review

Genetic dissection of mammalian fertility pathways

Martin M. Matzuk*†‡# and Dolores J. Lamb†§

Departments of *Pathology, †Molecular and Cellular Biology and ‡Molecular and Human Genetics, and §Scott Department of Urology, Baylor College of Medicine, Houston, TX 77030, USA

#e-mail: mmatzuk@bcm.tmc.edu

The world's population is increasing at an alarming rate and is projected to reach nine billion by 2050. Despite this, 15% of couples world-wide remain childless because of infertility. Few genetic causes of infertility have been identified in humans; nevertheless, genetic aetiologies are thought to underlie many cases of idiopathic infertility. Mouse models with reproductive defects as a major phenotype are being rapidly created and discovered and now total over 200. These models are helping to define mechanisms of reproductive function, as well as identify potential new contraceptive targets and genes involved in the pathophysiology of reproductive disorders. With this new information, men and women will continue to be confronted with difficult decisions on whether or not to use state-of-the-art technology and hormonal treatments to propagate their germline, despite the risks of transmitting mutant genes to their offspring.

Despite advances in assisted reproductive technologies, infertility is a major health problem worldwide. Approximately 15% of couples are unable to conceive within one year of unprotected intercourse. The fertility potential of a couple is dependent on the coordinated and combined functions of both male and female reproductive systems. Anatomic defects, gametogenesis dysfunction, endocrinopathies, immunologic problems, ejaculatory failure and environmental exposures are significant causes of infertility. Although several infertility disorders are associated with defined genetic syndromes (for example, cystic fibrosis and Turner's Syndrome[1,2]), almost a quarter of clinical infertility cases of either sex are idiopathic in nature, in part as a result of a poor understanding of the basic mechanisms regulating fertility. It is thought that genetic defects underlie many of these unrecognized pathologies. On the basis of over 200 infertile or subfertile genetic mouse models (see Supplementary Information Table; also see ref. 3), it is not surprising that the diagnosis of idiopathic infertility is common in the clinic[4,5].

In this review, we discuss causes of mammalian infertility with an emphasis on the genetic basis of fertility defects in humans and mice. Animal models have defined key signalling pathways and proteins involved in reproductive physiology[6]. Mouse models have been produced by spontaneous mutations, fortuitous transgene integration, retroviral infection of embryonic stem cells, ethylnitrosurea (ENU) mutagenesis and gene targeting technologies[3,7,8]. These mutations affect all aspects of reproduction, including ovarian development and function, testis determination, spermatogenesis, sperm function, genital tract development and function, sexual behaviour, fertilization and early embryonic development, and therefore have contributed much to our understanding of infertility. For example, male infertility is observed in the spontaneous mutant models hypogonadal (*hpg*)[9] and testicular feminization (*tfm*)[10] and in models created by transgene integration, such as the *kisimo* mouse model (which arose by transgene disruption of the *Theg* gene[11]) and retroviral disruption of the *Bclw*[12], *Mtap*[13] and *Spnr*[14] genes. These models are improving our knowledge of the genetic basis of mammalian infertility and suggest that in the future, clinical technologies must advance to enable analysis of many more genes when an infertile couple enters the clinic. Currently, karyotype analysis, sequence analysis of the cystic fibrosis transmembrane conductance regulator gene and Y chromosome deletion analysis (for males) are the only genetic tests commonly offered to infertile patients[4,5].

Where it all begins

Reproductive development and physiology are evolutionarily conserved processes across eutherian mammalian species and many other vertebrates, including marsupials[15], amphibians, reptiles, birds and fish[16–19]. Several genes required for vertebrate fertility are also highly conserved in evolution, with orthologues in *Drosophila melanogaster* (for example, *vasa* (DDX4), *fat facets* (DFFRY) and *boule* (DAZ)[20–22]). Propagation of the vertebrate germline requires development of the gonads, the site of future gamete production. The indifferent gonad forms during foetal development, primordial germ cells enter the gonad primordium and the tissue eventually differentiates along a female (ovarian) or male (testicular) pathway; this differentiation dictates the formation of the secondary sex organs[19]. Although there may be spatiotemporal variations of these processes in different species (for example, in mice and humans, gonadal sex determination occurs *in utero*, whereas in marsupial mammals, it occurs after birth), they eventually yield ovaries that produce eggs or testes that generate spermatozoa.

Defects in sexual differentiation pathways can cause infertility in mice and humans of both sexes (Fig. 1)[23–25]. In 1959, through the analysis of human XO (Turner syndrome) females and XXY

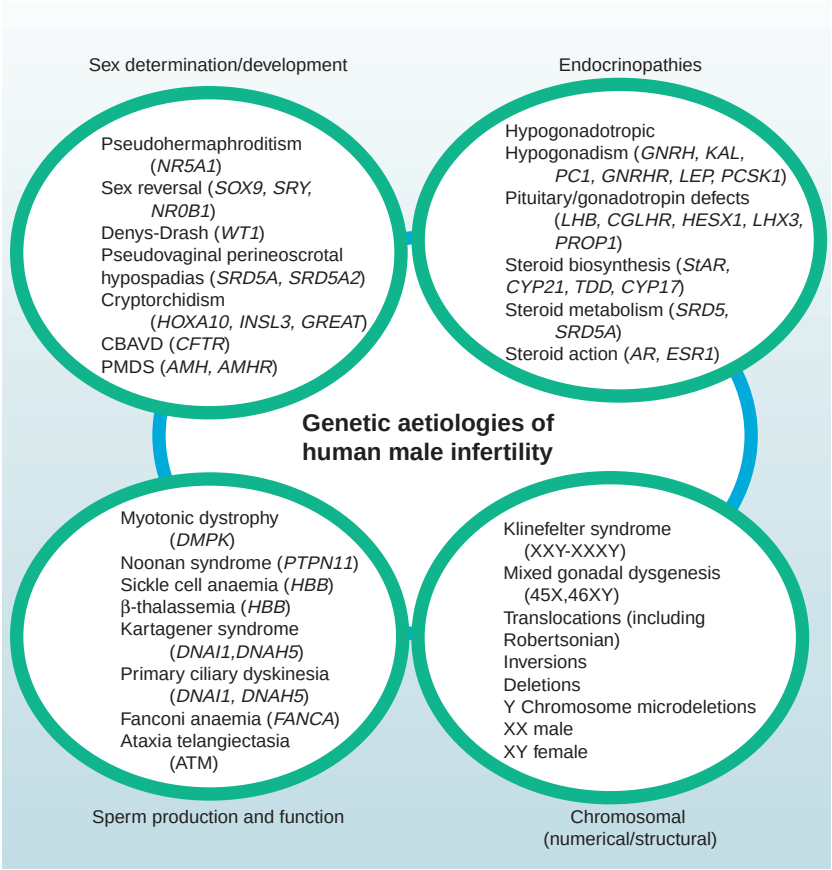

Figure 1 **Genetic aetiologies of human male infertility.** Developmental disorders causing human male infertility result from a failure of gonadal development or testis determination, endocrinopathies, well-known genetic syndromes, and numerical and structural chromosomal abnormalities (translocations, deletions and inversions).

(Kleinfelter syndrome) males, as well as XO and XX female mice and XY male mice, it was concluded that the Y chromosome was male determining[26–28]. Subsequent chromosomal and genetic studies of humans and mice with sex reversal syndromes and infertility revealed that many XX males have translocations of a small piece of the Y chromosome, so that the sex-determining region Y gene (*SRY*) results in testis development. Similarly, XY females often have inactivating mutations in the *SRY* gene, resulting in the development of ovaries[29,30]. A critical role of *Sry* in sex determination was confirmed by showing that the expression of an *Sry* transgene in an XX mouse causes testis formation, and physical and behavioural sex reversal[31]. Most *SRY* mutations disrupt the high mobility group (HMG) box of the SRY protein; not surprisingly, this region is highly conserved among different species[32]. HMG box-containing proteins typically bind and significantly bend DNA and function as transcription factors or facilitators of transcription. Several genes upstream and downstream of SRY in the sex determination pathway are now known (reviewed in ref. 23). For example, XY female sex reversal correlates with a duplication of the human X-linked gene *DAX1* (ref. 33) or haploinsufficiency of the autosomal *SOX9* gene[32,34–36]. Interestingly, whereas an extra Y chromosome (that is, XYY) has little effect on human male fertility because of the selected loss of the extra Y during spermatogenesis[37], Klinefelter (XXY–XXXXY) males account for 10–15% of azoospermic patients[38].

From a distance, they will come

In both sexes, the primordial germ cells (PGCs) are defined histologically as alkaline-phosphatase-positive embryonic cells[39,40]. In the mouse, these cells divide rapidly under the influence of transforming growth factor-β (TGF-β) superfamily signals; knockout models lacking bone morphogenetic protein-4 (BMP-4) or BMP-8b, or the downstream cytoplasmic-to-nuclear relay proteins, SMAD1 and SMAD5, have defects in PGC development[41–44]. At mid-gestation, the PGCs begin one of the longest journeys of any mammalian cell, migrating from their origin at the base of the yolk sac, along the hind-gut, to eventually enter the genital ridge. Factors required for this migration in humans are unknown, although chemoattractants and cell adhesion factors have been implicated[45]. In the mouse, mutations in Kit receptor (KITR) and Kit ligand (KITL) genes block PGC migration, causing infertility, but not altering sexual differentiation[46].

Few known human mutations result in a reduction of the PGC or follicle pool, although girls with Turner's syndrome (partial or complete X-chromosome monosomy), have streak (remnant) gonads with no oocytes. Many Turner's syndrome cases with ovarian failure seem to be caused by loss of the short arm of the X-chromosome[2]. Among the candidate Turner's syndrome ovarian failure genes are *ZFX, BMP15, UBE1* and *USP9X* (ref. 2). An absence of *Zfx* in mice results in a loss of germ cells and subfertility in both sexes[47]; loss of *BMP15* in sheep causes a block at the primary follicle stage and infertility[48]. Studies in XO mice suggest that abnormal chromosomal segregation contributes to germ cell problems[49], indicating that multiple factors are responsible for these human ovarian abnormalities.

Death in the germline

In females, quiescent primordial follicles (a non-growing oocyte surrounded by squamous granulosa cells) form during prenatal life in humans and post-natally in mice. Recruitment of these follicles during

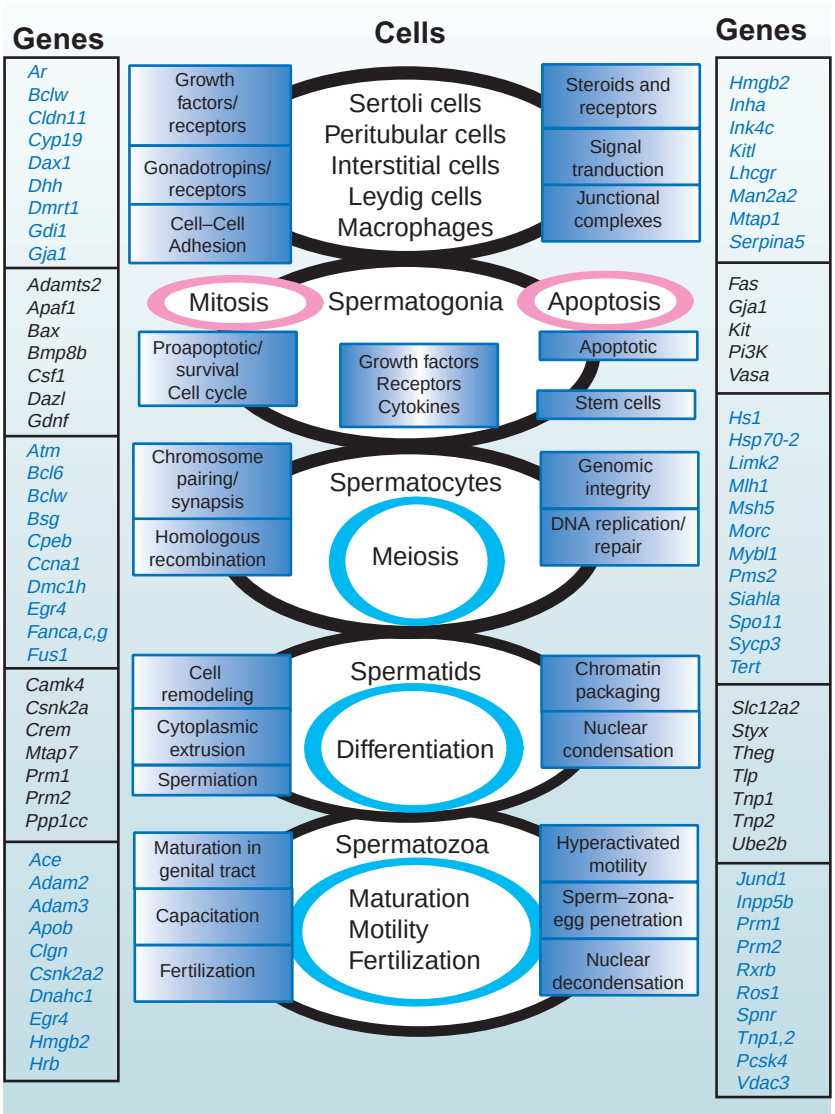

Figure 2 **Genes involved in the regulation of male reproduction in the mouse.** Spermatogenesis requires a complex interaction of the various cellular compartments of the testis (seminiferous epithelium containing spermatogenic cells, Sertoli cells and peritubular myoid cells, the interstitial cell compartment containing the steroidogenic Leydig cells, macrophages, and other interstitial cells, and the vasculature). Targeted mutation of the genes shown affects specific testicular cell types and reproductive function, resulting in male infertility or subfertility in the mouse (detailed in the Supplementary Information Table).

ovarian folliculogenesis permits further growth and development of oocytes. In males, spermatogenesis is characterized by three specific functional phases: proliferation, meiosis and spermiogenesis. The proliferative phase in the testis begins early in embryonic development, and with the exception of a brief period when spermatogonia arrest during late foetal and early postnatal life, they proliferate actively throughout life. Spermatogonial stem cells were one of the first recognized examples of adult stem cells capable of rejuvenating spermatogenesis after toxic insult[50,51]. In contrast, the formation of primordial follicles in females defines a finite endowment of oocytes. Between the time of ovary development and reproductive sequence, there is a precipitous drop in the number of oocytes. In humans, seven million foetal germ cells at 20 weeks are reduced to two million oocytes at birth, and eventually to 300,000 at puberty[52,53]. Thus, factors that prenatally and postnatally regulate germ cell survival in the ovary can prolong the reproductive lifespan.

The spermatogonia proliferation rate, the highest in the body, is well regulated; thus, it is not surprising that genes involved in growth (for example, *Kit*, *Csf* and *Bmp8b*) and apoptosis are also required for normal spermatogonia (Fig. 2). This stage of spermatogenesis is also noteworthy for its inefficiency; in rats, 75% of spermatogonia do not survive to become mature sperm[54]. A balance of anti-apoptotic members of the BCL2 family (that is, BCL2, BCL6, BCLX, and BCLW) and the pro-apoptotic BAX protein is extremely important in the regulation of germ cell survival prenatally and postnatally in both sexes, and in response to toxins in the ovary[55–57]. It is possible that defects in this delicate balance of cell proliferation and cell death contribute to the clinical pathology of hypospermatogenesis (all cellular elements of the testis are present, but at low cellularity). In the mouse, an absence of BCLX results in a complete loss of germ cells before birth in both males and females; furthermore, a lack of BCLW results in a partial reduction of PGCs in females, whereas an absence of BCL2 results in only decreased oocyte numbers postnatally[12,58,59]. Absence of either BAX or BCLW causes male infertility, and absence of BCL6 causes male subfertility, again suggesting that a balance of apoptotic/anti-apoptotic factors is necessary for normal spermatogenesis. In contrast, a prolonged female reproductive lifespan occurs in the absence of BAX in mice, consistent with its pro-apoptotic role[60]. Polycyclic aromatic hydrocarbons in cigarette smoke and air pollution bind to the aryl hydrocarbon receptor to stimulate transcriptional activation of *Bax*, thereby enhancing apoptosis and oocyte loss[61]. In the future, factors that inhibit the BAX pathways or stimulate the anti-apoptotic pathways could prolong the reproductive lifespans of women.

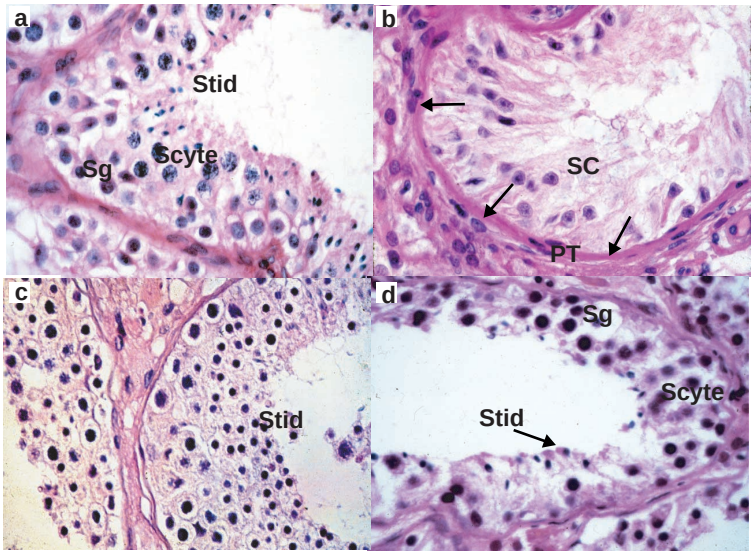

Figure 3 **Spermatogenic failure in the human.** Spermatogenesis in the human is characterized by six stages that are present in a mosaic fashion in the seminiferous tubule. **a**, Normal human spermatogenesis with Sertoli cells (SC) spermatogonia (Sg) towards the basal portion of the tubule, spermatocytes (Scyte) and maturing spermatids (Stid) located towards the lumen of the tubule. The tubules are surrounded by peritubular myoid cells (PT). The interstitial area contains the steroidogenic Leydig cells that secrete testosterone. **b**, An example of the most severe testicular pathology, with a total absence of germ cells and a Sertoli-cell-only pathology. Mild thickening of the peritubular layer is also observed (arrows; peritubular fibrosis). **c**, A late maturation arrest. The most mature cell type present is the round spermatid. **d**, An example of hypospermatogenesis, where all cell types are present in the testis, but with a low level of cellularity within the seminiferous tubule.

Meiosis, recombination, the integrity of the genome and more death

Meiosis is a process of cell division that is unique to germ cells and is required for the production of healthy haploid gametes (reviewed in greater detail in ref. 62). This process is evolutionarily important for both the integrity and diversity of species, as recombination of homologous chromosomes occurs during prophase of the first meiotic division, helping to orient chromosomes on the meiotic spindle, as well as introducing genetic variability. Male spermatogenesis is initiated postnatally (in mice at postnatal day 7) and is a continuous process producing spermatozoa. Proliferating spermatogonia differentiate and enter meiosis as spermatocytes. In contrast, oogenesis is initiated prenatally (in mice at embryonic day 13), arrests initially at the diplotene stage of meiotic prophase, resumes with the preovulatory luteinizing hormone (LH) surge and arrests again after the first polar body is released before fertilization.

Despite sexual dimorphism in meiosis, many regulators of the process are common to the germ cells of both sexes. In the absence of these proteins, prophase arrest and accompanying germ cell death occur in male and female germ cells. Infertility in both sexes is observed in knockout mice lacking the recombination and DNA damage/mismatch repair proteins, SPO11, DMC1, ataxia telangiectasia (ATM), MSH4, MLH1, and MSH5 (refs 63–74). Mutations in *ATM* and Fanconi anaemia (*FA*) complementation-group-protein genes result in fertility defects in humans and mice of both sexes (Figs 1,2). ATM is involved in DNA metabolism and cell cycle checkpoint control[75], whereas FA is a hereditary chromosomal instability syndrome[76]. FA men are hypogonadal, oligospermic and rarely fertile; FA women can experience premature ovarian failure in their 20s. Several FA mouse models have been created and display reduced fertility[77]. Thus, similar mechanisms for germline monitoring are conserved in mammals and in both sexes.

When the germline 'proofreading' system goes awry in an otherwise 'normal' individual, there are major consequences. Despite a normal somatic karyotype, sperm collected from oligospermic men exhibit an increased frequency of chromosomal abnormalities[78,79]. Aneuploidy is the most common genetic abnormality in humans[80], and the common trisomies (for example, trisomy 21 (Down's syndrome and trisomy 18)) arise primarily in the children of ageing women through non-disjunction defects during the first meiotic division[81]. These findings are exemplified in mice lacking synaptonemal complex protein 3 (SYCP3), which functions in synapsis (pairing) of the homologous chromosomes during meiosis. *Sycp3* knockout males are infertile; females are subfertile, exhibiting loss of aneuploid embryos[82,83]. Interestingly, germline deletions resulting in Duchenne muscular dystrophy (DMD) more often arise during oogenesis, whereas DMD point mutations result more commonly from spermatogenic failure[84]. This suggests that some proofreading mechanisms during male and female gametogenesis may differ (see also ref. 80).

Hormones take control

After sexual maturity, all stages of spermatogenesis (male) and folliculogenesis (female) are observed, the end result in each case being gamete production. Hypothalamic pituitary control of gonadal somatic cells is critical for fertility in all mammals and in both sexes (Fig. 3; reviewed in refs 85–88). Gonadotropin releasing hormone (GnRH) from the hypothalamus regulates the pituitary gonadotrope production of follicle stimulating hormone (FSH) and LH, α–β heterodimers that share a common α subunit with placental human chorionic gonadotropin (hCG) and pituitary thyroid stimulating hormone (TSH). Spontaneous deletion of the hypothalamic GnRH-encoding sequences in mice (that is, *hpg*),

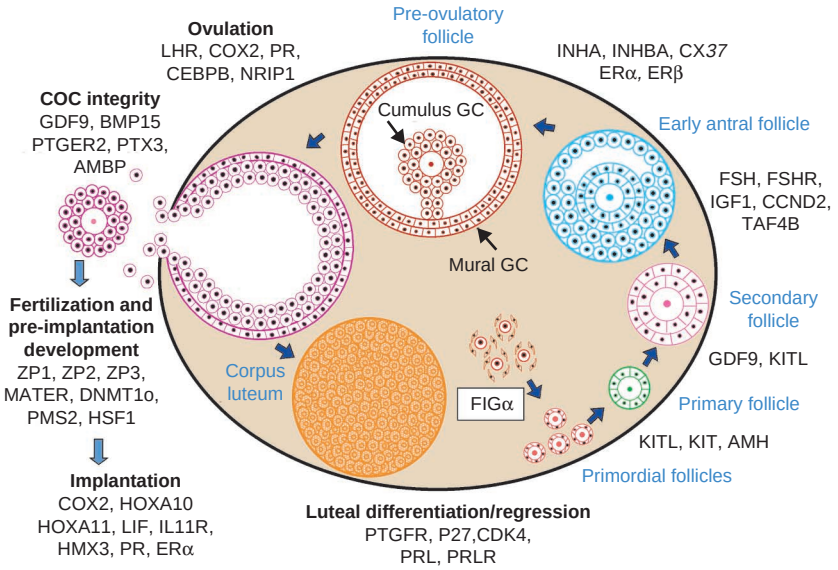

Figure 4 **Female fertility proteins.** Knockout mouse models have defined key proteins that function at various stages of follicle formation, folliculogenesis, ovulation, and post-ovulatory events. FIGα is required for primordial follicle formation, and several proteins are needed for oocyte and granulosa cell (GC) growth and differentiation, ovulation, and the integrity of the cumulus oocyte complex (COC) (reviewed in the Supplementary Information Table).

mutation of the human GnRH processing enzyme gene (*PC1*), disruption of developmental migration of the GnRH neurons in human Kallman Syndrome, or mutation of the GnRH receptor gene (expressed in gonadotropes), results in hypogonadotropic hypogonadism (HHG) and infertility (Fig. 1). Loss-of-function mutations in the pituitary-expressed FSHβ genes and gonadal-expressed FSH receptor genes decrease testis size and spermatozoa counts in men and male mice, and cause a block in folliculogenesis and infertility in women and female mice. This emphasizes the conservation and importance of these signalling pathways. Similarly, pituitary gland development and downstream steroidogenic pathways are conserved in humans and mice and are critical for fertility in both sexes. For example, a homozygous *Prop1* missense mutation causes multiple pituitary defects in the *Ames* dwarf mouse, including defects in gonadotrope differentiation and infertility in all female and most male mice. Similarly, human *PROP1* mutations cause combined pituitary hormone deficiency, including HHG and infertility (Fig. 1).

Members of the steroid receptor superfamily and their transcriptional coactivators (for example, AR, ER, PR, RXRβ, SF1, DAX1, and SRC1) are pivotal in the regulation of reproductive function. Disruption of any of the genes involved in androgen biosynthesis, metabolism and action negatively impact male development, spermatogenesis and function. Spontaneous mutations of the X-linked androgen receptor gene in XY mice (that is, *tfm* (*testicular feminization*)) and humans result in individuals with abnormal testes, no ductal system and external female genitalia[10,89] (http://ww2.mcgill.ca/androgendb). Absence of steroid 5α reductase, which converts testosterone to dihydrotestosterone, results in external female genitalia, prostate absence in XY humans and developmental disruption of the male ductal system (that is, seminal vesicles and prostate)[90,91]. Similarly, mutations in the orphan nuclear receptors, steroidogenic factor-1 (SF1) and DAX1, have been described in mice and humans; mutations in *DAX1* cause almost universal HHG in adult humans (Fig. 1).

Not surprisingly, oestrogen and progesterone are key to early folliculogenesis and corpus luteum maintenance of early pregnancy in the female[92]. Targeted deletion of oestrogen receptor α in mice revealed that it is also required for male fertility and for male and female sexual behaviour[93]. Similarly, the progesterone receptor (also required for female fertility) is important in sexual behaviour in the mouse[94]. In the evaluation of the infertile couple, assessment of circulating hormone levels (FSH, LH, testosterone, prolactin and free testosterone in the male; FSH, LH, oestradiol and progesterone levels in the female) can provide important information concerning the function of the hypothalamic–pituitary–gonadal axis and the presence of endocrinopathies.

Spermatogenesis has many unique players

Spermatogenesis requires not only the appropriate hormonal milieu, but also autocrine, paracrine and juxtacrine signalling between the various testicular compartments. The testis is composed of an interstitial cell compartment with androgen-producing Leydig cells, and the seminiferous tubule containing Sertoli cells, peritubular myoid cells and germ cells. Whereas follicles are recruited each cycle to enter the ovulatory pathway in females, all stages of spermatogenesis are present at any one time in different tubules within the testis. Thus, the wave of spermatogenesis resulting in development of mature sperm is a spatial cycle rather than a temporal one. The importance of growth factors and cytokines, their receptors and signal transduction pathways to gametogenesis cannot be underestimated. For example, deletion of mouse *Desert hedgehog* (*Dhh*) affects testicular development, resulting in anastomatic seminiferous tubules and an absence of adult Leydig cells. Similarly, the insulin-like growth factor (*Igf1*) null male mouse is characterized by vestigal vas deferens, prostate and seminal vesicles, caused by a steroidogenic Leydig cell defect.

Once male germ cells complete meiosis to achieve a haploid chromosomal complement, they are called spermatids. Spermatids undergo a process of cellular differentiation known as spermiogenesis, progressing from round to elongating to

elongated spermatids, culminating in the development of spermatozoa. Many male-specific genes are involved in this extensive cellular remodelling and concomitant condensation of the chromatin (for example, *Tnp1*, *Tnp2*, *Prm1*, *Prm2*, *Theg* and *Hsp60-2*; see Fig. 2). In common with many of the Y chromosome genes that encode RNA-binding proteins and are implicated in human infertility, an absence of similar proteins in the mouse, such as STYX, disrupts spermatid development[95]. After spermiogenesis and release of the spermatozoa from the Sertoli cells into the seminiferous tubule lumen, acquisition of motility occurs during transit through the epididymis and capacitation occurs in the oviduct (fallopian tubes) of the female genital tract. Both of these processes are required for effective penetration of the zona pellucida and egg.

In the evaluation of the infertile male, a semen sample is ordered to determine sperm count, motility and morphology. Some laboratories perform sperm function tests that predict defects in sperm–zona or egg interaction, or in penetration. However, semen analysis is not a definitive test of the fertility potential of an individual unless there are no sperm in the ejaculate. This is also true in mice. For example, FSH-β mutant mice exhibit reduced sperm counts, but fertility is normal[96]. Conversely, many mouse models are infertile and demonstrate abnormal sperm function, sperm motility (for example, *ApoB*, *CatSper*, *Dnahc1*, *Hmgb2* and *Ros* knockout mice), or morphology (for example, *Tnp2*, *Tnp1* and *Sperm1* knockout mice) with no detrimental effect on sperm count. In addition, targeted deletion of the *Ace*, *Adam2*, *Adam3*, *calmegin*, *Pc4*, *Spam1*, *Spnr*, *Trg26*, *Jdf2* or *Mdhc7* genes results in normal sperm count, motility and morphology; however, sperm function is defective (Fig. 2). As a majority of unexplained cases of infertility in human males result from spermatogenic defects (Fig. 3), the homologues of the above-described mouse genes are actively being pursued for their possible roles in human infertility.

Chromosomal abnormalities are observed in 5.8% of infertile males[97] and more commonly involve sex chromosomes (4.2%), as opposed to autosomes (1.5%; Fig. 1). In addition to *SRY*, other Y chromosome genes are required for spermatogenesis. This became obvious in the XX *Sxr* male mouse and the XX *Sry* transgene-positive male mouse, which are sex-reversed, but display spermatogenesis blocks[31]. Similarly, a region at Yq11 that is deleted in several infertile men was termed the azoospermia factor (*AZF*) region[98]. In general, the reported incidence of deletions in this region in severe oligospermic/azoospermic men is 10–18% and varies depending on the stringency of diagnostic classification[99]. This region (now further subdivided into *AZFa*, *AZFb* and *AZFc*), contains several genes involved in spermatogenesis, including deleted in azoospermia (*DAZ*)[100,101]. In the mouse, disruption of the testis-expressed *Dazla* homologue gene on chromosome 17 abrogates gamete production. Other putative evolutionarily conserved spermatogenesis genes have been mapped to Y chromosome regions commonly deleted in infertile men (reviewed in ref. 99). Mutations in the human gene *USP9Y* (ubiquitin-specific protease 9, Y chromosome or DFFRY), a homologue of the *D. melanogaster* development *Fat facets* gene[21], cause infertility[102]. Functional analysis of additional Y chromosome genes in the mouse has been complicated by the presence of multiple copies or X-chromosome homologues, as well as technical difficulties related to the low efficiency of Y chromosome homologous recombination in embryonic stem cells. However, it is expected that the recent and exciting technological breakthroughs achieved by Bishop and colleagues[103], who developed a method for successful gene targeting of the Y chromosome in embryonic stem cells, and the development of an embryonic stem cell line[104] that will facilitate germ line transmission of Y chromosome targeted genes, will rapidly translate into an enhanced understanding of the role of specific Y chromosome genes in male reproductive function.

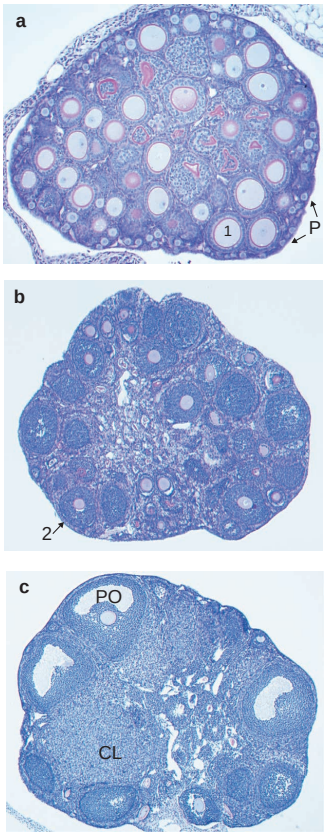

Figure 5 **Mouse knockout models to study folliculogenesis. a**, Targeted mutation of the oocyte-secreted growth factor, *Gdf9*, results in an early folliculogenesis block, resulting in an ovary with only primordial (P) and primary (1) follicles[108]. **b**, Absence of the endocrine hormone, FSH, results in a later block at the secondary (2) to antral follicle transition[96]. **c**, These knockout models contrast with wild-type ovaries that contain pre-ovulatory (PO) follicles and corpora lutea (CL). Primary- to preovulatory-stage oocytes are surrounded by a zona pellucida (magenta).

No crosstalk in females, no folliculogenesis progression

Although several proteins are involved in ovarian folliculogenesis, meiosis, and oocyte survival, oocyte–somatic cell crosstalk is especially critical for release of a fertilizable egg (Fig. 4 and ref. 105). Without the helix-loop-helix protein factor in the germline α (FIGα), pre-granulosa (somatic) cells fail to form a monolayer around individual primordial oocytes, resulting in rapid germ cell depletion from the neonatal mouse ovary and sexual infantilism[106]. Similarly, oocyte growth during folliculogenesis is regulated by signalling of

granulosa KITL to the oocyte-expressed KITR[46]. KITL expression is controlled by both hormonal (FSH) and oocyte (growth differentiation factor-9 (GDF-9)) factors[107]. In the absence of the TGF-β superfamily ligand GDF-9 in mice[108] or its close oocyte-specific homologue, BMP-15, in sheep[48], an arrest in folliculogenesis at the primary follicle stage is observed (Fig. 5). FSH has no effect on the 'arrested' primary follicles of *Gdf9* knockout ovaries[108], suggesting that GDF-9 allows the granulosa cells to grow and acquire the competence to respond to FSH. Absence of GDF-9 results in elevated levels of KITL[109], which signals back to markedly increase oocyte size[108]. These findings were confirmed by studies showing that recombinant GDF-9 downregulates levels of *Kitl* mRNA[107]. In addition to oocyte factors, FSH[96] functions with IGF-1 (by stimulating cyclin D2 and oestrogen synthesis)[110,111] to regulate the growth of the follicle through the pre-ovulatory stage (Fig. 5). In pre-ovulatory follicles, LH, in conjunction with the oocyte-secreted proteins GDF-9 and BMP-15, signals to somatic cells to initiate ovulation of a healthy cumulus-oocyte complex (COC). Thus, important crosstalk between somatic cells and oocytes, as well as endocrine signalling, is necessary for normal folliculogenesis and ovulation.

Post-fertilization, *Mater* (maternal antigen that embryos require) and several other genes (including *Dnmt1o*, *Pms2* and *Hsf1*) (Fig. 4), have been identified by knockout mouse studies as maternal effect (oocyte-synthesized) genes that are essential for development[112]. The human homologue of *Mater* has been identified and may be a candidate gene for premature ovarian failure[113]. Similarly, several uterine proteins are required for implantation (Fig. 4 and ref. 114). Thus, these studies have pinpointed multiple putative diagnostic targets in women who present with infertility.

In women, several syndromes — including ovarian failure and infertility — are attributed to autosomal recessive mutations[88,115]. Blepharophimosis/ptosis/epicanthus inversus syndrome, the only autosomal dominant disorder associated with premature ovarian failure (POF), is caused by mutations in the forkhead transcription factor gene (*FOXL2*)[116]. Expansion of a CGG trinucleotide repeat of the Xq27.3 fragile X mental retardation gene (*FMR1*) to over 200 repeats is the most common heritable cause of mental retardation. The unstable premutation *FMR1* allele (60–199 CGG repeats) causes POF in 21% of heterozygote carriers and increased twin pregnancies[117]. Furthermore, 2% of sporadic cases and 14% of familial cases of POF are associated with the premutation allele. The pathophysiology of the premutation allele in POF is unknown, but this finding clearly represents a step forward in identifying a genetic locus for POF. To date, all other identified single gene autosomal dominant or recessive mutations with isolated infertility in humans affect steroidogenic or gonadotropin pathways, often in both sexes. However, many candidate genes await analysis in human idiopathic infertility cases.

Descent of the testis and problems with sperm transit

Testis determination and gametogenesis are necessary, but not sufficient, for male fertility, as testicular descent down the inguinal canal into the scrotum, in addition to the development of the genital tract and penis, are also critical. Mutations of the mouse genes *Insl3, Great* (G-protein coupled receptor that affects testicular descent; a possible relaxin receptor) and *Hoxa10* (refs 118–122) result in male infertility secondary to cryptorchidism. The second phase of testicular descent requires androgens and a functional androgen receptor. In humans, cryptorchidism results from anti-Mullerian hormone (AMH) deficiency caused by obstruction of the genital tract.

Gonadal sex determines secondary duct differentiation. In females, the Müllerian duct differentiates into the oviducts, uterus and upper portion of the vagina; in males, the Wolffian duct differentiates into epididymis, vas deferens and seminal vesicles[19,23,123]. The Müllerian duct regresses in response to prenatal production of testicular AMH, and Wolffian duct development requires testosterone. Differentiation of the prostate and male external genitalia is driven by dihydrotestosterone, a product of the conversion of testosterone by 5-α reductase. Mutations in genes that affect steroidogenesis (for example, P450 aromatase (*CYP19*) and 5-α reductase) and steroid signalling pathways (for example, oestrogen receptor α (*ERα*) and androgen receptor) have deleterious effects on genital tract development and function in the male. Thus, it follows that pseudohermaphroditism occurs as a result of defects in genes involved in gonad formation (for example, *SF1* and *WT1*). Mutations of the AMH or AMH receptor genes result in persistence of Müllerian duct syndrome (PMDS), resulting in obstructive azoospermia and fertility defects in men, male dogs and mice (Fig. 1 and ref. 123).

One to two per cent of infertile men present with obstructive azoospermia caused by congenital bilateral absence of the vas deferens (CBAVD), as a result of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene[1,124]. These CBAVD patients successfully father offspring because microsurgical epididymal sperm aspiration yields 'normal' sperm for *in vitro* fertilization (IVF). Male fertility also may be compromised by epididymal, ejaculatory or erectile dysfunction, as well as by other congenital anomalies.

New technologies and perspectives

Genome, gene and cDNA sequences are being deposited into public databases (for example, the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) or the Wellcome Trust Sanger Institute (http://www.sanger.ac.uk)) with amazing speed. Furthermore, programs to search these databases, such as BLAST (http://www.ncbi.nlm.nih.gov/blast) and the Unigene database at NCBI, are helping scientists to use this wealth of information. In particular, sequences unique to mammalian germ cells have been identified using an *in silico* subtraction (electronic database) approach[125]. For example, *GASZ* (germ-cell-specific and ankyrin repeat, sterile α-motif and basic leucine-zipper-containing protein) was identified as a

novel evolutionarily-conserved germ cell-expressed gene lying adjacent to the *CFTR* gene in human, chimpanzee, baboon, cow, rat and mouse[126]. Functional expression and sequence data is also being collated into collections, such as the Ovarian Kaleidoscope database (http://ovary.stanford.edu), the Male Reproductive Genetics database (http://mrg.genetics.washington.edu/home.html) and the GermOnline database (http://germonline.igh.cnrs.fr). With the use of microarrays for expression analysis of reproductive tissues[127], these 'bits' of data will increase exponentially. Therefore, there is an urgent need for bioinformatics advances to facilitate compiling and sorting through this wealth of *in silico* information for future applications in the clinic.

Technological procedures and advances in the clinic are also wrought with some controversies and dilemmas. Of particular importance, the application of assisted reproductive technologies (ART) for severe male and female factor infertility serves to not only overcome sterility, but bypasses natural barriers to the inheritance of defective genes. This results in considerable concern that genetic defects will be transmitted to the next generation. Among these controversial treatments are ICSI (intracytoplasmic sperm injection), cytoplasmic (ooplasmic) transfer, round spermatid nuclear injection (ROSNI or ROSI) and reproductive cloning (described in more detail by Schatten in this issue[128]).

These technologies have prompted significant debates concerning their morality and safety. However, even a United States government moratorium on human cloning has not deterred renegade scientists overseas from actively engaging in this research, which could result in a potentially disastrous outcome.

Conclusions

To date, diagnosis of infertility in the clinic has been hindered by our relatively poor understanding of the underlying molecular mechanisms. Although investigators have attempted to translate findings in animal models to humans by searching for gene mutations/deletions in idiopathic infertility patients, in general, these investigations have not been fruitful. Given the large number of candidate evolutionarily conserved 'fertility' genes yet to be discovered or identified from mutant mouse studies, and the overall complexity of the reproductive system in general, proper diagnosis and treatment of these patients will await the development of more sophisticated and rapid technologies. Finally, if mutation of a gene in mice or humans results in infertility, the protein product of that gene may be a future target for novel contraceptives that are designed to transiently or permanently cause infertility. ❑

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Online Table: Mouse mutations causing reproductive defects. Only single mutant defects are described. Fertility defects of unknown gene origin are not described. M, male; F, female; Hetero, heterozygote phenotype

Mutant gene	Sex Affected	Reproductive Phenotype	Fertility Status	References
Acrosin (*Acr*)	Males	Sperm are capable of binding and penetrating the zona pellucida	Delayed fertility	1
Activin receptor-type IIA (*Acvr2*)	Both	Antral follicle block in females; small testes, delayed fertility in males	Infertility (F) Subfertility (M)	2
Activin/inhibin βB subunit (*Inhbb*)	Female	Delivery and nursing defects	Subfertility	3
Acyl-CoA synthetase 4 (ACS4; *Facl4*)	Female (Hetero)	Enlarged uteri with prostaglandin accumulation	Subfertility	4
Adamts1 (a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 1)	Female	Cystic formations in uteri; defects in preovulatory follicle development	Subfertility	5
Adamts2 (procollagen N-proteinase)	Male	Defects in spermatogenesis; marked decrease in sperm within testes tubules	Infertility	6
ADP-ribosylation factor-like 4 (*Arl4*)	Male	Significantly reduced testicular weights and sperm counts	Normal fertility	7
Alpha 1 microglobulin/bikunin (*Ambp*; Urinary trypsin inhibitor)	Female	Defects in ovulation and cumulus-oocytes complex (COC) integrity	Subfertility	8
Angiotensin-converting enzyme (*Ace*)	Male	Compromised ability of sperm to fertilize ova	Subfertility	9
Androgen receptor (*Ar*; *tfm* or Testicular feminization)	Male	Feminized external genitalia; hypogonadal; cryptorchidism with a block in spermatogenesis	Infertility	10
Anti-Müllerian hormone (*Amh*)	Both	Uteri development in males causes obstruction and secondary infertility; females exhibit early delpetion of primordial follicles	Secondary Infertility	11,12
AMH receptor (*Amhr2*)	Male	Uteri development in males causes obstruction and secondary infertility	Secondary Infertility	13
Apaf1 (Apoptotic protease activating factor 1)	Male	Spermatogonial degeneration	Variable lethality; Infertility	14
Apolipoprotein B (*Apob*)	Male (Hetero)	Decreased sperm count, motility, survival time, and ability to fertilize ova	Infertility	15
Aryl-hydrocarbon receptor (*Ahr*)	Female	Early development of primordial follicles; decreased numbers of antral follicles	Subfertility	16,17
Ataxia telangiectasia (*Atm*)	Both	Germ cells degenerate; disruptions evident in meiosis I	Infertility	18,19
ATP-binding cassette transporter 1 (*Abca1*)	Female	Placental malformations leading to impaired embryo growth, embryo loss and neonatal death	Subfertility	20

Basigin (*Bsg*)	Both	Defects in fertilization and implantation (F); block in spermatogenesis at metaphase I (M)	Partial lethality; Infertility	21,22
Bax (Bcl2-associated X protein)	Both	Premeiotic arrest of spermatogenesis; increased oocytes and primordial follicles postnatally	Infertility (M)	23,24
Bcl2 (B-cell leukemia/ lymphoma 2)	Female	Fewer oocytes/primordial follicles in the post-natal ovary	Subfertility	25
Bcl6	Male	Apoptosis in metaphase I spermatocytes	Subfertility	26
BclX (*Bcl2l*) hypomorph	Both	PGCs are lost by E15.5	Infertility	27
*Bclw (*Bcl2l2, *Bcl2-like 2)*	Both	Late meiotic arrest with loss of germ cells (M) and reduced PGC survival (F)	Infertility (M) Subfertility (F)	28
Bone morphogenetic protein 4 (*Bmp4*)	Both	Absent primordial germ cell (PGC) population; defect in PGC development	Lethal	29
Bmp8a	Male	Degeneration of germ cells and epididymis	Progressive Infertility	30
Bmp8b	Both	Reduced or absent PGCs (developmental defect); Postnatal male germ cell proliferation/differentiation defect and spermatocyte apoptosis	Subfertility/ Infertility	31
Bmp15	Female	Defects in cumulus-oocyte complex (COC) formation and ovulation	Subfertility	32
BMP receptor, type IB (*Bmpr1b*)	Female	Defects in estrous cyclicity, cumulus expansion, and endometrial gland development	Subfertility	33
Calmegin (*Clgn*)	Male	Defect in sperm-zona pellucida binding	Infertility	34
Camk4 (calcium/calmodulin-dependent protein kinase IV)	Male	Impaired chromatin packaging during spermiogensis	Infertility	35
cAMP-responsive element modulator (*Crem*)	Male	Defective spermiogenesis with aberrant post-meiotic gene expression	Infertility	36,37
cAMP-specific phosphodiesterase type 4 (*Pde4d*)	Female	Diminished sensitivity of the granulosa cells to gonadotropins	Subfertility	38
Casein kinase IIα 1 (*Csnk2a2*)	Male	Globozoospermia (no acrosomal cap)	Infertility	39
Caspase-2 (*Casp2*)	Female	Decreased apoptosis of female germ cells	Increased Fertility	40
CatSper (putative sperm cation channel)	Male	Defects in motility and fertilization	Infertility	41
CD9 antigen (*Cd9*)	Female	Sperm-egg binding defect	Subfertility	42
Cell division cycle 25 homolog B (*Cdc25b*) (Cdc25b phosphatase)	Female	Oocytes are arrested in meiotic prophase, with defects in maturation promoting factor activity	Infertility	43
Centromere protein B (*Cenpb*)	Both	Males are hypogonadal and have low sperm counts; females have strain-dependent uterine epithelium defects	Subfertility (F)	44,45

C/EPBβ (CCAAT/enhancer-binding protein β)	Female	Reduced ovulation and block in CL differentiation	Infertility	46
Claudin 11 (*Cldn11*; Osp-11)	Male	No tight junctions between Sertoli cells	Infertility	47
Colony stimulating factor (macrophage) (*Csf1*)	Both	Males have reduced testosterone; females have implantation and lactation defects	Subfertility	48
Colony stimulating factor (granulocyte-macrophage) (*Csf2*)	Both	Mean litter size decrease with disproportionate loss of males pups (F); maternal effects most pronounced in intercrosses with knockout males	Intercrossing Subfertility	49
Connexin 37 *(Gja4*; Cx37*)*	Female	Defects in late folliculogenesis and oocyte meiosis	Infertility	50
Connexin 43 *(Gja1*; Cx43*)*	Both	Small ovaries and testes; decreased numbers of germ cells from E11.5	Neonatal lethality	51
Cpeb (cytoplasmic polyadenylation element binding protein)	Both	Disrupted germ cell differentiation and meiosis I synaptonemal complex formation	Infertility	52
Cut-like 1(*Cutl1*; CDP/Cux) truncation mutant	Male	Severely reduced male fertility	Subfertility	53
Cyclin A1 (*Ccna1*)	Male	Block in spermatogenesis before the first meiotic division	Infertility	54
Cyclin D2 (*Ccnd2*)	Both	Failure of granulosa cell proliferation (F); males fertile with decreased testis size	Infertility (F)	55
Cyclin dependent kinase 4 (*Cdk4*)	Female	Defects in the hypothalamic-pituitary-gonadal axis	Infertility	56
Cyclooxygenase 2 (*Ptgs2)*	Female	Defects in ovulation and implantation	Most Infertile	57,58
Cyp11a (Cytochrome P450, 11a, cholesterol side chain cleavage)	Both	Males feminized with female external genitalia, underdeveloped sex organs; gonads degenerate	Lethal	59
Cyp19 (Cytochrome P450, 19, aromatase)	Both	Early spermatogonial arrest, Leydig cell hyperplasia, and defects in sexual behavior (M); folliculogenesis block and ovulation defects (F)	Progressive Infertility (M); Infertility (F)	60-62
Cyp40 (P450 25-hydroxyvitamin D-1α–hydroxylase)	Female	Uterine hypoplasia and absence of CL	Infertility	63
Cyritestin (*Adam3*)	Male	Altered sperm protein expression and adhesion defects during fertilization	Infertility	64,65
Dax1 (*Nr0b1*)	Male	Progressive degeneration of the germinal epithelium	Infertility	66
Dazl (Deleted in azoospermia-like)	Both	Reduced germ cells; differentiation failure and degeneration of germ cells	Infertility	67
Desert hedgehog (*Dhh*)	Male	Complete absence of mature sperm; defects in Sertoli-to-Leydig cell	Infertility	68,69

		signaling		
Dmc1h (Disrupted meiotic cDNA 1 homolog)	Both	Defects in chromosome synapsis in meiosis; female germ cells degenerate during embryogenesis	Infertility	70,71
Dnmt1o (DNA methyltransferase)	Female	Embryos of knockout females die during gestation due to imprinting defects; maternal effect gene	Subfertility	72
DNA polymerase λ	Male	Immotile spermatozoa	Lethality; Infertility	73
Doublesex and mab-3 related transcription factor 1 (*Dmrt1*)	Male	Defects in post-natal testes differentiation; disorganized seminiferous tubules and absence of germ cells	Infertility	74
Dynein heavy chain 7 (*Dnahc1*)	Male	Defects in sperm flagellar motility	Infertility	75
Early growth response 1 (*Egr1*; NGFI-A) targeted *lacZ* insertion	Both	Lack of LH (M); downregulation of LHR, not remedied with gonadotropin treatment (F)	Infertility	76
Early growth response 1 (*Egr1*) targeted *neo* insertion	Female	LH insufficiency; loss of estrous cyclicity, no CL; rescued by treatment with gonadotropins	Infertility	77
Early growth response 4 (*Egr4*)	Male	Germ cells undergo apoptosis during pachytene stage	Infertility	78
ELKL motif kinase (*Emk*)	Both	β-gal gene trap insertion creates a null allele; homozygotes intercrossed are not fertile	Intercrossing Infertility	79
Empty spiracles homolog 2 (*Emx2*)	Both	Defective development of gonads and urogenital tracts	Lethal	80
Estrogen receptor α (ERα)(*Esr1*)	Both	Females have hemorrhagic ovarian cysts and uterine defects, decreased lordosis response; males develop disruptions of the seminiferous epithelium due to abnormal epididymal function, no ejaculations	Infertility	81-84
Estrogen receptor β (ERβ)(*Esr2*)	Both	Females are subfertile; males are fertile, but develop prostate hyperplasia	Subfertility	85
Fanconi anemia complementation group A (*Fanca*)	Both	Hypogonadism, reduced fertility, more dramatic and progressive in females	Subfertility	86
Fanconi anemia complementation group C (*Fancc*)	Both	Hypogonadism, compromised gametogenesis	Subfertility	87,88
Fanconi anemia complementation group G (*Fancg*)	Both	Hypogonadism, compromised gametogenesis	Subfertility	89
Fertilin β *(Adam2)*	Male	Altered sperm protein expression and adhesion defects during fertilization	Infertility	65,90
Fibroblast growth factor 9 (*Fgf9*)	Male	XY male-to-female sex reversal; phenotype ranges from testicular hypoplasia to complete sex reversal	Lethal	91
Figla or FIGα (Factor	Female	No primordial follicles develop at birth	Infertility	92

in the germline α)		and oocytes die		
Fragile-X mental retardation syndrome 1 homolog (*Fmr1*)	Male	Macroorchidism		93
FSH hormone β-subunit (*Fshb*)	Both	Female pre-antral block in folliculogenesis; males decreased testis size	Infertility (F)	94
FSH receptor (*Fshr*)	Both	Female pre-antral block in folliculogenesis; males decreased testis size	Infertility (F)	95
Fus1 (translocated in liposarcoma; TLS)	Male	Defects in spermatocyte chromosome pairing	Infertility	96
β1,4-Galactosyltransferase	Both	Male infertility; defects in sperm-egg interaction; females exhibit defects in delivery and lactation	Variable lethality	97,98
γ-Glutamyl transpeptidase (*Ggtp*)	Both	Both males and females are hypogonadal and infertile; phenotype corrected by feeding mice N-acetylcysteine	Infertility	99,100
Gdi1 (guanosine diphosphate dissociation ihibitor 1; Rho GDIα)	Both	Impaired spermatogenesis, vaculolar degeneration in males; post-implantation pregnancy defects in females	Infertility	101
Glial cell line-derived neurotrophic factor (*Gdnf*)	Male (Hetero)	Depletion of stem cell reserves; spermatogonia differentiate	Fertile	102
Glycoprotein hormone α-subunit (*Cga*)	Both	Hypogonadal due to FSH and LH deficiency	Infertility	103
Gpr106 (G protein-coupled receptor 106)	Male	*Crsp* males homozygous for transgene integration exhibit a high intraabdominal position of the testes, complete sterility	Infertility	104
Growth differentiation factor-7 (*Gdf7*)	Male	Defects in seminal vesicle development	Infertility	105
Growth differentiation factor-9 (*Gdf9*)	Female	Folliculogenesis arrest at the one-layer follicle stage	Infertility	106,107
Growth hormone receptor (*Ghr*)	Female	Delayed puberty and prolonged pregnancy		108
H19	Female (Hetero)	Loss of maternal allele in developing embryos causes somatic overgrowth due to loss of IGF2 imprinting		109
Heat shock protein 70-2 (*Hsp70-2*)	Male	Meiosis defects and germ cell apoptosis	Infertility	110
Heatshock transcription factor 1 (*Hsf1*)	Female	Maternal effect gene; pre- and post-implantation defects	Infertility	111,112
Hepatocyte nuclear factor (HNF-1α)(transcription factor 1; *Tcf1*)	Both	Infantile uterus; normal ovarian histology (F); vestigial vas deferens, seminal vesicles and prostate, impaired spermatogenesis, no mating behavior	Infertility	113

		(M)		
High mobility group box 2 (*Hmgb2*)	Male	Sertoli and germ cell degeneration and immotile spermatozoa	Subfertility	114
Histone H2A family, member X (*H2afx*)	Male	Pachytene stage arrest in spermatogenesis; defects in chromosome segregation and MLH1 foci formation	Infertile	115
Histone 3.3A gene (*H3f3a*) insertional mutation	Male	β-gal gene trap insertion creates a hypomorphic allele; homozygous males have reduced copulatory activity and fewer matings result in pregnancy	Subfertility	116
Homeobox A10 (*Hoxa10*)	Both	Variable infertility; males have cryptorchidism and females have frequent embryo loss prior to implantation	Progressive infertility (M); subfertility (F)	117
Homeobox A11 (*Hoxa11*)	Both	Females have uterine defects; males have malformed vas deferens and undescended testes	Infertility	118
Hrb (HIV-1 Rev binding protein) (RAB/Rip)	Male	Round-headed spermatozoa lack an acrosome (globozoospermia)	Infertility	119
Inhibin α (*Inha*)	Both	Granulosa/Sertoli tumors, gonadotropin hormone-dependent	Infertility (F) Secondary infertility (M)	120,121
Inositol polyphosphate-5-phosphatase (*Inpp5b*)	Male	Sperm have reduced motility and reduced ability to fertilize eggs; defects in fertilin β processing	Infertility	122
Insulin-like growth factor 1 (*Igf1*)	Both	Hypogonadal and infertile; disrupted spermatogenesis and vestigial ductal system, defects in mating behavior (M); impaired antral follicle formation (F)	Infertility	123
Insulin-like growth factor 2 receptor (*Igf2r*); T-associated maternal effect (*Tme*) mutation	Female (Hetero)	Mutation of maternal allele in pups causes developmental defects and embryonic/perinatal death	Lethality; maternal effect	124
Insulin-like hormone 3 (*Insl3*)	Both	Bilateral cryptorchidism results in abnormal spermatogenesis in males; female subfertility associated with irregular estrous cycles	Subfertility	125
Insulin receptor substrate 2 (*Irs2*)	Female	Small, anovulatory ovaries with reduced numbers of follicles	Infertility	126
Interleukin 11 (*Il11*)	Female	Compromised implantation and decidualization	Infertility	127
JunD (*Jund1*)	Male	Anomalous hormone levels and sperm structural defects	Infertility	128
Kit ligand (*Kitl*)	Both	*steel* defect mutation causes defect in PGC migration/survival; *panda*	Infertility	129,130

		mutation causes blocks in folliculogenesis in females		
Kit receptor (*Kit*)	Both	*White spotting* null mutation causes PGC defects	Infertility	131
Leptin (*Lep*; *ob/ob*) mutant	Both	Obese and infertile with hypogonadotropic hypogonadism	Infertility	132,133
Leptin receptor (*Lepr*; *db/db*) mutant	Both	Obese and infertile with hypogonadotropic hypogonadism	Infertility	134
Leukemia inhibitory factor (*Lif*)	Female	Failed implantation	Infertility	135
Limk2 (LIM motif-containing protein kinase 2)	Male	Degeneration of spermatogenic cells in the seminiferous tubules; increased apoptosis		136
Lipase, hormone sensitive (HSL) (*Lipe*)	Male	Multiple abnormalities in spermatogenesis	Infertility	137,138
Luteinizing Hormone Receptor (*Lhcgr*)	Both	Underdeveloped sex organs and infertility in both males and females; spermatogenesis arrested at round spermatid stage; preantral folliculogenesis block	Infertility	139,140
Man2a2 (α-mannosidase IIx)	Male	Defect in adherence of spermatogenic cells to Sertoli cells; germ cells prematurely released from the testis	Mostly infertile	141
Mater (maternal antigen that embryos require)	Female	Development beyond the two-cell stage is blocked; Maternal effect gene	Infertility	142
Mlh1 (MutL homologue 1)	Both	Meiotoc arrest and genomic instability	Infertility	143,144
Mos (Moloney sarcoma oncogene)	Female	Parthenogenetic activation, cysts and teratomas	Subfertility	145,146
Msh4 (MutS homologue 4)	Both	Prophase I meiotic defects apparent at the zygotene/pachytene stage; germ cells lost within a few days post-partum	Infertility	147
Msh5 (MutS homologue 5)	Both	Zygotene/pachytene meiotic defects with aberrant chromosome synapsis and apoptosis	Infertility	148,149
Microtubule-associated protein (*Mtap7*)(E-MAP-115) insertional mutation	Male	Abnormal microtubules in germ cells and Sertoli cells	Infertility	150
Morc (microrchidia) insertional mutation	Male	Early arrest in meiosis and germ cell apoptosis	Infertility	151
Mybl1 (A-myb) myeloblastosis oncogene-like 1	Male	Germ cell meiotic arrest at the pachytene stage	Infertility	152
$Na^{(+)}$-$K^{(+)}$-$2Cl^{(-)}$ cotransporter (NKCC1) solute carrier family 12, member 2 (*Slc12a2*)	Male	Low spermatid counts and compromised sperm transport	Infertility	153
Neuronal Helix-Loop-Helix 2 (*Nhlh2*)	Both	Males are infertile and hypogonadal; females are fertile when reared with	Infertility	154

		males		
Neuronal insulin receptor (NIR)	Both	Hypothalamic hypogonadism; impaired spermatogenesis and follicle maturation	Infertility	155
Nitric oxide synthase 3, endothelial cell (*Nos3*; eNos)	Female	Compromised ovulation, delayed meiotic progression from metaphase I	Subfertility	156
Nuclear receptor co-activator (*Ncoa1*); steroid receptor coactivator-1 (SRC1)	Both	Decreased responsiveness to steroid hormones in uterus, mammary glands (F), testes and prostate (M)	Fertile	157
Nuclear receptor co-repressor RIP40 (*Nrip1*)	Female	Ovulation defect; ovaries accumulate luteinized, unruptured follicles	Infertility	158
Nuclear receptor subfamily 5, group A, member 1 (*Nr5a1*); Steroidogenic factor-1 (SF-1)	Both	Gonadal agenesis in both sexes	Lethal	159
Otx1(orthodenticle homolog 1)	Both	Prepubescent dwarfism and hypogonadism; progressive recovery of follicular development and sperm development and fertility	Delayed fertility	160
Ovo	Male	Reduced fertility and underdeveloped genitalia	Subfertility	161
P2X1 receptor (*P2rx1*)	Male	Oligospermia and defective vas deferens contraction	Infertility	162
$p18^{Ink4c}$ (*Cdkn2c*)	Male	Leydig cell hyperplasia and reduced testosterone production	Fertile	163
$p19^{Ink4d}$ (*Cdkn2d*)	Male	Testicular atrophy and germ cell apoptosis	Fertile	164
$p27^{Kip1}$ (*Cdkn1b*)	Both	CL differentiation failure and granulosa cell hyperplasia (F); males fertile with testicular hyperplasia	Infertility (F)	165,166
$p57^{Kip2}$ (*Cdkn1c*)	Both	Surviving mice show sexual immaturity	Mostly lethal	167
PAC_1; adenylate cyclase activating polypeptide 1 receptor 1 (*Adcyap1r1*)	Female	Prolonged and irregular diestrous phase	Subfertility	168
PC4 (testicular germ cell protease) (*Pcsk4*)	Male	Sperm have impaired fertilization ability	Infertility	169
Pentraxin 3 (*Ptx3*)	Female	Defects in cumulus-oocyte complex (COC) integrity and ovulation	Subfertility	170
Phosphatidylinositol 3'-kinase (*Pi3k*)	Male	Defects in proliferation and increased apoptosis of spermatogonia	Infertility	171
Phosphatidylinositol glycan, class A (*Piga*)	Chimeric Male	Abnormal testes, epididymis and seminal vesicles	Variable Infertility; no allele transmission	172
Pit1 (pituitary specific transcription factor 1)	Both	*Snell* dwarf mice have multiple anterior pituitary hormone deficiencies and	Infertility	173

		hypogonadism		
Polyomavirus enhancer activator 3 (*Pea3*)	Male	Normal mating behavior, but males do not set plugs or release sperm	Infertility	174
Postmeiotic segregation increased 2 (*Pms2*)	Both	Abnormal chromosome synapsis in meiosis (M); female knockout zygotes have microsatellite instability in both maternal and paternal genomes; Maternal effect gene	Infertility (M)	175,176
Progesterone receptor (*Pgr*)	Female	Defects in ovulation, implantation, sexual behavior, and mammary gland development	Infertility	177
Prolactin (*Prl*)	Female	Females are infertile with irregular estrus cycles	Infertility	178
Prolactin receptor (*Prlr*)	Both	Compromised ovulation, fertilization and preimplantation development in knockouts (F); defects in maternal behavior in knockouts and heterozygotes (F); variable infertility and subfertility in males	Infertility (F); Subfertility (M)	179,180
Prop1 (paired like homeodomain factor 1; prophet of *Pit1*)	Both	*Ames* dwarf mice have multiple anterior pituitary hormone deficiencies and hypogonadism	Infertility	181
Prostaglandin E2 EP2 receptor (*Ptger2*)	Female	Decreased fertilization and defects in cumulus expansion	Subfertility	182-184
Prostaglandin F receptor (*Ptgfr*)	Female	Females do not undergo parturition; failed luteolysis	Infertility	185
Protamine 1 (*Prm1*)	Chimeric Male	Protamine haploinsufficiency; abnormal spermatogenesis	Infertility	186
Protamine 2 (*Prm2*)	Chimeric Male	Protamine haploinsufficiency; abnormal spermatogenesis	Infertility	186
Protease inhibitor protease nexin-1 (PN-1) knockout (*Serpine2*)	Male	Abnormal seminal vesicle morphology and altered semen protein composition	Subfertility	187
Protein kinase A, catalytic subunit α (*Prkaca*)	Male	Most mice die; few viable mice have sperm motility defects	Mostly lethal	188
Protein phosphatase 1 catalytic subunit γ (*Ppp1cc*)	Male	Defects in spermiogenesis	Infertility	189
Protein phosphatase 1 regulatory subunit 1B (*Ppp1r1b*)(DARPP-32)	Female	Knockouts exhibited defects in progesterone facilitated sexual receptivity	Not reported	190
Puromycin-sensitive aminopeptidase (*Psa*)	Female	Lack of CL formation and prolactin production cause early pregnancy loss	Infertility	191
Retinoic Acid Receptor alpha (*Rara*)	Male	Complete arrest and degeneration or germ cell depletion	Infertility	192
Retinoic acid receptor γ (*Rarg*)	Male	Squamous metaplasia of the seminal vesicles and prostate	Infertility	193
Retinoid X receptors (*Rxrb*)	Male	Germ cell maturation defects and tubular degeneration	Infertility	194
Ros1 (c-ros	Male	Sperm motility defects	Infertility	195,196

protoncogene)				
Scavenger receptor, class B1 (*Srb1*)	Female	Defects in oocyte maturation and early embryo development due to abnormal lipoprotein metabolism	Infertility	197-199
Serpina5 (Serine proteinase inhibitor A 5; Protein C inhibitor)	Male	Sertoli cell destruction	Infertility	200
SH2-B	Both	Males have small testes and reduced sperm count; females have small, anovulatory ovaries with reduced numbers of developing follicles	Subfertility (M) Infertility (F)	201
Smad1 (MAD homolog 1; *Madh1*)	Both	Developing embryos lose PGCs	Lethal	202
Smad5 (MAD homolog 5; *Madh5*)	Both	Developing embryos lose PGCs	Lethal	203
Sp4 trans-acting transcription factor (*Sp4*)	Male	Defects in reproductive behavior	Infertility	204
Spam1 (sperm adhesion molecule) mutations	Female	Sperm defects in hyaluronic-acid binding	Subfertility	205
Sperm-1	Male	Defect in haploid sperm function	Subfertility	206
Sperm mitochondrion-associated cysteine-rich protein (SMCP)	Male	Defects in sperm motility and migration into the oviduct; defects in fertilization	Subfertility and Infertility	207
Spermatid perinuclear RNA-binding protein (*Spnr*) insertional mutation	Male	Defects in seminiferous epithelium and spermatogenesis	Subfertility	208
SPO11 homolog (*Spo11*)	Both	Defects in meiosis; oocytes lost soon after birth	Infertility	209,210
Steroid 5α-reductase type 1 (*Srd5a1*)	Female	Defects in parturition	Infertility	211,212
Steroidogenic acute regulatory protein (*Star*)	Both	Males have female external genitalia; both sexes die of adrenocortical insufficiency	Lethal	213
Styx (phosphoserine/ threonine/tyrosine interaction protein)	Male	Defects in round and elongating spermatid development	Infertility	214
Superoxide dismutase 1 (*Sod1*)	Female	Folliculogenesis defect; failure to maintain pregnancy	Subfertility	215,216
Sycp3 (synaptonemal complex protein 3)	Both	Defects in chromosome synapsis during meiosis; germ cell apoptosis in males; embryonic loss in females due to aneuploidy	Infertility (M) Subfertility (F)	217,218
Taf4b (TAF4B RNA polymerase II, TATA box binding protein-associated factor; TAFII105)	Female	Defects in follicular development, oocyte maturation/fertilization	Infertility	219
TATA-binding protein-like protein (*Tlp*;	Male	Post-meiotic spermiogenesis block (defective acrosome formation in early	Infertility	220,221

TRF2)		stage spermatids)		
Telomerase reverse transcriptase (*Tert*)	Both	Progressive infertility in both sexes; females have few oocytes and uterine abnormalities	Progressive Infertility	222
Theg (*kisimo*) (Transgene integration)	Male	Abnormal elongated spermatids; asthenospermia	Infertility	223
Thyroid stimulating hormone β (*Tshb*; *hyt/hyt*) mutant	Female	Hypothyroid; females show continuous dioestrus, and poor response to gonadotropin-induced superovulation	Infertility	224
Tial1 (cytotoxic granule-associated RNA binding protein-like 1)	Both	PGCs lost by E13.5	Infertility	225
Tnp1 (transition protein 1)	Male	Abnormal chromosome condensation, sperm motility	Subfertility	226
Tnp2 (transition protein 2)	Male	Abnormal chromosome condensation	Subfertility	227
Tumor necrosis factor type I receptor (*Tnfrsf1a*)	Female	Enhanced prepubertal response to gonadotropins; early ovarian senescence	Subfertility	228
Ube2b (E2B ubiquitin-conjugating enzyme; HR6B)	Male	Alterations in sperm chromatin structure, an incomplete meiotic arrest, abnormal sperm morphology	Infertility	229
Ubiquitin-like DNA repair gene HR23B (*Rad23b*)	Male	Most knockouts die during development or shortly after birth; surviving mice have multiple abnormalities and male sterility	Variable lethality; Infertility	230
Ubiquitin protein ligase E3A (*Ube3a*; E6-AP ubiquitin protein ligase)	Both	Testicular hypoplasia, defects in spermatogenesis and prostate gland development (M); ovarian hypoplasia, defects in ovulation and uterine development (F)	Subfertility	231
Ubiquitin protein ligase seven in absentia 1A (*Siah1a*)	Male	Block in spermatogenesis and germ cell apoptosis; failure to complete transition to telophase of meiosis I	Partially lethal; Infertility	232
VASA homolog (*Ddx4*; DEAD box polypeptide 4)	Male	Defective proliferation/differentiation of PGCs	Infertility	233
Vitamin D receptor (*Vdr*) knockout	Both	Defects in estrogen biosynthesis in males and females; elevated serum gonadotropins	Infertility	234,235
Voltage-dependent Anion Channel Type 3 (*Vdac3*)	Male	Immotile sperm; axonemal defects with sperm maturation	Infertility	236
Wilms tumor homolog (*Wt1*)	Both	Gonadal agenesis	Lethal	237
Wip1 (p53-induced phosphatase)	Male	Runting and testicular atrophy	Subfertility	238
Wingless-related MMTV integration site 4 (*Wnt4*)	Female	Ovaries depleted of oocytes; Müllerian ducts do not form	Infertility	239

Wnt7a	Both	Females show abnormal development of oviducts and uterus; males do not have Müllerian duct regression	Infertility	240
Zfx (Zinc finger protein X-linked)	Both	Reduced germ cell numbers; males have reduced sperm, but are fertile; females subfertile	Subfertility (F)	241
Zona pellucida protein 1 (*Zp1*)	Female	Defects in fertilization	Subfertility	242
Zp2	Female	Fragile oocytes with defects in developmental competence	Infertility	243
Zp3	Female	Fragile oocytes	Infertility	244,245

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