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REVIEW

Mouse models in male fertility research

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Limited knowledge of the genetic causes of male infertility has resulted in few treatment and targeted therapeutic options. Although the ideal approach to identify infertility causing mutations is to conduct studies in the human population, this approach has progressed slowly due to the limitations described herein. Given the complexity of male fertility, the entire process cannot be modeled *in vitro*. As such, animal models, in particular mouse models, provide a valuable alternative for gene identification and experimentation. Since the introduction of molecular biology and recent advances in animal model production, there has been a substantial acceleration in the identification and characterization of genes associated with many diseases, including infertility. Three major types of mouse models are commonly used in biomedical research, including knockout/knockin/gene-trapped, transgenic and chemical-induced point mutant mice. Using these mouse models, over 400 genes essential for male fertility have been revealed. It has, however, been estimated that thousands of genes are involved in the regulation of the complex process of male fertility, as many such genes remain to be characterized. The current review is by no means a comprehensive list of these mouse models, rather it contains examples of how mouse models have advanced our knowledge of post-natal germ cell development and male fertility regulation.
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INTRODUCTION

Infertility is a major medical problem worldwide. In the Western societies, one in eight couples of reproductive age is infertile (http://www.endotext.org/male/male7/maleframe7.htm). Of these, male factors are the sole or a contributing cause of the infertility in >40% of cases.[1] Causes of male infertility may be broadly classified into four categories: (i) defective sperm production; (ii) reproductive tract obstruction; (iii) inflammation; and (iv) sexual disorders, for example, erectile dysfunction and ejaculatory failure.[2,3] Approximately half of the causes of male infertility result from defective sperm production, for example, complete blockade of spermatogenesis, low sperm counts, abnormal sperm motility, morphology or function.[3] Although the etiology of most cases remains unknown, there is increasing evidence that a significant percentage of male infertility is caused by genetic defects including chromosome aberrations, gene mutations and single-nucleotide polymorphisms.[4–11]

THE COMPLEXITY OF MAMMALIAN MALE FERTILITY REGULATION

Following the crucial process of in utero sex determination, the establishment of male fertility comprises a series of complex and highly structured steps involving cell division, differentiation and cell–cell interactions. The production of fully functional spermatozoa in mammals takes place within the seminiferous tubules of the testis and is under strict hormonal regulation. Several key hormones secreted by the hypothalamus (gonadotrophin-releasing hormone), pituitary gland (luteinizing hormone and follicle-stimulating hormone) and hormones secreted by the testis (for example, androgens, oestrogens, inhibins and follistatin) are involved in the regulation of spermatogenesis.[12–15] Deregulation of hormonal regulation has been shown to contribute to male/female infertility in human[15] and mouse models.[4,16]

Mammalian spermatogenesis can be separated into three categories of cellular events: the proliferative phase (spermatogonia), the meiotic phase (spermatocytes) and the differentiation phase or spermiogenesis (spermatids), followed by a series of post-testicular maturation processes required for fully functional spermatozoa (capable of motility and fertilization). In addition to germ cells, the testis contains two other specialized cell types, Sertoli and Leydig cells, which support spermatogenesis.[12] Sertoli cells form the blood–testis barrier through the presence of intercellular tight junctions, which provides the isolated environment necessary for development of spermatocytes and spermatids. Sertoli cells also provide paracrine support to all adult germ cell types.[17] Leydig cells in the interstitial tissue of the testis are uniquely positioned to provide testosterone to the seminiferous tubules to drive spermatogenesis.

In simple eukaryotes, such as flies and worms, it is estimated that over 1000 genes are involved in the regulation of male fertility.[18,19] In mice, it has been calculated that the male germ cell transcriptome comprises more than 30 000 transcripts.[20] The somatic cells of the testis and those involved in endocrine regulation of spermatogenesis will contain many others. Theoretically, defects in any one of these genes could lead to infertility.

CHALLENGES IN DEFINING MALE INFERTILITY GENES IN HUMANS

Two common approaches used to evaluate the function of a gene are reverse genetic and forward genetic approaches (Figure 1). In a reverse

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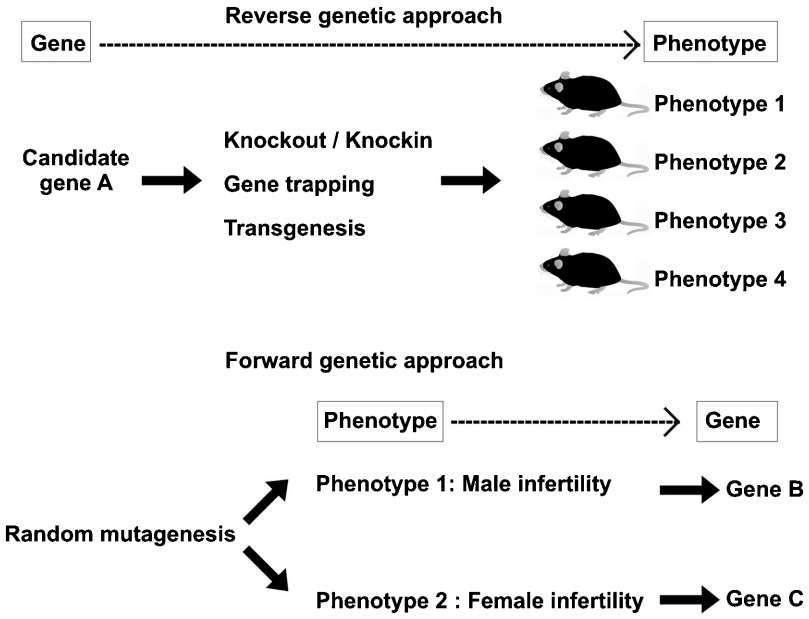

Figure 1 Reverse and forward genetic approaches for *in vivo* gene functional analyses in mouse models. The reverse approach begins by selecting a candidate gene of interest followed by evaluating its *in vivo* function using gene manipulation techniques and animal model production. Once generated, animal models are used for phenotypic characterization to define pathological abnormalities. In many cases, candidate genes have multiple roles in different tissues/organs; thus, the animal models often give rise to more than one phenotypic defect. By contrast, the forward genetic approach is initiated by the creation of animal models exhibiting a phenotypic defect of interest followed by defining the genetic alteration responsible for the phenotypic defects.

genetic approach, the functional analysis of a gene begins by altering gene expression (that is, complete or partial inactivation or ectopic expression), followed by an assessment of the phenotypic consequences. In contrast, a forward genetic approach, or phenotypic-driven approach, starts by the relatively undirected generation of and identification of phenotypic variants of interest. The underlying mutation is identified subsequently. The latter approach is a powerful method for revealing completely novel genes that function in a particular process as it does not require fore knowledge of a gene's function.

Cytogenetic and molecular studies have revealed that chromosome abnormalities including deletions (for example, the azoospermia factor region on the long arm of the Y chromosome), translocations and chromosome aneuploidies, occur with an increased incidence in infertile men compared with fertile men.[4–11] Although recent advances in molecular biology and the completion of human genome sequence have enabled the prediction of candidate genes within the deleted or translocated regions, the precise functions of the majority of these genes have yet to be determined. In addition, infertility-causing genes and risk factors are identified *via* mutation screening of candidate genes in infertile cases compared with fertile control men. However, the progress of this approach has been slow for a number of reasons, including: (i) inadequate numbers of patient and control groups for case–control studies; (ii) insufficiently detailed clinical/phenotypic information; (iii) the large genetic diversity in human populations that may contribute to different phenotypic outcomes (effects of modifier genes); (iv) the design of the mutation screens, for example, the majority of mutation screens focus on protein-coding regions of candidate genes that can eliminate or bias against the identification of intronic mutations; and (v) follow-up *in vivo* by gene functional analyses in humans remains problematic due to the limited availability of human samples and the associated ethical restrictions. The use of mouse models, however, does not have such problems and, as such, a substantial amount of fertility research is performed in the mouse.

The identification of genes required for spermatogenesis, post-testicular sperm maturation, capacitation and fertilization is imperative if we hope to obtain a complete understanding of male fertility. The outcomes of these studies have ramifications for the diagnosis and treatment of human male infertility as well as the development of the much needed male-based contraceptives for humans and animals. Male-based contraceptives, using both hormonal and immunological approaches, have been under trial with limited success.[15,21] Recent focus has been on identifying targets for non-hormonal contraception by taking advantage of the specific cellular and physiological processes unique to the reproductive organs. The main goal is to interfere in a highly specific manner in key developmental or functional processes. This requires the identification/characterization of many of the unknown male fertility regulators. With the exception of the spermatogonial stem cells, the temporary disruption of testicular germ cells is attractive as upon cessation of treatment it should require only one wave of spermatogenesis to restore germ cell production and fertility.

DIFFERENT TYPES OF MOUSE MODELS

Given the complexity of male fertility, the entire process cannot be fully modeled *in vitro*. As such, animal models provide a viable alternative for experimentation. Mice are the most commonly used animal models in biomedical research, including reproductive biology, because of their short reproductive cycle with a large litter size and relatively cheap housing conditions. Most importantly, mice are genetically very similar to humans[22] and their embryos are relatively easy to manipulate at the genetic level. Moreover, mouse spermatogenesis and oogenesis are comparable to humans. Over several decades, many types of mouse models have been made available for biomedical research, including knockout/knockin and transgenic models (using the reverse genetic or candidate gene approach). More recently, chemical-mutagenized mutant mouse models (using the forward genetic

approach) have been increasingly used to reveal disease-associated genes, including causes of male infertility.[23–28]

Knockout/knockin mice and gene-trapped mice

The most common approach used to define a gene function *in vivo* is gene ablation by homologous recombination in embryonic stem (ES) cells, known as 'knockout' or 'gene targeting'.[29,30] This strategy is designed for the evaluation of a gene function on the basis of the complete elimination (null allele) or partially elimination of a candidate gene function (that is, deletion of particular domain(s) of the encoded protein).

Generally, targeting constructs are designed to carry a selectable marker to enable the selection of correctly targeted ES clones (for example, kanamycin/neomycin resistance gene), flanked by 5′ and 3′ homology regions of the target gene (Figure 2). Targeted ES cells are injected into blastocysts of a different mouse strain to produce chimeric progeny, which are subsequently mated with wild-type mice to generate heterozygous knockout mice, that is, carrying one deleted allele and one wild-type allele. Homozygous knockout mice, that is, carrying two deleted alleles, are generated by heterozygous intercrosses.[31,32] Once generated, the phenotypic characterization is performed to define pathological defects due to the complete or partial loss of the targeted gene function.

In addition to the generation of null alleles, specific disease-causing mutations can be precisely introduced into the mouse genome by a 'knockin' approach. The production of knockin mice requires two rounds of homologous recombination in ES cells in order to eliminate the remaining sequence of targeting vectors from the targeted locus. Although the generation of knockin mice is more complicated than knockout mice, this approach enables the creation of mouse models carrying specific disease-causing mutations rather than a gene deletion that completely eliminates the protein function. For example, such alleles could carry mutations observed within the human population.

The production of null alleles by which the targeted genes are inactivated in every cell in the animal throughout its life can be very valuable in defining *in vivo* gene function. This approach, however, has a limitation due to a large percentage of knockout mice exhibiting unexpected embryonic/neonatal lethality, which limits the analysis of the gene function in later stages of life. In some instances, the gene may contribute to different aspects of health at different stages, for example, different functions in embryo stage compared with neonate and adult stages. In the case of infertility and contraceptive research,

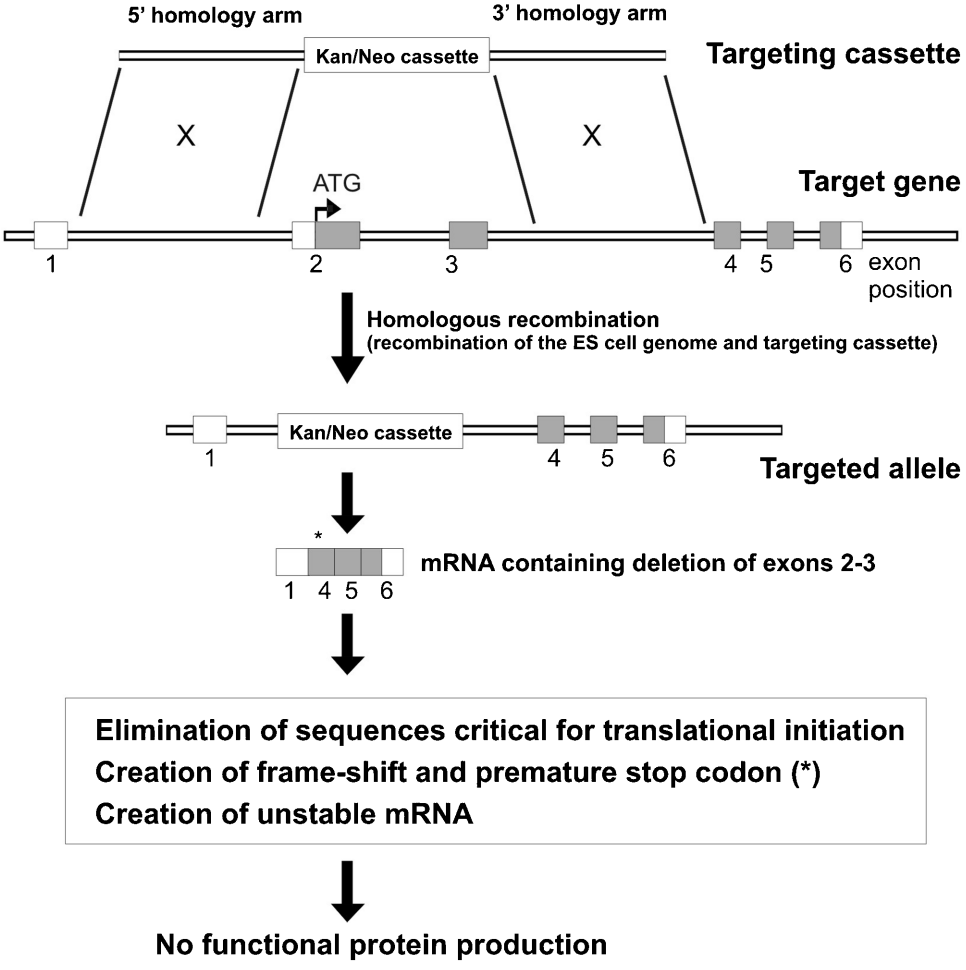

Figure 2 Gene targeting strategy for the generation knockout mice. A targeting cassette is designed to contain homology arms flanking a drug selectable marker (for example, kanamycin/neomycin) to facilitate the identification of targeted ES clones. Depending upon the design of the targeting cassette, a null allele can be generated by: (i) elimination of sequences critical for translational initiation; (ii) creation of a frame shift in the coding region and premature stop codon (indicated by *); and (iii) creation of unstable mRNA. Untranslated regions are depicted by unfilled boxes; protein-coding regions are depicted by filled boxes; ATG: initiation codon. ES, embryonic stem.

this is a serious limitation. To overcome this limitation, the ablation of a target gene at a precise time and/or specific tissue can be achieved by using the conditional knockout approach, which allows the mouse to develop up to a certain stage of development prior to inactivation of the gene. This approach utilizes the site-specific recombination systems, Cre/LoxP or Flp/FRT.[33]

In addition to knockout and knockin approaches, a high throughput method for random gene disruption, known as 'gene trapping', has been developed.[34] This approach is based on the use of a gene-trapping vector containing a conserved 3′ acceptor splice site, a eukaryotic drug-selectable marker (and/or markers for gene expression analysis) and transcriptional termination (poly A) signal to randomly insert and interrupt gene transcription in the ES cell genome (Figure 3). If the insertion of a gene trap cassette occurs within an intron of a gene, it will result in alternative splicing *via* the use of the 3′ acceptor splice site within the cassette. The transcription of the trapped gene will terminate at the poly A signal and result in the production of truncated protein or no protein depending upon where the cassette is inserted. Insertion in an intron prior to the ATG start site is likely to generate null alleles.[34]

Through the use of high-throughput technologies, ES clones containing thousands of different trapped genes have been produced. The collection of ES gene trap cell lines has been made publically available and centralized through the International Gene Trap Consortium, which aims to generate a library of mouse mutant ES cells covering most of the genes in the mouse genome.[35–37] Furthermore, researchers should be aware of the existence of the International Knockout Mouse Consortium (http://www.knockoutmouse.org), which aims to mutate all protein-coding genes in the mouse genome using a combination of gene trapping and gene targeting in C57BL/6 mouse ES cells.[38] The International Knockout Mouse Consortium includes the following programs: the Knockout Mouse Project (http://www.knockoutmouse.org/about/komp) (USA), the European Conditional Mouse Mutagenesis Program (http://www.eucomm.org),[39] the North American Conditional Mouse Mutagenesis Project (http://www.norcomm.org/index.htm) (Canada) and the Texas A&M Institute for Genomic Medicine (http://www.tigm.org) (USA).

Transgenic mice

A transgenic organism refers to an organism that has had its genome modified to carry a copy (or more than one copy) of a piece of DNA of foreign origin. These models enable researchers to evaluate *in vivo* phenotypic defects as a result of ectopic expression of a gene, for example, overexpression of a wild-type gene and expression of a dominant-negative form to diminish the normal function of a gene. The production of transgenic mice is achieved by direct injection of a purified fragment of a transgene of interest into fertilized oocytes (Figure 4). Following injection, the transgene is inserted into the genome of fertilized oocytes, which are subsequently used to generate transgenic mice. The fact that transgene integration usually occurs randomly, and the expression level of transgenes can be influenced by positional effect and transgene copy number.

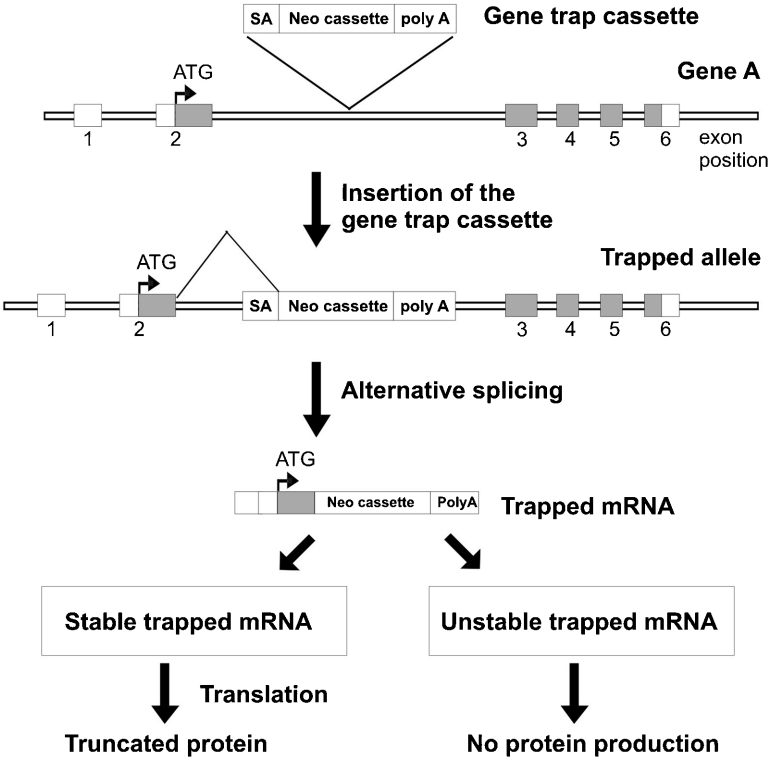

Figure 3 A high-throughput gene trapping approach. A gene trap cassette is designed to contain the features necessary for its integration into the ES cell genome and termination of transcription of the trapped allele. These features include SA site and poly A signal. If inserted within an intron of a gene, the native splicing pattern is affected by the SA, resulting in fusion of the upstream exons with the trapped cassette. Transcription of the trapped mRNA is subsequently terminated at the poly A site. If inserted within the 5′ UTR, the trapped mRNA will produce no functional protein. If insertion occurs within an intron downstream of the translational start site of a gene, a truncated protein (fused with the trapped cassette) may be produced depending upon the stability of the trapped mRNA. ES, embryonic stem; poly A, polyadenylation; SA, slicing acceptor; UTR, untranslated region.

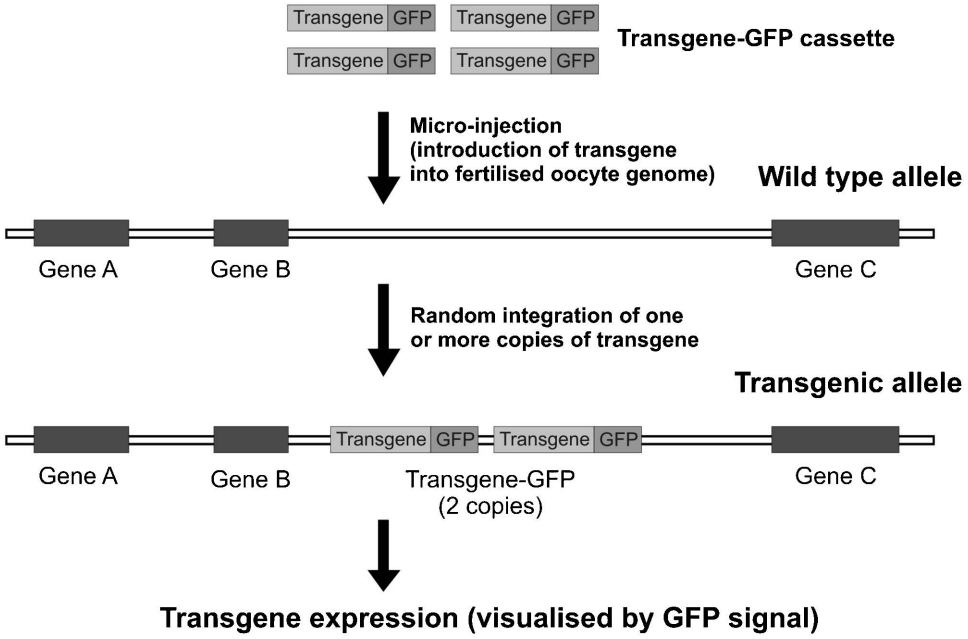

Figure 4 Transgenesis strategy. A transgene cassette is designed to contain features necessary for gene expression, for example, promoter and polyadenylation signal. Additional markers such as fluorescent protein tags (for example, GFP and YFP) can be included to facilitate the detection of transgene expression. The transgene is introduced into the genome of fertilized mouse oocytes. Integration of the transgene occurs randomly and multiple copies of the transgene may be integrated in the same chromosomal location or on different chromosomes. GFP, green fluorescent protein; YFP, yellow fluorescent protein.

Chemical-induced point mutant mice

To facilitate unbiased gene discovery in the mouse, a forward genetic-based approach using the potent mutagen, *N*-ethyl-*N*-nitrosourea (ENU) has been developed.[40–42] ENU primarily acts as an alkylating agent, transferring its ethyl group to any of a number of nucleophilic nitrogen or oxygen sites on each of the four deoxyribonucleotides.[40–42] The transferred ethyl group constitutes a DNA adduct that, during cell proliferation and DNA replication, results in heritable mutations. ENU induces the highest mutation rate of any agent tested in mice (on average 0.0015 point mutations per locus per gamete in the C57BL/6 strain). An ENU mutagenesis program involves injecting adult male mice with a series of ENU intraperitoneal injections. Following a transient period of sterility (10–12 weeks, depending on mouse strain and doses of ENU used), owing to the depletion of differentiated spermatogonia, mice are subjected to controlled breeding schemes to propagate the induced mutations. Ultimately descendents of these mice are screened for a phenotype of interest and linkage analysis used to identify the underlying mutation. Mutations are often introduced onto one mouse strain and offspring are outcrossed to a different strain to enable mapping of the mutated chromosomal region. Outbreeding can occur either during the generation of mice for phenotypic screening (Figure 5) or once a phenotype of interest has been identified. Linkage analysis (the localization of the causal mutation to a relatively small region in the whole genome) can be done using a number of techniques. The majority, however, exploit small differences in genomic DNA sequence between individual strains of mice. Once the linkage region is defined, the mutation is identified through the sequencing of candidate genes. Candidate genes within the linkage interval are generally selected based on gene expression profile, predicted protein function and available mouse/human disease phenotypes. Most recently, mutation identification without the need for candidate gene selection has been achieved by whole genome sequencing technologies (O'Bryan MK, pers. commun.). For more details on an ENU mutagenesis approach used to discover male fertility regulators, readers are referred to Refs 23–28.

In contrast to a candidate gene approach (knockout/knockin and transgenesis), ENU mutagenesis provides a powerful tool for the discovery of previously unsuspected genes within biological processes. In addition to defining gene function, critical domains/regions of the mutated protein can be identified. The first large-scale ENU mutagenesis began in 1997.[43,44] Currently, there are at least 20 ENU consortiums around the world.[45] Such programs have revealed many critical genes for human diseases, including male infertility.[46–49]

MOUSE MODELS FOR MALE INFERTILITY

In recent years, the identification of infertility genes spanning all aspects of spermatogenesis, sperm maturation, capacitation and fertilization has been accelerated by mouse model studies.[4,50–52] At least 400 fertility essential genes have been modeled in mice. The majority of these genes were identified using knockout models and to a lesser extent, knockin, transgenic and ENU-mutagenized models. The current review is by no means a comprehensive list of these mouse models, rather it contains examples of how mouse models have advanced our knowledge of male fertility regulation. Herein, we focus on single-gene defects that affect the process of spermatogenesis and post-testicular maturation and fertilization. Defects in genes involved in hormonal regulation of spermatogenesis are not included in the review. Readers are referred to a comprehensive list of these genes in Refs 4 and 16.

Premeiotic defects

In mammals, although spermatogenesis begins in fetal life, germ cells undergo mitotic arrest prior to regaining their proliferative and differentiative capacity at puberty. The stem cells involved in the process of spermatogenesis are called spermatogonia. Spermatogonia differentiate and undergo numerous cycles of mitosis at the basement of

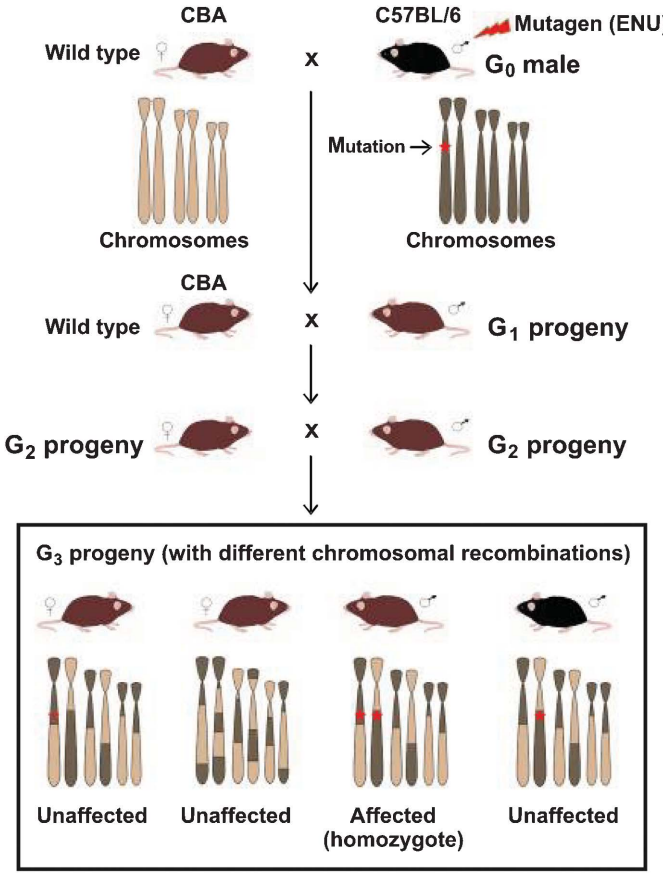

Figure 5 Example of a three-generation breeding scheme used to identify recessive mutations. To screen for recessive mutations, a three-generation breeding scheme is required. Founder male mouse (referred to as generation 0 (G_0)) of an inbred strain (for example, C57BL/6) is injected with ENU. The ENU-treated male is subsequently mated with wild-type females of a different inbred strain (for example, CBA) to produce G_1 offspring. G_2 progeny can be produced from G_1 littermate intercrosses or from G_1×wild-type CBA crosses (as shown here). Finally, G_3 progeny are generated from G_2 littermate intercrosses and/or G_2 females×G_1 fathers. During each step of crossing, each progeny will have different combinations of chromosomes from the ENU-treated mouse strain and the strain used for subsequent outcrossing. These differences enable researchers to map the region containing the ENU-induced mutation causing phenotypic defects of interest (indicated by *). In this case, mutations are introduced into the C57BL/6 genome. CBA, cytometric bead array; ENU, *N*-ethyl-*N*-nitrosourea.

seminiferous tubules. In mice, spermatogonia begin to proliferate (as well as self-renew) and differentiate at ~4 days after birth and divide continuously through mitosis to give rise to spermatocytes. Premeiotic defects can lead to the complete disruption of spermatogenesis and result in a phenotype equivalent to that of the 'Sertoli cell-only' syndrome seen in ~15.7% of men with no sperm in their ejaculates.[53] Several genes involving mouse spermatogonia self-renewal, apoptosis and cell cycle regulation have been shown to contribute to male infertility. Examples of such genes are listed in Table 1.

Meiotic defects

Meiosis is a special cell division whereby diploid parental cells produce genetically diverse haploid sperm (or eggs). The reduction in chromosome number is achieved by one round of DNA replication followed by two successive rounds of chromosome segregation (meiosis I and II). Meiosis I involves the segregation/separation of homologous chromosomes from each other, whereas meiosis II involves the segregation of sister chromatids and therefore resembles mitosis.[61] Male germ cells in the mouse testis enter meiosis in the second week of life. During meiosis, germ cells are termed spermatocytes. Spermatocytes pass through G_1 and S phase and subsequently enter the meiotic prophase, during which time chromosome condensation and the formation of DNA double-strand breaks (DSBs) (leptotene spermatocytes), followed by the initiation of pairing (synapsis) between homologous chromosomes (zygotene spermatocytes) occur. The completion of synapsis of homologous chromosomes and the repair of DSBs, using homologous chromosomes as templates, occur from the midzygotene through the pachytene spermatocyte periods. The culmination of this process results in the reciprocal exchange of genetic information between homologues, and is completed through the midpachytene to diplotene periods.[62] Genetic exchange takes place through the formation of DSBs followed by a crossover (synapsis) of genetic material between homologous chromosome pairs. This leads to the reassortment of maternal and paternal alleles and the production of genetically diverged haploid sperm. The formation and repair of meiotic DSBs is a pivotal process that drives genetic diversity.

Due to the complexity of the meiotic process, a large number of genes are proposed to be involved in its regulation. Defects in this process can lead to meiosis failure, the production of aneuploid gametes and infertility. Furthermore, gamete aneuploidy can result in embryonic death or developmental defects in the offspring.[62] A list of some crucial meiotic genes identified by the use of mouse models is shown in Table 2. Many of these genes are involved in the initiation of programmed DSB formation, meiotic recombination, DSB repair and

Table 1 Examples of genes essential for premeiotic germ cell development implicated by mouse model studies

Gene	*Proposed function*	*Knockout phenotype*	*Fertility status*	*Reference*
Etv5 (*Erm*) (Ets variant gene 5)	Transcription factor	Azoospermia; failed to maintain spermatogonia	Male infertility	54
Bax (BCL2-associated X protein)	Regulation of apoptosis	Premeiotic germ cell arrest	Male infertility	55
Pi3k (phosphoinositide-3-kinase)	Phosphatidylinositol 3′-kinase signaling pathway	Impaired spermatogonia proliferation and increased apoptosis of spermatogonia	Male infertility	56
Nanos2 (Nanos homolog 2 (*Drosophila*))	Germ cell differentiation	Germ cell apoptosis and complete loss of spermatogonia	Male infertility	57[a], 58
Ddx4 (*Vasa*) (DEAD (Asp–Glu–Ala–Asp) box polypeptide 4)	Germ cell proliferation and differentiation	Impaired premeiotic germ cells differentiation and increased apoptosis of spermatogonia	Male infertility	59
Dazl (Deleted in azoospermia-like)	Germ cell proliferation and differentiation	Azoospermia; few spermatogonia enter meiosis, and those that do fail to proceed beyond pachytene	Male/female infertility	60

[a] Transgenic mouse model.

Table 2 Examples of genes essential for meiosis implicated by mouse model studies

Gene	*Proposed function*	*Knockout phenotype*	*Fertility status*	*Reference*
Spo11 (Sporulation protein, meiosis-specific, SPO11 homolog (*S. cerevisiae*))	Programmed DSB formation	Impaired DSB formation and recombination initiation	Male/female infertility	63
Mei1 (Meiosis defective 1)	Programmed DSB formation	Impaired DSB formation and recombination initiation	Male/female infertility	64[a]
Atm (Ataxia telangiectasia mutated homolog (human))	DSB sensing and repairing	Impaired chromosomal synapsis and chromosome fragmentation	Male/female infertility	65
Dmc1 (Dosage suppressor of mck1 homolog, meiosis-specific homologous recombination (yeast))	Meiotic recombination	Impaired chromosomal synapsis	Male/female infertility	66
H2afx (H2A histone family, member X)	Assembly of specific DSB repair complexes	Meiosis arrested at pachytene stage	Male infertility/female subfertility	67
Trip13 (Thyroid hormone receptor interactor 13)	Meiotic recombination and DSB repair	Impaired DSB repair	Male/female infertility	68
Mlh1 (MutL homolog 1 (*E. coli*))	Mismatch repair	Impaired meiotic recombination	Male/female infertility	69
Mlh3 (MutL homolog 3 (*E. coli*))	Mismatch repair	Impaired chromosomal synapsis	Male/female infertility	70
Pms2 (Postmeiotic segregation increased 2 (*S. cerevisiae*))	Mismatch repair	Impaired chromosomal synapsis	Male infertility	71
Msh4 (MutS homolog 4 (*E. coli*))	Mismatch repair	Impaired chromosomal synapsis	Male/female infertility	72
Msh5 (MutS homolog 5 (*E. coli*))	Mismatch repair	Impaired chromosomal synapsis and synaptonemal complexes formation	Male/female infertility	73
Exo1 (Exonuclease 1)	Mismatch repair	Dynamic loss of chiasmata and apoptosis	Male/female infertility	74
Cdk2 (Cyclin-dependent kinase 2)	Cell cycle regulation	Meiosis arrest at prophase I and atrophy of the testes and ovaries	Male/female infertility	75
Ccna1 (Cyclin A1)	Cell cycle regulation	Meiosis prophase I arrest and increased germ cell apoptosis	Male infertility	76
Fkbp6 (FK506-binding protein 6)	Homologous chromosomes synapsis	Meiosis arrest at pachytene stage	Male infertility	77
Psmc3ip (*Hop2, Tbpip*) (Proteasome (prosome, macropain) 26S subunit, ATPase 3, interacting protein)	Homologous chromosomes synapsis	Meiosis arrest	Male/female infertility	78
Dnmt3l (DNA (cytosine-5-)-methyltransferase 3-like)	DNA methylation	Meiosis arrest and epigenetic defects	Male infertility; heterozygous progeny of homozygous females die by midgestation	79,80
Siah1 (Seven in absentia 1A)	Protein ubiquitination and degradation	Meiosis prophase I arrest	Male infertility/female subfertility	81
Prdm9 (*Meisetz*) (PR domain containing 9)	Histone H3 methyltransferase that controls epigenetic events required for meiotic prophase	Impaired chromosomal synapsis and sex body formation	Male/female infertility	82
Rec8 (Rec8 homolog (yeast))	Synaptonemal complex formation	Abnormal synaptonemal complexes	Male/female infertility	83
Sycp1 (Synaptonemal complex protein 1)	Synaptonemal complex assembly, meiotic recombination, and XY body formation	Impaired chromosomal synapsis	Male/female infertility	84
Sycp2 (Synaptonemal complex protein 2)	Synaptonemal complex assembly and chromosomal synapsis	Impaired axial element formation	Male infertility/female subfertility	85
Sycp3 (Synaptonemal complex protein 3)	Axial/lateral elements and synaptonemal complex formation and chromosomal synapsis	Impaired axial/lateral elements and synaptonemal complexes formation	Male infertility/female subfertility	86
Syce1 (Synaptonemal complex central element protein 1)	DSB repair	Impaired DSB repair	Male/female infertility	87
Syce2 (Synaptonemal complex central element protein 2)	Synaptonemal complex formation, homologous recombination and DSB repair	Impaired chromosomal synapsis and sex body formation	Male/female infertility	88
Smc1b (Structural maintenance of chromosomes 1B)	Segregation of chromosomes	Impaired sister chromatid cohesion formation and chromosomal synapsis	Male/female infertility	89
Eif4g3 (Eukaryotic translation initiation factor 4γ, 3)	Translational regulation	Meiotic arrest; spermatocytes failed to exit prophase *via* G2/MI transition	Male infertility	49[a]

[a] Chemical-mutagenized point mutant mouse models.

Abbreviations: DSB, double-strand break; *E. coli*, *Escherichia coli*; *S. cerevisiae*, *Saccharomyces cerevisiae*.

cell cycle regulation, and play a pivotal role in both male and female meiosis.

Postmeiotic or spermiogenesis defects

Upon the completion of meiosis, haploid germ cells termed 'round spermatids' are produced and subsequently undergo a series of differentiation processes collectively known as 'spermiogenesis' to give rise to highly polarized spermatozoa. Spermiogenesis involves dramatic morphological alterations, which include chromatin reorganization and condensation, acrosome formation, cytoplasmic removal, sperm tail assembly and spermiation.

During sperm head formation, the characteristic shape of the sperm in each species is formed through the coordinated repackaging of the chromatin and the sculpting of the sperm head through processing involving the acroplaxome–manchette complex. Specifically, histones that package DNA in the majority of cell types are removed and replaced first with transition proteins (TPs) and then protamines (Prms) to form a highly condensed and transcriptionally inactive nucleus. As evidence in both TP (*Tp1* and *Tp2*) and Prm (*Prm1* and *Prm2*) knockout models, the attainment of the correct ratio of these proteins is essential for the formation of 'normal' looking sperm and for fertility.[90–93] In agreement with mouse model studies, clinical association data suggest that attainment of the correct ratio of Prms is essential for human male fertility.[94,95]

In parallel with the changes in nuclear packaging, mounting data suggest that the elongating spermatid nucleus is shaped through processes involving the acroplaxome–manchette.[96] The acroplaxome is an F-actin-containing series of loop-like structures that form around the elongating spermatid and appear to be involved in the progressive extrusion of the head, as well as in the anchoring of the growing acrosome.[97,98] In addition, the acroplaxome is coupled to the manchette, which in turn is composed of a marginal ring around the nucleus and a fringe-like microtubule structure that extends into the distal cytoplasm. Several mouse models, and microscopic studies, strongly suggest that these structures are integral to the formation of normal sperm head shape.[99–101] Data also suggest that the manchette is involved in the transport of proteins required for sperm tail formation in a process known as intramanchette transport.[100–102]

Mouse models have also been critically important in defining the mechanisms of sperm tail formation and have revealed some surprising phenomena and the etiology of several significant human pathologies.[52] During the last few years in particular, research has begun to illustrate the importance of axoneme function, not only for sperm motility but also for cilia function in a range of somatic tissues and many aspects of human health. The axoneme is a 9+2 microtubule structure that runs as a core through the sperm tail (modified flagella).[103] The same structure is conserved in all motile cilia (and flagella) from all kingdoms ranging from trypanosomes and *Chlamydomonas*, to mice and humans. While Afzelius *et al.*[104] recognized several decades ago that abnormally formed axonemes lead to both sterility and disorders in other ciliated tissues, including the lung, brain and kidney in a syndrome known as primary ciliary dyskinesia, it is only in the last decade that some of the underlying genes have been revealed.[105–107] In addition, and largely through the use of mouse models, it is now recognized the abnormal cilia (sperm tail) function/formation at least in mice can be induced by a range of other genes involved in sperm tail/cilia development or in transducing environmental signals to the axoneme. Such genes and models include *Tektin 2* (*Tekt2*), which is involved in dynein arm formation in the axoneme;[108] *Alms1*,[109,110] and several other genes involved in basal body function and in initiating the growth of the axoneme; the *Catsper* genes, which are involved in Ca^{2+} regulation and onset of hyperactivated motility;[111–114] *Slo3*, which is involved in K^+ flow and in regulating capacitation and hyperpolarization;[115] and *Gapds* and *Pgk2*, which are involved in the establishment of glycolytic pathways on the sperm tail accessory structures and the generation of ATP for axoneme function.[116,117] For a comprehensive review of mouse models with abonormal sperm tail function, readers are referred to Escalier.[52]

Spermiation is a hormonally regulated process by which spermatozoa are released into the lumen of the seminiferous tubules. This involves the detachment of elongated spermatids from the Sertoli cells and the removal of most of the cytoplasm within the nascent spermatozoa as residual bodies.[118,119] Several knockout models of genes involving in chromatin packing, nuclear condensation, cytoplasmic exclusion, and signal transduction and protein transport have been shown to contribute male infertility (Table 3).

Post-testicular maturation and fertilization defects

Sperm appear morphologically mature following spermiation; however, they do not gain the capacity for fertilization until they have undergone transit through the epididymis in a poorly understood process known as 'epididymal maturation'. Epididymal maturation is dependent upon factors produced by the epididymis and is characterized by extensive post-translational protein modifications, changes in membrane composition and the progressive attainment of functional competence.[120–123] For a more extensive review of these important processes, readers are referred to Refs 124–126.

Subsequent to epididymal maturation, sperm must undergo the process of capacitation in the female reproductive tract (or in media) to be fully capable of fertilization. Capacitation refers to a series of signal transduction and protein modification processes that occur during a period of time following the ejaculated sperm into the female reproductive tract. Capacitation confers upon sperm the ability to bind the zona pellucida of the oocyte and undergo the acrosome reaction. Sperm capacitation correlates with a massive increase in the tyrosine phosphorylation of many sperm tail and head proteins,[127–129] an increase in membrane fluidity, cholesterol efflux and an increase in intracellular Ca^{2+} and cyclic AMP concentration.[130–132] Capacitation is also correlated with the ability of sperm to manifest hyperactivated motility and for them to bind to the outer vestments of the oocyte during acrosome reaction.[133–135] Hyperactivated motility, which is characterized by high-amplitude asymmetrical bending of the sperm tail, is proposed to be important for the sperm to detach itself from the mucosal membrane of the oviduct, where they are temporarily stored, and also to penetrate the zona pellucida of the oocyte during the acrosome reaction.[135] Ion channels including the CatSper channels play a central role in sperm hyperactivation *via* the regulation of Ca^{2+} flow.[111–114]

The acrosome reaction is also characterized by a Ca^{2+} influx that triggers fusion of a number of sites of the initial sperm–zona pellucida interaction.[136,137] This interaction involves complementary, but as yet incompletely defined, sites on the sperm outer acrosomal membrane and ZP3 on the egg plasma membrane.[138–140] The fusion allows development of vesicles, containing hydrolytic enzymes, which are released in a process called acrosomal exocytosis to break down the egg cumulus mass. This exposes the inner acrosomal membrane so that it can bind the zona pellucida and an appropriately reacted sperm enters the perivitelle space. The sperm and the plasma membrane of the egg bind and fuse.[141–143]

Several genes have been implicated to play a role in post-testicular maturation, capacitation and fertilization using mouse model studies

Table 3 Examples of genes essential for spermiogenesis, epididymal maturation, capacitation and fertilization implicated by mouse model studies

Gene	*Proposed function*	*Knockout phenotype*	*Fertility status*	*Reference*
Spem1 (Sperm maturation 1)	Spermatid cytoplasmic removal	Deformed sperm head and tail structure and impaired spermiogenesis	Male infertility	144
Gopc (Golgi-associated PDZ and coiled-coil motif containing)	Acrosome biogenesis	Fragmented acrosomes; globozoospermia (round-headed spermatozoa)	Male infertility	145
Prm1 and *Prm2* (Protamine 1 and 2)	Sperm chromatin condensation	Haploinsufficiency effect; sperm derived from spermatogenic cells with one copy of the mutated allele are immotile and unable to fertilize eggs	Chimera male/female infertility	90
Tnp1 (Transition protein 1)	Sperm chromatin condensation	Subtle sperm morphology abnormalities and reduced motility	Male subfertility	91
Tnp2 (Transition protein 2)	Sperm chromatin condensation	Impaired chromatin condensation, sperm head abnormalities and reduced sperm motility	Male subfertility	92,93
Tssk6 (*Sstk*) (Testis-specific serine kinase 6)	Chromatin remodeling	Abnormal sperm head morphology, reduced DNA compaction in spermatozoa and impaired sperm motility	Male infertility	146
Cadm1 (*Tslc1*) (Cell adhesion molecule 1)	Cell adhesion, differentiation and apoptosis	Spermatid maturation arrest, degenerated and sloughed off into the lumen	Male infertility	147,148
Pvrl2 (*Nectin 2*) (Poliovirus receptor-related 2)	Cell adhesion	Abnormal sperm head and sperm tail midpiece structure, impaired zona binding, and lack of oocyte penetration	Male infertility	149
Csnk2a2 (Casein kinase 2, alpha prime polypeptide)	Sperm head morphogenesis	Oligospermia (low sperm counts) and globozoospermia	Male infertility	150
Rara (Retinoic acid receptor, alpha)	Ligand-induced transcription factor	Sloughing of immature germ cells in the lumen of seminiferous tubules	Male infertility	151
Pafah1b1 (*Lis1*) (Platelet-activating factor acetylhydrolase, isoform 1b)	Acrosome and tail biogenesis	Impaired acrosome and tail formation; the basal tail cuff at the point of insertion but fail to add axonemes to elongate the tail structure	Male infertility	100[a]
Meig1 (Meiosis-expressed gene 1)	Sperm tail biogenesis, assembly of the axonemes/flagella	Absent sperm flagella, abnormal sperm head, disrupted microtubular organelle essential for sperm head and flagella formation	Male infertility	101
Tekt2 (*Tektin-t*) (Tektin 2)	Sperm tail biogenesis, dynein arm formation in the axoneme	Bending of the sperm flagella and marked defects in motility and primary ciliary dyskinesia	Male infertility	108[a]
Adcy10 (*sAC*) (Adenylate cyclase 10)	Sperm motility	Normal sperm counts and morphology with severely impaired motility	Male infertility	152
Gapds (Glyceraldehyde-3-phosphate dehydrogenase, spermatogenic)	Generation of energy supply for sperm motility	Normal sperm counts and morphology with impaired motility (loss of forward progression motility)	Male infertility	117
Pgk2 (Phosphoglycerate kinase 2)	Generation of energy supply for sperm motility	Impaired progressive motility	Male subfertility	116
Agfg1 (*Hrb*) (ArfGAP with FG repeats 1)	Acrosome biogenesis	Globozoospermia	Male infertility	153
Pick1 (Protein interacting with C kinase 1)	Vesicle trafficking from the Golgi apparatus to the acrosome	Fragmented acrosomes Globozoospermia	Male infertility	154
Vdac3 (Voltage-dependent anion channel 3)	Ion transport for sperm tail formation	Normal sperm counts, abnormal sperm tail structure and markedly reduced motility	Male infertility	155
Catsper1, *Catsper2*, *Catsper3*, *Catsper4* (Cation channel, sperm associated 1–4)	Sperm motility and fertilization (ion channels)	Normal sperm counts, normal morphology, markedly reduced motility and unable to fertilize intact eggs	Male infertility	111–114
Capza3 (Capping protein (actin filament) muscle Z-line, alpha 3)	Spermatid cytoplasmic extrusion	Excess cytoplasm and impaired motility	Male infertility	46[b]
Dnahc1 (Dynein, axonemal, heavy chain 1)	Cilia and flagella function; axoneme movement	Normal sperm counts, normal morphology, markedly reduced forward motility	Male infertility and primary ciliary dyskinesia	156
Izumo1 (Izumo sperm–egg fusion 1)	Membrane fusion	Defect in sperm–egg fusion	Male infertility	157
Akap4 (A kinase (PRKA) anchor protein 4)	Sperm tail assembly (scaffold protein)	Normal sperm counts, normal morphology, markedly reduced motility	Male infertility	158
Pcsk4 (Proprotein convertase subtilisin/kexin type 4)	Acrosome reaction and sperm–egg interaction	Impaired fertilization	Male subfertility	159
Kcnu1 (*Slo3*) (Potassium channel, subfamily U, member 1)	Capacitation and acrosome reaction (ion transportation)	Impaired motility, abnormal sperm morphology (hairpin shape), impaired sperm capacitation and acrosome reaction	Male infertility	115
Adam3 (Cyritestin)	Cell adhesions (plasma membrane protein)	Defect in sperm to bind to the zona pellucida	Male infertility	160
Clgn (Calmegin)	Folding and transport of integral membrane proteins (testis-specific chaperone)	Impaired sperm–zona pellucida adhesion	Male infertility	161
Adam2 (Fertilin-β)	Cell adhesions (plasma membrane protein)	Impaired sperm–egg membrane adhesion, sperm–egg fusion, migration from the uterus into the oviduct, and binding to the egg zona pellucida	Male subfertility	162

[a] Gene-trapped mouse models.
[b] ENU mutagenized mouse model.

(Table 3). In contrast to premeiotic and meiotic defects, the majority of genes critical for postmeiotic and post-testicular defects exhibit male-specific infertility defect.

FACTORS TO BE CONSIDERED WHEN EVALUATING MOUSE MODELS

Although the use of animal models is ideal for *in vivo* gene function analysis, phenotypic defects observed in genetically modified animal models can influenced by different genetic backgrounds and environmental factors. It is important that these factors are appropriately addressed to prevent misinterpretation of results.

In the case of knockout/knockin mice, the ES cells and the recipient blastocyst are often obtained from animals carrying genes of different coat colors to facilitate the initial selection of chimeric progeny. The most commonly used ES cells are those derived from the mouse strain 129, which has an agouti coat color. Once targeted, these ES cells are microinjected into blastocysts obtained from a mouse strain with different coat colors (for example, C57BL/6 mice, which have a black coat color). Offspring with a high degree of agouti coat color, as an indication of the transmission of 129 ES cell-derived gene, are mated to produce heterozygous knockout mice. These mice are intercrossed to generate homozygous null progeny, which give rise to a cohort of mice on a mixed genetic background (for example, 129 and C57BL/6). Similarly, mice generated through genome-wide ENU mutagenesis may be on a mixed genetic background as a consequence of the founder mouse strain used for ENU treatment and a mouse strain used for outcrossing for linkage analysis and mutation identification being different. In many cases, the initial phenotypic characterization of the knockout/knockin and point mutant mutagenized mice is performed on a mixed genetic background, which can result in a range of phenotypic variations. Genetic modifiers (for example, single-nucleotide polymorphisms and flanking gene effect) are proposed to be a critical factor that contributes to the phenotypic diversity.[163]

To minimize the effect of genetic background variations, knockout mice generated by 129 ES, for example, cells can be backcrossed to produce a congenic line, that is, a strain that differs from another in the region of the gene of interest and a short-linked chromosomal segment around the gene. The production of a congenic line involves breeding of the knockout mice with the strain of mice used for the initial chimera breeding (or a new inbred strain of choice) for at least 10 generations. This conventional backcrossing approach can take up to 3 years to generate 99% of the recipient genome at the tenth backcrossing (N10). Recently, a new high-speed congenic approach using round spermatids retrieved from immature males (22–25 days of age) and polymorphic marker analysis was reported.[164] This approach has been successfully used to produce congenic line within a 6-month period. An alternative approach to avoid congenic line production is to use C57BL/6 ES cells for the initial gene targeting. Although homologous recombination in the C57BL/6 ES cells is lower than that of the 129 strain, it has been shown that a significant number of targeted ES clones are produced using C57BL/6 ES cells.[165]

In addition to genetic background, several environmental factors can have a profound influence on the observed phenotype in inbred and congenic strains. These factors include viral and bacterial infections, exposure to loud sounds, and dietary and maternal effects.[166]

INTERNATIONAL MOUSE PHENOTYPING CONSORTIUMS

Researchers should be aware of the existence of resources that provide access to data and procedures for mouse phenotyping. These include the European Mouse Phenotyping Resource (EuroPhenome: http://www.europhenome.org), the European Mouse Phenotyping Resource of Standardised Screens (EMPReSS: http:empress.har.mrc.ac.uk) and the European Mouse Disease Clinic (EUMODIC: http://www.eumodic.org).[167,168] Within our lab, we broadly categorize the type of male infertility using the strategy outlined in Borg *et al.*[169]

EMPReSS provides comprehensive standard operating procedures (SOPs) covering the main physiological systems for mouse phenotypic screens. To date, ~150 SOPs have been developed. Additionally, SOPs for histology, pathology and gene expression analyses are available. EuroPhenome provides phenotyping data from SOPs contained in the EMPReSS database together with the development of novel mouse phenotyping approaches. EUMODIC aims to perform primary phenotyping of 650 mouse mutant lines derived using the European Conditional Mouse Mutagenesis Program.[39] Mouse lines with phenotypic defects of interest will undergo specialized phenotypic assessment. Data from the EUMODIC project are being integrated within the EuroPhenome web interface. These publically available resources will enable researchers to precisely assess the phenotypic defects of mouse models.

CONCLUSION

The availability of complete genome sequences of human, mouse and a number of organisms has opened up a new era of gene functional analysis. Using mouse models, reproductive biologists have successfully revealed hundreds of genes essential for fertility and many are in the process of being identified and characterized. The next challenge is the translation of the knowledge obtained from studies on the mouse into the human infertility clinic to serve the ultimate goals of: (i) improving the existing infertility treatments; (ii) providing alternative choices of treatment; (iii) developing precise diagnostics; and (iv) formulating contraceptives for males with minimal side effects.

COMPETING FINANCIAL INTERESTS

The authors declare no conflict of interest.

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