CD46 (in the sperm/egg plasma-membrane interaction). Despite the large number of these proteins, there is little reason to suspect functional redundancy among these receptors. Indeed, antibody-blocking experiments indicate that most or all of these molecules are essential for fertility, possibly indicating functional interactions between these molecules. Another kind of functional interaction may occur between these molecules and the biosynthetic machinery that produces them, and targeted gene disruptions suggest that these interactions may also be required for fertility.

As in other tissues, newly synthesized membrane proteins in the germ line emerge through the ER membrane and undergo folding and glycosylation. These events are mediated by resident ER chaperones and modifying enzymes, which also perform "quality control," recognizing and retaining proteins that are not in their final folded conformation. Evidence is accumulating that many disease-causing mutations and modifications exert their effects by alteration of protein folding (for reviews, see Thomas et al. 1995; Ashkenas and Byers 1997). Interactions between nascent proteins and ER chaperones also appear to be crucial for the later stages of sperm maturation.

The soluble ER-chaperone protein calreticulin is remarkable for its effects on cellular adhesion in fibroblasts and other cell types. Calreticulin associates with the cytoplasmic domains of integrin alpha-subunits, and this interaction influences integrin-mediated cell adhesion to the extracellular matrix. Embryonic fibroblasts from calreticulin-deficient mice are severely impaired in integrin-mediated adhesion, despite the fact that the expression of integrins on the surface of these cells is unaffected. Expression of recombinant calreticulin cDNA in homozygous knockout ES cells rescues integrin-mediated adhesion. Coppolino et al. (1997) have demonstrated that calreticulin is an essential modulator both of integrin adhesive functions and of integrin-initiated signaling. It is not known whether calreticulin plays a similar role in modulating the adhesion of sperm to oocytes, but this chaperone is expressed in the acrosome and in the Golgi apparatus of mature sperm. Because calreticulin is an essential gene, calreticulin-deficient sperm are not available. Nonetheless, the principal that transient interactions with chaperones can alter the function of adhesive receptors seems to apply to sperm maturation, as suggested by the phenotype of another targeted mutation in the mouse.

Calnexin is a ubiquitous ER chaperone that plays a major role in quality control, by retaining incompletely folded or misfolded proteins. Unlike several other ER chaperones, such as BiP and calreticulin, calnexin is an integral membrane protein. Calmegin is a testis-specific ER protein that is homologous to calnexin. We have shown that calmegin binds to nascent polypeptides during spermatogenesis, and, by targeted disruption of its gene, we have analyzed its physiological function. Homozygous-null male mice are nearly sterile, even though spermatogenesis is morphologically normal and mating is normal. In vitro, sperm from homozygous-null males do not adhere to the egg extracellular matrix, the ZP (see Greenhouse et al. 1998 [in this issue]), and this defect may explain the observed infertility (Ikawa et al. 1997). Analysis of two-dimensional gels of sperm surface or total proteins indicates that sperm from *Calmegin* −/− mice is indistinguishable from wild-type sperm. We have further examined the surface expression of most of the candidate proteins, for evidence of mediation of sperm-egg interaction, using immunostaining or flow cytometry, and we have yet to detect a quantitative or qualitative difference between mutant and wild-type sperm. This may indicate, by analogy with the *Calreticulin*-mutant mouse, that calmegin is required for sperm proteins to attain their normal conformations or to form essential quaternary interactions. To confirm the phenotype of the calmegin disruption, we examined whether the infertility of the knockout mice could be rescued by expression of a calmegin transgene. Among transgenic mouse lines, only those that expressed the transgene regained their fertilizing ability (M. Ikawa and M. Okabe, unpublished data). Transgenic mice that express either calmegin or a calmegin-calnexin chimeric protein may provide crucial insights into interactions between sperm surface proteins and ER chaperones.

Targeted Disruption of Genes Expressed in the Testis

A large number of genes that are known to be expressed in the testis or in maturing spermatocytes have been disrupted in the mouse, to test their function in

male fertility. Surprises in this field have been numerous, as the following examples show.

Proteinases: Acrosin and Fertilin

Acrosin is a stored serine proteinase that is released from sperm in the acrosome reaction. Acrosin, produced from an inactive precursor, pro-acrosin, by proteolytic clipping, is the major such secreted proteinase, and it has been predicted to play a key role in fertilization, by degrading the ZP locally to permit access of sperm to the egg surface. However, male mice homozygous for a targeted mutation in the *Acrosin* gene are fertile, despite a complete lack of acrosin activity in their sperm (Baba et al. 1994). This unexpected phenotype may indicate that other sperm-derived proteinases are sufficient to degrade the ZP.

Fertilin is a heterodimeric sperm plasma-membrane protein. Both of its subunits belong to the MDC (metalloproteinase-like, disintegrin-like, cysteine-rich) family of surface proteins, which contain a metalloprotease and a disintegrin domain. The disintegrin domain of fertilin-β has been reported to mediate binding to the egg cell surface, whereas a "fusion peptide" derived from the α subunit is believed to participate in the fusion of the two gametes. Mice deficient in the *fertilin-β* gene produce morphologically normal sperm with impaired binding to the ZP, as well as binding ability to eggs (C. Cho, personal communication).

Peptide-Processing enzymes: ACE and PC4

ACE is a membrane-bound dipeptidyl carboxy-peptidase that generates the vasoconstricting peptide angiotensin II and that inactivates the vasodilating peptide bradykinin. The gene encoding ACE consists of two homologous regions and encodes both a somatic- and a testis-specific isoenzyme. Female mice deficient for both forms of ACE were found to be fertile, but the fertility of homozygous male mutants was greatly reduced (Krege et al. 1995). The cause of the male sterility was demonstrated to be a defect in sperm migration within the oviducts, as well as a decreased ability to bind to the ZP. This phenotype in the male was not an indirect effect of a blood-pressure decrease caused by the loss of somatic ACE expression: mutant males that lack only the somatic form of ACE were found to be fertile. The male-specific infertility of animals lacking the testis-specific ACE is curious, because angiotensin itself does not seem to be required for spermatogenesis. Indeed, male mice lacking angiotensinogen are reported to have normal fertility (Tanimoto et al. 1994). No other substrate of ACE in the testis has been proposed.

PC4 is a member of the proprotein convertase (PC) family of serine proteases, which are implicated in the processing of a variety of prohormones, proneuropeptides, and cell-surface proteins. In rodents, PC4 transcripts have been detected in spermatocytes and round spermatids exclusively, suggesting a reproductive function for this enzyme. As expected, the in vivo fertility of homozygous mutant males was severely impaired, but no spermatogenic abnormality was evident. In vitro, the fertilizing ability of PC4-null spermatozoa was also significantly reduced (average litter size 6.9, compared with 0.8 in wild-type and −/− mice). Interestingly, if the average litter size is calculated only on the basis of successful matings, the figure increases to 3.3 in knockout mice. Calmegin-deficient mice are similar in this regard. Their average litter size is 0.02, compared with 8.2 in wild-type mice, but one successful mating resulted in 2 pups. This indicates that, in some females (or in some ejaculates), the defect in fertility is not as severe as the average value would suggest. Anecdotal evidence suggests that this pattern may occur in humans as well. Many supposedly infertile couples find, to their surprise, that they are suddenly able to conceive.

A Signaling Molecule: c-ros

c-ros is a receptor-type tyrosine kinase that is expressed in a small number of epithelial-cell types, including those of the caput epididymis. Targeted mutations of *c-ros* cause male but not female infertility. Sperm isolated from c-ros −/− mice appear normal and can fertilize eggs efficiently in vitro. Remarkably, sperm derived from *c-ros* −/− spermatocytes have their in vivo fertilization capacity restored if they pass through the wild-type epididymal epithelium of a chimeric mouse (Sonnenberg-Riethmacher et al. 1996). Thus, whereas spermatogenesis does not appear to be affected in c-ros–deficient mice, sperm maturation is impaired, because of a defect in a signaling pathway in epididymal cells. It has been suggested that glycosylphosphatidylinositol-anchored proteins are transferred to the sperm surface during epididymal transit (Kirchhoff et al. 1997), and it may be that c-ros is required for such transfer. However c-ros may act in this tissue, the infertility of these sperm seems to be explained by their failure to reach the oviduct, despite the fact that they are produced in normal numbers. This phenotype is reminiscent of that of *Ace* knockout animals, and it may indicate a failure of some form of "homing" mechanism in both classes of mutants.

Prospects for the Future

It is clear that a man can be diagnosed as infertile if he is azoospermic or oligospermic. However, there are many infertile men who have semen parameters within the normal range but who show diminished binding of sperm to the ZP; still others may be deficient either at

one or more of the later steps of sperm maturation or in the expression or activation of stored sperm components. The sheer diversity of mouse mutations that cause male infertility may be daunting, but work in this system now provides a number of candidate genes that may be relevant to infertility in men. The prediction of men's fertility on the basis of their genotype could be possible in the future, but a more immediate application of this knowledge is likely to occur in the design of novel forms of contraception. For each of the many genes that are required for the development of active sperm (table 1), there may be one or more treatments developed that can mimic the observed effects of mutations on sperm synthesis or function.

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