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Mouse models for identifying genes modulating fertility parameters

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Fertility can be defined as the natural capability of giving life. It is an important factor both for human medicine, where ~10% of the couples call for the services of assisted reproductive technologies, and for species of economic interest. In particular, in dairy cows, the recent years have seen a kind of competition between milk production and fertility, and genes improving fertility are now considered as parameters to be selected for. The study of fertility pathways is nevertheless made difficult by the strong impact of environmental factors on this parameter, as well as by the number of genes potentially involved (as shown by systematic transcriptome analysis studies in the recent years). One additional level of complexity is given by the fact that factors modulating fertility will probably be sex specific. The usage of mouse models has been one of the solutions exploited for tackling with these difficulties. Here, we review three different approaches using mice for identifying genes modulating fertility in mammals: gene invalidation, positional cloning and in vitro *mutagenesis. These three approaches exploit specific characteristics of the mouse, such as the possibility of controlling precisely the environment, an excellent genetic characterization and the existence of genomic and molecular tools equalled only in humans. Many indications suggest that at least some of the results obtained in mice could be easily transposed to the species of interest.*

Keywords: fertility, mice, gametogenesis, cattle, humans

Introduction

Inherent complexity of the sexual reproduction and its implications in evolution

Mammalian reproduction encompasses several highly convoluted phases: gamete production, gamete encounter and fusion, early development, implantation and post-implantation developmental stages. These intertwined phases involve the modification and put on gear thousands of genes. This fact, which is not *a posteriori* surprising, was nevertheless considerably underestimated before the setting up of pan-genomic approaches of gene expression analysis carried out from the early events of sex determination (Nef *et al*., 2005; Beverdam and Koopman, 2006; Cederroth *et al*., 2007) to implantation (Giudice, 2003; Talbi *et al*., 2006). Any improper regulation in either phase of the highly complex network of genes involved may lead to infertility. The selection pressure applied to reproduction is huge and if fitness is defined as the capability of producing living offspring, then fertility is an obvious target for selection to play with. Such selection pressure is presumably an important reason why, despite the complexity of the reproductive system, things seem to work pretty well.

Cost/benefit of sexual reproduction: the example of high-producer dairy cows

From an evolutionary point of view, two contradictory trends seem to be at work in mammalian reproduction: an irreducible complexity for fabricating male and female gametes in order to allow the fecundation and the vital necessity of the system for the mere survival of the species. In this context, another parameter can be taken into account concerning the instance of energy and resource allotment. Indeed sexual reproduction is a costly method of survival, especially in female mammals, where the normal follow-up of fecundation consists of long-lasting hosting of one or several greedy foetuses. This essential biological and evolutionary condition is made even more severe by the fact that parturition in most mammalian species is followed by the care of the offspring during weeks, months or even years.

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All these drawbacks are therefore to be counter-balanced by an increased fitness. Recently, the true cost of prolificacy has been illustrated by dairy cows producing ever higher amounts of milk (from 2500 kg in 1945 to almost 9000 kg a year today). This evolution has been particularly rapid in the last 20 years and it is estimated at 3000 kg from 1986 to 2006. In parallel and sometimes controversially, infertility has started to be a significant problem in cattle farming, evocating a potential antagonistic relation opposing fertility and high production, albeit up to now such a negative relationship has not been strictly demonstrated (Lopez-Gatius *et al.*, 2006; Garcia-Ispierto *et al.*, 2007).

Elucidating the genetic control of reproductive functions is a difficult challenge

Heritability of reproductive parameters. The heritability of reproduction parameters remains relatively elusive but most reports suggest that it is either very low (0.05 or less) or weak (less than 0.20) (Bergmann and Hohenboken, 1992; Jamrozik *et al.*, 2005; Bormann *et al.*, 2006). Two likely reasons for this may be either a very strong influence of external factors (light, temperature, hormonal and dietary status) on the phenotype, which makes it difficult to isolate the genetic component of the observed variance, or a very high underlying genetic complexity.

General statements on human infertility treatments. In humans, infertility affects up to 10% of the couples in industrialized countries (Feng, 2003) and the complexity of the underlying biological disorders remains largely unknown at the molecular level. The responses brought to the patients involve technological approaches based upon two essential techniques: *in vitro* fecundation (IVF) and intra-cytoplasmic sperm injection (ICSI). These approaches are meant to alleviate or escape the problems encountered during gamete production, gamete encounter and fusion, early development and implantation. Such therapeutics, generally based upon gamete and/or embryo manipulations, bring immediate solution to patient's infertility but moves the possible genetical basis of the fertility problem to the next generation (Cram *et al.*, 2000; Meschede *et al.*, 2000).

Experimental models for a better understanding of fertility and its control

The following two points: (i) poor level of knowledge about the genetic bases of infertility/low fertility in humans and domestic species and (ii) high variation in the measured parameters (litter size, sperm characteristics, etc., inducing a lot of problem while trying to measure heritabilities) lead to the conclusion that a model where genetic variability is limited and where the environment can be tightly controlled has a high potential to make knowledge progress on this difficult subject. Classically, rodent models are consistently exploited to infer mechanisms transposable to other mammals. This is particularly the case for the laboratory mouse, characterized by its short generation time, high prolificacy and small size (Figure 1). The debate of transporting data from mice to humans or other species is controversial and never-ending. However, it should be recognized that many advances on mammalian physiology and genetics originate from mouse studies.

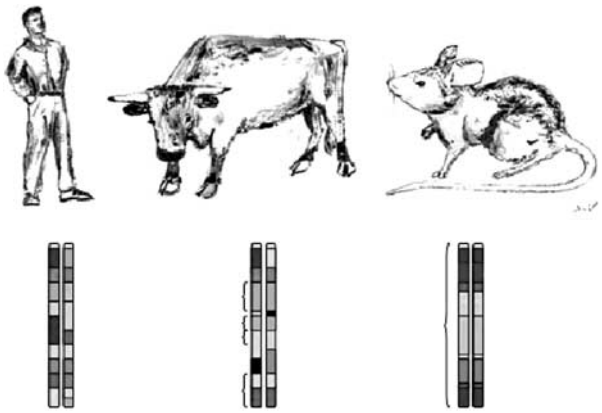


Figure 1 One of the main interests of using mice as animal models is the fact that they are available as strains, meaning that their chromosome content is homozygous at every locus. Intra-strain crosses are largely comparable to the mating of cloned animals, which, genetically speaking, should yield only identical animals, but the rare events of point mutations. By contrast, outbred species such as humans or species, which have been heavily selected for agricultural traits, have a strong level of heterozygosity. In the figure, homozygous regions are shown by brackets. For traits displaying a low heritability, either because they are genetically ill-defined or because environmental factors play a major role, crosses between mice species are very useful, since environmental conditions can be very precisely controlled, and because the genetic diversity is obtained by crossing existing strains, or by analyzing recombinant congenic strains.

Reproduction has not escaped this reality and several research groups around the world use laboratory mice to evaluate fertility parameters. One of the important characteristics of laboratory mice is that they exist as strains, a collection of genetically identical animals. These strains show complete homozygosity at all genomic loci, of course higher than in clones, which can be heterozygous at numerous loci. This situation lessens considerably the potential influences of individual genetic heterogeneity compared with other species.

Different types of mouse models have been constructed corresponding to different strategies for identifying gene(s) governing phenotypic traits: (1) Knocked-out (KO) mice in which a gene of interest has been invalidated. Analyzing the resultant phenotype of these KO mice makes it possible to directly know the function of this gene. (2) Mice in which diverse aleatory mutations have been chemically induced (*N*-ethyl-*N*-nitrosourea (ENU) mice). (3) Mice in which a certain degree of polymorphism has been introduced by successive crosses between different mouse strains or species. Indeed, variability is the major tool that geneticist can use; therefore, one of the recurrent problems of lab mice crosses is the low level of genetic diversity of the starting material, generally exclusively congenic homozygous *Mus musculus* lab mice. This has conducted mice geneticists to perform crosses

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between different strains in order to restore at least part of the necessary diversity. In a recent study, physiological parameters quantitatively governed by specific genes were studied in a multi-cross generated from eight different lab mouse strains (Valdar *et al.*, 2006).

Gene invalidation experiments in mice

Most of the information relating to fertility gene effects in mice originate from gene invalidation experiments. A recent review emphasizes the use of gene KO mice for understanding fertility (Roy and Matzuk, 2006). KO phenotypes have given categorical data at different levels about numerous pathways involved in fertility. These data may be summarized as follows essentially according to the classification of Roy and Matzuk (2006).

Germ line specifications: Primordial germ cells (PGC) derive from cells of the proximal epiblast after induction by the extra-embryonic ectoderm. More precisely, PGCs originate from the internal wall of the allantois, migrating inside a nascent gonad composed of three independent somatic cell lines (Koopman, 1999). Several molecular actors fixing the fate of these PGCs have been identified by gene invalidation experiments. Among those, transforming growth factor beta (TGFβ) family members appear heavily over-represented. This is the case for ligands such as Bmp4 (Lawson *et al.*, 1999), Bmp8b (Ying *et al.*, 2000) and Bmp2 (Ying and Zhao, 2001) as well as for their receptors (de Sousa Lopes *et al.*, 2004). Defects in TGFβ signalling pathway, by intracellular effectors such as Smad1 and Smad5, also have a reducing effect on the PGC number (Chang and Matzuk, 2001; Tremblay *et al.*, 2001). The Znf repressor *Prdm1* has been shown to have a specifying effect on the PGC (Vincent *et al.*, 2005). Systematic analyses of the PGC transcriptome by differential screening revealed genes that contribute to their determination and specificities. To this respect, Fragilis, an interferon-inducible transmembrane protein, seems to play a pivotal function together with the Stella transcription factor (Saitou *et al.*, 2002).

PGC proliferation and survival: In the Pin1$^{-/-}$ mice, PGCs were allocated properly at the base of the allantois, but their cell expansion was progressively impaired, resulting in a markedly reduced number of PGCs at 13.5 dpc (Atchison *et al.*, 2003). *Oct4*, a major gene of the POU family involved in the inner cell mass/trophectoderm choice, is also involved in PGC proliferation (Niwa *et al.*, 2000). Other important genes are *Nanos3* (Forbes and Lehmann, 1998), *Sdf1* and its receptor Cxcr4 (Molyneaux *et al.*, 2003; Holt *et al.*, 2006). PGCs are maintained owing to several growth factors such as LIF and bFGF (Farini *et al.*, 2005). Other less-documented transcription factors may have a very important incidence, such as Zfp148, the loss of which leads to a complete disappearance of PGCs at the heterozygous state (Takeuchi *et al.*, 2003). It is hypothesized that this gene mediates p53 function by preventing its phosphorylation during germ cell development.

Sex determination: In mice, mutations in many genes such as *AMH* (Behringer *et al.*, 1994) and its receptor (Jamin *et al.*, 2002), *Wnt4* (Vainio *et al.*, 1999), have been found to lead to at least a partial phenotype of sex reversal, similar either to a persistent Mullerian duct syndrome (PMDS) or to a masculinization with Leydig cells developing in the ovarian context. For a review of these genes, see for instance Vaiman and Pailhoux, (2000) and Vaiman (2003).

Preantral folliculogenesis: It may be sometimes difficult to separate genes involved in the early steps of folliculogenesis from genes involved in sex determination. This is illustrated by the recent analysis of the combined double KO of *Wnt4* and *FoxL2* conducting to XY sex reversal. Interestingly, either gene cannot reproduce such a clear-cut phenotype in mice (Ottolenghi *et al.*, 2007). The disruption of *Fig*α (Soyal *et al.*, 2000), *Wnt4* (Vainio *et al.*, 1999), *Nobox* (Rajkovic *et al.*, 2004) and *Gdf9* (Dong *et al.*, 1996) explain to various extents the onset of poor or absent fertility. Other species than mice also helped in the identification of new genes involved in folliculogenesis. This is the case of the sheep model in which different kinds of mutations in *FecX* (*Bmp15*) (Galloway *et al.*, 2000) and *BmpR1B* (Mulsant *et al.*, 2001) displayed variable phenotypes from total infertility to superfertility in terms of ovulation rate. Furthermore, several important genes for female fertility are located on the X chromosome (for a review, see Vaiman, 2002).

Spermatogenesis: KO at all steps of spermatogenesis has been shown to disrupt fertility. A relatively exhaustive list was given by Roy and Matzuk (2006). Briefly, for the transition from spermatogonia to spermatids, the role of *Kit* was early recognized (Yoshinaga *et al.*, 1991). More recently, the role of *Cks* in the differentiation timing between spermatogonia and spermatids was shown (Donovan and Reed, 2003). Plzf, a zinc finger transcription factor, was shown to be essential for the turnover and renewal of stem cells in the testis (Buaas *et al.*, 2004). Recently, it was shown that Sohlh1 (testis and ovary expressed basic helix–loop–helix (bHLH) transcription factor) is essential for spermatogonia differentiation (Ballow *et al.*, 2006). In invalidated testis from *Sohlh1*$^{-/-}$ mice, the testis transcription factors Etv5, Taf4b, Zfp148 and Plzf were normally expressed, while the bHLH Tohlh2 transcription factor was increased while Lhx8 and Ngn3 were repressed. These examples illustrate the fact that impaired fertility can result from various breakpoints in the complex cascade of sperm cell differentiation. Defective spermiogenesis is also a potential cause for infertility, relating to alterations of protamine genes (*Prm1*, *Prm2*) and transition proteins in the extreme DNA compaction of the sperm nucleus. A recent study made possible the identification by proteomics of not less than 132 proteins important for DNA compaction, chromosome segregation and fertility (Chu *et al.*, 2006). The final flagellar structure of sperm is the key to normal sperm movement. In a similar manner, many mouse mutants display an asthenozoospermia phenotype. Some genes affecting motility have been described such as *Catsper1* (Ren *et al.*, 2001), *Catsper* 2 (Quill *et al.*, 2003), other members of the same family (Jin *et al.*, 2007), dynein (Neesen *et al.*, 2001) and the SlcA28 anion transporter (Toure *et al.*, 2007),

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whose mutations induce an abnormal morphology of the flagellum.

Endocrine regulation of gametogenesis: GnrH deletion in mice leads to a condition where neither LH nor FSH are produced (hypogonadism), inducing infertility (Mason *et al*., 1986). Similarly, KO of *Fshb*, *Fshr*, *Lhb* and *Lhr* (Kumar *et al*., 1997; Dierich *et al*., 1998) have been produced. Although the disruption of FSH pathways induces the formation of small testes, the males are fertile while the females are sterile. In these KOs, folliculogenesis is blocked before the antrum formation. Contrary to FSH, the LH pathway is essential for both sexes. Other steroid hormones are crucial for reproductive function in mammals since the estrogen beta receptor (*Esrb*) disruption leads to subfertility in female and males in which serious defects of spermatogenesis are observed (Lubahn *et al*., 1993; Dupont *et al*., 2000). The invalidation of the progesterone receptor alpha (*Pra*) results in uterine defects, preventing normal implantation. Physiologically, once prepared by progesterone, the uterus is able to synthesize catecholestrogens from ovarian estrogens or from the blastocyst, these molecules appearing as essential for a successful implantation (Paria *et al*., 1998 and 2001). Numerous studies were performed on the androgen receptor (AR) KO mice (Chang *et al*., 2004; De Gendt *et al*., 2005), demonstrating its essential role for testosterone metabolism and androgen signalling during spermatogenesis. Consequently, the normal function of this molecule is necessary for male fertility. Additional roles of the AR in female fertility pathways have been shown (Hu *et al*., 2004; Walters *et al*., 2007).

This non-exhaustive list emphasizes the importance of gene invalidation in mice for identifying genes involved in controlling fertility. However, many KO mice harbor extreme phenotypes, sometimes missing complete systems such as genitalia, head, spinal cord, etc. In these cases, the KO will act in an on/off manner and induce, of course, a complete sterility when, in fact, many infertility phenotypes appear as quantitative deficiencies. In these cases, KO mice may not be ideal, suggesting that more subtle models could be helpful to deal with the variability observed in humans or livestock.

Genetic mapping techniques for infertility phenotypes in the mouse model

In humans, the identification of genetic loci quantitatively modulating fertility has not been a subject of research. Similarly, in mice, the number of studies aiming at identifying such loci is reduced. Nevertheless, quantitative variations can be important to explain the continuum of inter-individual differences, sometimes leading to infertility. In human male fertility, it is known that the number of spermatozoa is an important parameter, since below a given threshold (around 5 million sperm/ml), human males are almost sterile. The follow-up of quantitative parameters impacting on fertility has been much more consistent in cattle, where the economical relevance of such traits has a great importance. Several recent studies taking into account fertility parameters alongside other production traits illustrate this point (Kirkpatrick *et al*., 2000; Lien *et al*., 2000; Boichard *et al*., 2003; Ashwell *et al*., 2004; Schnabel *et al*., 2005). To the best of our knowledge, only four studies in cattle focused exclusively on fertility traits (Holmberg and Andersson-Eklund, 2006; Guillaume *et al*., 2007; Holmberg *et al*., 2007; Schulman *et al*., 2008), while the other studies involve the phenotyping of other production traits. Loci involved mainly in female fertility were identified (see Table 1). Today, no gene could be identified by this preliminary approach of positional cloning. This is explained by the paucity of molecular tools available in ruminants compared with humans and mice.

Therefore, for the cases where mice quantitative trait loci (QTL) appear at orthologous positions with cattle QTL, it is clear that *M. musculus* is the experimental species on which to work to identify causal mutation(s). Several different genomic characteristics have been exploited in different mouse models, and always present a combination of advantages and drawbacks. In mice, laboratory strains originate mainly from a small pool of ancestors belonging to the *M. musculus* species. For this reason, the different strains are genetically highly similar, an issue that sometimes constitutes a serious problem for addressing the genetic bases of complex phenotypes, due to the lack of informative markers. This lack of genetic polymorphisms can be overcome by crossing laboratory mice with wild mice or by creating original mutations with ENU mutagen. The use of such various models has been described for the dissection of several complex traits, such as the genetics of obesity (Brockmann and Bevova, 2002), but most models have not been exploited for the positional cloning of genes involved in fertility.

Since mouse strains are completely homozygous, polymorphism is generally partially restored by crossing two laboratory strains or even two mouse species. The development of inter-specific hybrids strains is made possible by the possibility of getting offspring from mice belonging to two species in the early stages of speciation (Guenet and Bonhomme, 2003). In this case, generally, the female offspring is fertile whereas the male is sterile, showing a strong disruption of steroidogenic functions (Chubb and Nolan, 1987). The origin of this hybrid breakdown has been traced back to meiotic defects (Matsuda and Chapman, 1991; Matsuda *et al*., 1992; Hale *et al*., 1993). To date, nine loci inducing male hybrid sterility have been mapped on chromosomes X (*Hstx1*, *Mhstq2* and *Hst3*) and 17 (*Mhstq1*, *Hst1*, *Hst4*, *Hst5*, *Hst6* and *Hst7*). Some of them have been mapped so closely that it is difficult to be certain that they correspond to different genes. In several cases, candidate genes potentially involved in the sterility phenotype have been proposed. For instance, the *Hst6* locus encompasses the gene *Dnahc8*, which seems to be at least partially responsible for a phenotype of sperm tail curvature (Samant *et al*., 2005). This locus is nevertheless complex since in the same region, two candidate genes (*Tsga2* and *Tctex5*) might play synergistic roles in the generation of motility

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Table 1 *Quantitative trait loci (QTL) modulating fertility parameters in cattle*

Phenotype	QTL mapping	Candidate gene	Animal model	Reference
Female side				
Ovulation rate	BTA5 (107 cM) BTA7 (5 cM) BTA7 (57 cM) BTA19 (65 cM)		USDA twinning herd	Kirkpatrick *et al.* (2000) Mammalian Genome
Twinning rate	BTA5 (70 to 80 cM) BTA7 (105 cM) BTA12 (10 cM) BTA23 (30 cM)	 Bola, Cyp21	Norwegian cattle	Lien *et al.* (2000) Mammalian Genome
Female fertility (success/failure of each insemination)	BTA1 (49 to 100 cM) BTA7 (116 to 122 cM) BTA10 (95 cM) BTA20 (75 cM) BTA21 (56 cM)		French dairy or mix breeds grand-daughter design	Boichard *et al.* (2003) Genetics Selection Evolution
Pregnancy rate	BTA6 (122 cM) BTA14 (11 cM) BTA16 (81 cM) BTA18 (14 cM) BTA27 (62 cM) BTA28 (48 cM)		American and Israeli Holstein families	Ashwell *et al.* (2004) Journal of Dairy Science
Pregnancy rate	BTA1 (9 cM) BTA14 (44 to 62 cM)		Cooperative dairy DNA depository	Schnabel *et al.* (2005) Animal Genetics
Ovulation rate	BTA14 (59 cM)		USDA twinning herd	Gonda *et al.* (2004) Animal Genetics
Calving performance	BTA6 (55.4 cM)		Swedish dairy cattle (10 grand-daughter families)	Holmberg and Andersson-Eklund (2006) Journal of Dairy Science
Calving performance (maternal)	BTA6 (35.5 cM) BTA13 (38.6 cM) BTA15 (22.1 cM)			
Stillbirth (maternal)	BTA7 (34.6 cM) BTA11 (45 cM)			
Nb inseminations (heifer)	BTA9 (145 cM)	CGA (hypothesis in this paper)		
Non-return at 56 days	BTA9 (145 cM) BTA15 (22.1 cM) BTA20 (19.1 cM)	CGA (hypothesis in this paper)		
Nb inseminations (cow)	BTA11 (62 cM) BTA29 (2 to 21 cM)			
Heat intensity score	BTA13 (91.4 cM)			
Non-return at 56 days	BTA3 (26 cM)		French dairy or mix breeds. Granddaughter design	Guillaume *et al.* (2007) Animal Genetics
Non-return at 90 days	BTA3 (26 cM)			
Non-return at 282 days	BTA3 (26 cM) BTA7 (110 cM)			

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abnormalities and the sperm–egg interaction aberration observed in hybrids. Nevertheless, these features can be regarded as functionally unrelated traits (Pilder *et al.*, 2007).

Using more classical QTL mapping approaches, several preliminary localizations of fertility-modulating genes have been achieved in mice, but are still very imprecise in most studies, sometimes an entire chromosome. Such QTL-containing regions have been identified for female (Krewson *et al.*, 2004; Peripato *et al.*, 2004; Rocha *et al.*, 2004; Liljander *et al.*, 2006) and male fertility parameters (Zídek *et al.*, 1998; Le Roy *et al.*, 2001; Oka *et al.*, 2004). For instance, two QTLs affecting meiosis on chromosomes 4 and 13, two QTLs for relative testis weight on chromosomes 4 and X and several QTLs for degenerated germ cells and multi-nuclear giant cells (Bolor *et al.*, 2006). Effects on fertility have been thoroughly analyzed for chromosome X loci in two recent studies based on mouse subspecies (Oka *et al.*, 2004 and 2007) in which parameters of sperm morphology and function were assessed. The results displayed a QTL responsible for testis weight as well as two QTL for sperm head morphology. In the 2007 study, the authors demonstrated the importance of epistatic interactions between various chromosome loci for achieving a normal fertility (Oka *et al.*, 2007). Only one recent study (L'Hote *et al.*, 2007), using recombinant congenic strains of mice, made it possible to restrict the gene location to a small interval of 1 to 2 cM. In this study, we proposed using inter-specific recombinant congenic strains (IRCS) in order to rapidly map genes modulating fertility to short chromosome intervals. These mice have been generated over 20 years ago in the Pasteur Institute by an initial cross between two evolutionary distant parental species (*M. musculus*, C57BL/6 × *Mus spretus*), followed by two backcrosses on C57BL/6. In total, 118 strains were originally raised by mating mice trios. Then, brother/sister crosses were performed during more than 30 generations. Finally, 53 IRCS strains survived this process. Each strain possesses on average 98% of C57BL/6 genome and 2% of *spretus* genome fixed at the homozygous state (see Figure 2). The positions of the *spretus* fragments (1 to 7 per strain) have been precisely determined from the analysis of around 700 single nucleotide polymorphisms (SNPs) and microsatellite markers (Burgio *et al.*, 2007). Using this model, fertility analysis has been addressed on the male side by measuring several sperm parameters having a known influence on fertility (L'Hote *et al.*, 2007). Eight QTL were identified and are currently under scrutiny to identify the underlying genes and potential causal mutations. The female side is addressed on the same model using *in vivo* high-frequency ultrasonography. Phenotypes of *in utero* lethality and embryonic resorption have been found and are currently analyzed in more detail searching to clone the relevant endometrial and placental genes (Laissue *et al.*, 2008). This IRCS model constitutes a unique opportunity to clone new genes involved in fertility in mammals. A summary of fertility-modulating genes is presented in Table 2.



Figure 2 The map status of the 53 interspecific recombinant congenic strains developed at the Pasteur Institute (Paris). Horizontal bars correspond to markers that have been genotyped yellow fragments are present in at least one of the strains in a *spretus* form. Pink fragments are fragments that can apparently be maintained only at the heterozygous state in at least one strain, despite the number of brother–sister crosses. In average, B6 and *spretus* genome diverge for one nucleotide every 80 bp, which insures a high level of heterozygosity and the existence of informative markers able to delineate precisely the *spretus* fragments (http://www.pasteur.fr/recherche/unites/Gfons/ircs/ircshome.htm).

Ethyl-nitrosourea mutagenesis and fertility analysis

Genetic polymorphisms can be induced in laboratory mice by ENU mutational treatment. ENU is an alkylating agent that is a powerful mutagen. In mouse spermatogonial stem cells, it produces single locus mutation at high frequencies (Russell *et al.*, 1979; Rinchik *et al.*, 1991).

ENU can induce many different types of alleles: Loss of function mutations, viable hypomorphs of lethal complementation groups, antimorphs and gain of function mutations. Mutations induced by ENU are not molecularly tagged, and have to be analyzed by linkage analysis and positional cloning. Mutations can be induced in living males or in embryonic stem cells culture. When the mutation is roughly mapped on a chromosome, different schemes exist to finely map the mutations and establish it at a homozygous state, using a deletion scheme, or balancer chromosomes to isolate mutations.

Several consortia have initiated systematic, genome-wide production of mouse mutants. The phenotypic analyses included reproduction traits. For example, the Jackson laboratory has created in 2005 'The Reproductive Genomics Program', and so far has established 40 mutations impairing male or female fertility. Infertility phenotypes include ovaries that develop poorly, ovaries with no oocytes, ovaries whose follicles do not mature properly, oocytes that die inside the follicles, oocytes that cannot complete meiosis and embryos that die before they reach the blastocyst stage. Aberrant male phenotypes include spermatocytes that do not develop defective sperm and sperm with a low fertilization rate. These mice mutants can be obtained from the Jackson laboratory.

A total of 89 mutations are present in databases, 24 of which have been precisely localized (Table 3) encompassing

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Table 2 *Quantitative trait loci (QTL) modulating fertility parameters in mice*

Name	Phenotype	QTL mapping	Candidate gene	Animal model	Reference
Male side					
Hom1	Increased circulating testosterone level decreased seminal gland weight increased testis weight	MMU17 (~19 cM)		A/Ph × B10.A-H2 <a> F2	Ivanyi P *et al.* (1972) Nature New Biology
Mors1	Decreased sterility in obese male animals	MMU1 (101.5 to 106.3 cM)		(C57BL/6J-Lep<ob>/Lep <ob> × BALB/cJ)F2	Ewart-Toland A *et al.* (1999) Endocrinology
Mors2	Decreased sterility in obese male animals	MMU3 (52.5 to 71.8 cM)			
Tesw1	Increase in testis weight	MMUX (~37.8 cM)	Ar	(C57BL/6By × NZB/BlNJ)F2	Le Roy *et al.* (2001) Genetics
Tesw2	Increase in testis weight	MMU10 (~57 cM)			
Tsvw1	Increased testis; seminal vesicles weight	MMU4 (~47.5 cM)	Lepr		
Hstx1	Abnormal testes histology, reduced testis weight; abnormal sperm head morphology	MMUX (~29.5 cM)	Ihpd	Interspecific consomic C57BL/6J-Chr X < PWD/Ph>	Storchova R *et al.* (2004) Mammalian Genome
Mhstq1	Reduced testes weight; abnormal sperm heads, no sperm tails	MMU17: (~16 cM)	Dnahc8	(C57BL/6J × *M. macedonius*)F1 × C57BL/10J backcross ≥ MMU17 congenic strain	Elliott RW *et al.* (2004) Mammalian Genome
Mhstq2	Reduced testes weight; most males do not produce sperm heads, in rare cases abnormal sperm heads are present	MMUX (~17 cM)		(C57BL/6J × *M. macedonius*)F1 × C57BL/10J backcross ≥ MMUX congenic strain	
Tesq1	Decreased testis weight	MMU13 (~10 cM)		(M16i × L6)F2	Rocha *et al.* (2004) Mammalian Genome
Tesq1	Decreased testis weight	MMU6 (~44.9 cM)			
Tesq1	Increase in testis weight	MMU10 (~69 cM)			
Spha1	Abnormal sperm head morphology	MMUX (8.8 cM)		Consomic (C57BL/6J-X <MSM>)	Oka *et al.* (2004) Genetics
Spha2	Abnormal sperm head morphology	MMUX (14.1 to 33.5 cM)	Tsga8 (Halapx), Nr0b1, Fmr1, and Sox3		
Spha3	Abnormal sperm head morphology	MMUX (43 to 70.5 cM)			
Tswt1	Decreased testis weight	MMUX (58 to 63 cM)			
Smtw1	Decreased testis weight	MMU4 (~61.6 cM)		(Smt × *Mus musculus molossinus*)F2	Bolor *et al.* (2006) Experimental Animals
Smtw2	Decreased testis weight	MMUX (~59 cM)			
Spmd1	Increased meiotic defect; multi-nuclear giant cells	MMU4 (~39.5 cM)			
Spmd2	Increased meiotic defect; increased germ cell degeneration	MMU4 (~25.8 cM)			
Spmd3	Increased germ cell degeneration	MMU7 (~22 cM)			
Spmd4	Increased germ cell degeneration	MMU13 (epistatic with spmd2)			
Ilx1	Partially restore fertility induce by Spha QTLs	MMU1 (D1MIT49 to D1MIT288)		Consomic (C57BL/6J-X <MSM>+ 1 <MSM>)	Oka *et al.* (2007) Genetics
Ilx2	Partially restore fertility induce by Spha QTLs	MMU11 (D11MIT52 to D11MIT69)			
Ilx3	Partially restore fertility induce by Spha QTLs	MMU11 (D11MIT71 to D11MIT236)			
Dss1	Decreased sperm survival	MMU6 (137.4 to 149.5 Mb)		IRCS	L'Hote *et al.* (2007) Genetics
Hpw1	High prostate weight	MMU19 (43 Mb and the telomere)	Mxi1		
Sh1	QTLs affecting sperm head shape	MMU6 (137 to 146 Mb)	Capza3 Tuba3b		

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Table 2 *Continued*

Name	Phenotype	QTL mapping	Candidate gene	Animal model	Reference
Sh2	QTLs affecting sperm head shape	MMU3 (144.17 to 146.94 Mb)	Spata1		
Sh3	QTLs affecting sperm head shape	MMU12 (106.3 to 120.5 Mb)			
Sh4	QTLs affecting sperm head shape	MMU11 (3.5 to 26.5 Mb)			
Ltw1	Decreased testis weight	MMU11 (3.5 to 26.5 Mb)			
Ltw2	Decreased testis weight	MMU6 (133.3 to 149.5 Mb)			
Female side					
	Offspring survival	MMU2 (peak: 69 cM)		F2 intercrosses SM/J and LG/J	Peripato *et al.* (2002) Genetics
	Offspring survival	MMU7 (centromere, peak: 0.5 cM)			
	Litter size	MMU7 (centromere, peak: 0.5 cM)		F2 intercrosses SM/J and LG/J	Peripato *et al.* (2004) Journal of Evolution Biology
	Litter size	MMU12 (45 + 8 cM)			
Fecq3	Litter size	MMU1 (peak: 9 cM)		Inbred high and poor breeder strains	Liljander *et al.* (2006) Genetics
Fecq4	Litter size	MMU9 (peak: 29 cM)			
Neogq1	Neonatal growth	MMU9 (peak: 42 cM)			
Pregq3	Pregnancy rate	MMU13 (26 cM)			
Pregq4	Pregnancy rate	MMU17 (44.5 cM)			
Bwq7	Maternal body weight	MMU1 (62 cM)			
Oriq2	Induced ovulation rate	MMU2 (31.7 cM)		C57BL/6J and A/J strains	Spearow *et al.* (1999a) Biology of Reproduction
Oriq3	Induced ovulation rate	MMU6 (28 cM)			
Oriq1	Induced ovulation rate	MMU9 (31 cM)	Cyp19		
Oriq5	Induced ovulation rate	MMU10 (6 cM)			
Oriq4	Induced ovulation rate	MMUX (17 cM)			
Popoa	Oocytes arrested at MI	MMU1 (58.7 cM)		*Mus musculus castaneus* × LT/SvKau	Everett *et al.* (2004) Reproduction
	Oocytes arrested at MI	MMU9 (48 cM)			
Estoq4	Diverse parameters	MMU1 (41 cM)		Intercross high and poor increase weight strains	Rocha *et al.* (2004) Mammalian Genome
Dfq1, *Espq1*, *Espq2*, *Espq4*, *Estoq1*, *Estoq2*, *Lfq1*, *Lfq2*	Diverse parameters	MMU2 (41.6 cM) (44.1cM) (66.8 cM) (50.1cM) (70.2 cM) (38.4 cM)			
Estq3, *Lfq3*	Diverse parameters	MMU10 (52.7) (50.2)			
Vgot1	Pubertal timing	MMU6 (68 cM)		Inbred A/J and C57BL mice (CSSs)	Krewson *et al.* (2004) Endocrinology
Vgot2	Pubertal timing	MMU13			

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Table 3 *ENU fertility mutant mice*

Name	Phenotype	Chromosomal position	Candidate gene/mutation	Reference
KitlSl-17H	Lower than normal numbers of oocytes observed at 12 days of age; at sexual maturity mice were fertile; by 7 months of age no oocytes were observed; delayed onset and reduction in numbers of male germ cells; depletion of differentiating male germ cells by 8 weeks of age	MMU 10 (99.44 to 99.53 Mb, +strand)	Kit ligand	Brannan CI *et al.* (1992) Genes and Development
Pgm3 mld1	While immature sperm is detected all mature sperm are pyknotic; irregardless of the genotype of the females, male mice never sire offspring	MMU 9 (86.35 to 8637 Mb, −strand)	Phosphoglucomutase 3	Greig KT *et al.* (2007) Molecular Cell Biology
Mitfmi-bcc2	Male and female are often sterile	MMU 6 (97.77 to 97.98 Mb, +strand)	Microphthalmia-associated transcription factor	Hansdottir AG *et al.* (2004) Genomics
Crsl	Females have vulva abnormalities; most females have clitoral hypoplasia	MMU 4	Undefined	Hawker K *et al.* (2005) International Journal of Audiology
repro31	Low/abnormal pre-implantation development with low 2-cells and low blastocysts; low seminal vesicle weight; abnormally shaped sperm heads with short tails or without tails; abnormal cells in epididymal lumen; low testis weight, very low epididymal sperm concentration; very low sperm motility	MMU 1	Undefined	JAX Reproductive Mutagenesis Program, MGI Direct Data Submission 2004–07
repro4	Very low testis weight; some germ cells appear apoptotic; no sperm recovered; arrest of germ cells during late pachytene stage of meiotic prophase	MMU 1	Undefined	
repro3	Low epididymal sperm concentration; all sperm have abnormal head and/or tail morphology; immobile sperm	MMU 10	Undefined	
repro15	Epididymal sperm with abnormal head morphology and no IVF success; within testes abnormally shaped spermatid heads are abundant, few elongated spermatids in some tubules	MMU 11	Undefined	
Agtpbp1pcd-6J	No sperm production was observed at 61 days of age	MMU 13 (59.45 to 59.56 Mb, −strand)	ATP/GTP-binding protein 1	
ferf1	Extremely low *in vitro* fertilization success rate	MMU 14	Undefined	
repro33	Low seminal vesicle weight and low epididymal sperm concentration; abnormal looped sperm tails; sperm wiggle but are without forward movement	MMU 16	Undefined	
repro5	Low epididymal sperm concentration; abnormal sperm head morphology; impaired sperm motility; oocytes commonly contain vacuolesand have low developmental competence	MMU 16	Undefined	
repro17	Low epididymal sperm concentration; abnormal sperm head shape and poor IVF success; low epididymal sperm motility	MMU 17	Undefined	
repro21	Low testis weight; low epididymal sperm concentration; abnormally shaped sperm heads; absent sperm tail; no sperm motility	MMU 17	Undefined	
repro7	Very small ovaries with no growing follicles; some seminiferous tubules have only spermatogonia and Sertoli cells present; low testis weight; no epididymal sperm; arrest of spermatogenesis at the pachytene spermatocyte stage	MMU 17	Undefined	

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Table 3 *Continued*

Name	Phenotype	Chromosomal position	Candidate gene/mutation	Reference
Npc1 nmf164	Mutants do not breed well	MMU 18 (12.33 to 12.37 Mb, −strand)	Niemann pick type C1	
repro30	Very low seminal vesicle weight; abnormal spermatogenesis; no epididymal sperm recovered; arrest of spermatogenesis predominantly at pachytene spermatocyte stage	MMU 2	Undefined	
repro26	Low testis weight abnormal hammer-head shape of elongating spermatid heads; very low epididymal sperm concentration; abnormally shaped sperm heads; no sperm tails; low epididymal sperm motility and poor IVF success	MMU 3 (∼9.45 cM)	Undefined	
repro8	Low seminal vesicle weight; some seminiferous tubules have only spermatogonia and Sertoli cells present; very low testis weight; no sperm recovered; arrest of germ cells during early meiotic prophase; abnormal spermiogenesis	MMU 4	Undefined	
repro10	Variable epididymal sperm concentration; spermatogenesis predominantly normal but some aberrant spermatid head morphology; low epididymal sperm motility and low IVF success	MMU 4 (∼32.05 cM)	Undefined	
repro25	Female infertility	MMU 4 (∼46.4 cM)	Undefined	
repro22	Absent ovarian follicles; small ovary;decreased testis weight; low testis weight; decreased male germ cell number; no epididymal sperm are recovered	MMU 4 (∼73.5 cM)	Undefined	
repro34	Low seminal vesicle weight; very low testis weight; no epididymal sperm; accumulation of meiotic division phase spermatocytes and degenerating round spermatids present in multi-nucleated cell bodies	MMU 5	Undefined	
repro57	Low seminal vesicle weight; very low testis weight; some germ cells appear apoptotic; no sperm are recovered; arrest of germ cells during late pachytene stage of meiotic prophase	MMU 5	Undefined	
repro29	Low seminal vesicle weight; abnormally shaped sperm heads with and without tails; low epididymal sperm concentration; abnormal spermatid head shape and low number of elongating spermatids; very low sperm motility	MMU 5	Undefined	
repro2	Low sperm concentration; some spermatids display abnormal hammerhead nuclear shapes and tail elongation is impaired; dysplasia of the fibrous sheath is frequently detected; many sperm lack heads and the remainder have abnormal head and/or tail morphology; sperm frequently display a short tail and in some cases tails terminating in a coil; immobile sperm	MMU 5	Undefined	
repro14	Tiny ovaries with no growing oocytes or follicles; very low testis weight; no sperm recovered; decreased oocyte cell number; arrest of spermatogenesis predominantly at pachytene spermatocyte stage or meiotic division stage	MMU 5	Undefined	

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Table 3 *Continued*

Name	Phenotype	Chromosomal position	Candidate gene/mutation	Reference
repro12	Low seminal vesicle weight; few elongated spermatids and abnormal round cells with condensed nuclei in many tubules; low testis weight; generally no epididymal sperm recovered; among sperm recovered, there is high frequency of morphological head abnormalities and low IVF success	MMU 5 (~4.5 cM)	Undefined	
repro32	Abnormally shaped sperm heads, low number of sperm with tails; low epididymal sperm concentration; giant multi-nuclated cell; *in vitro* fertilization has very low success; very low sperm motility	MMU 6	Undefined	
Clcn1adr-mto6J	Mice do not breed	MMU 6 (42.21 to 42.24 Mb, +strand)	Chloride channel 1	
repro23	Low testis weight; no epididymal sperm recovered; arrest of spermatogenesis predominantly at pachytene spermatocyte stage	MMU 7 (~13.0 cM)	Undefined	
repro13	Low sperm concentration was noted; a high frequency of abnormal sperm heads was noted; including elongated spermatid heads; the acrosome is present; a low rate of IVF success was observed	MMU 7 (~8.25 cM)	Undefined	
repro16	Abnormal cells in epididymal lumen; low epididymal sperm concentration; abnormal sperm head shape and poor IVF success; low epididymal sperm motility	MMU 8	Undefined	
repro20	Low testis weight; low epididymal sperm concentration; abnormally shaped sperm heads; short or absent sperm tail; low sperm motility	MMU 9	Undefined	
repro28	Low seminal vesicle weight and low epididymal sperm concentration; abnormally shaped sperm heads; kinky tails and absence of tails on sperm; low sperm motility	MMU 9	Undefined	
repro36	Tiny ovaries with no growing oocytes or follicles; low testis and seminal vesicle weight; arrest of germ cells during pachytene stage of meiotic prophase; azoospermia	MMU 9	Undefined	
repro46	Tiny ovaries with some follicle activity; hemorrhagic cysts present in some ovaries; low testis and seminal vesicle weight; arrest of germ cells during pachytene stage of meiotic prophase; azoospermia	MMU 9	Undefined	
repro24	Low epididymal sperm concentration; abnormally shaped sperm heads; poor epididymal sperm motility	MMU1 (~49.5 cM)	Undefined	
nmf240	Aspermiogenesis	UN	Undefined	
repro19	Decreased testis and seminal gland weight; abnormal sperm heads and short or absent tails; low sperm production	UN	Undefined	
repro47	Low seminal vesicle weight; very low epididymal sperm concentration; teratozoospermia; very low sperm motility	UN	Undefined	
repro48	Low seminal vesicle weight; low number of mature spermatids; no sperm recovered	UN	Undefined	
repro49	Low seminal vesicle weight; low epididymal sperm concentration; abnormal sperm heads with and without short tails; very low sperm motility	UN	Undefined	

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Table 3 *Continued*

Name	Phenotype	Chromosomal position	Candidate gene/mutation	Reference
repro50	Low seminal vesicle weight; low epididymal sperm concentration; abnormal sperm heads without tails; no sperm motility	UN	Undefined	
repro51	Low seminal vesicle weight; low epididymal sperm concentration; abnormal sperm heads with coiled and folded tails; low sperm motility	UN	Undefined	
repro52	Low seminal vesicle weight; low epididymal sperm concentration; low sperm motility but yields successful *in vitro* fertilization	UN	Undefined	
repro53	Low seminal vesicle weight; low epididymal sperm concentration; low sperm motility	UN	Undefined	
repro54	Low seminal vesicle weight; low epididymal sperm concentration; abnormally shaped sperm heads with coiled tails or tails without heads; low sperm motility	UN	Undefined	
repro27	Low testis weight; very low epididymal sperm concentration; abnormally shaped sperm heads; some sperm with no tails; low epididymal sperm motility	MMU 5	Undefined	
inft3	Female infertility	MMU 11	Undefined	Kile BT *et al.* (2003) Nature
inft7	Female infertility	MMU 11	Undefined	
inft2	Male infertility	MMU 11	Undefined	
inft5	Male infertility	MMU 11	Undefined	
inft6	Male subfertility probably due to cysts in multiple organs	MMU 11	Undefined	
inft4	Severely reduced epididymal sperm count, decreased testis weight; many sperm exhibited club-shaped heads; sperm with stumped, short tails were present; spermatogenic arrest at stage VII, with spermatids in step 8 of spermiogenesis; apoptotic nuclei were noted in spermatids and in the tubule lumen; absence of forwardly progressive motile sperm	MMU 11	Undefined	
inft8	Severely reduced epididymal sperm count; many sperm exhibited club-shaped heads; sperm with either stumped, short tails or looped tails were present; spermatogenic arrest at stage X, with spermatids in step 10 of spermiogenesis; apoptotic nuclei were noted in spermatids and in the tubule lumen; absence of forwardly progressive motile sperm	MMU 11	Undefined	
inft9	Severely reduced epididymal sperm count; many sperm exhibited club-shaped heads; sperm with either stumped, short tails or looped tails were present; spermatogenic arrest at stage X, with spermatids in step 10 of spermiogenesis; apoptotic nuclei were noted in spermatids and in the tubule lumen; absence of forwardly progressive motile sperm	MMU 11	Undefined (potentialy the same gene affected as in inf 4 and 8, because of absence of complementation between the three loci)	
gro25	Azoospermia	UN	Undefined	
gro26	Azoospermia (incomplete penetrance)	UN	Undefined	
ww1	Cryptorchism; small testes	UN	Undefined	
ww2	Small gonads	UN	Undefined	

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Table 3 *Continued*

Name	Phenotype	Chromosomal position	Candidate gene/mutation	Reference
Alms1L2131X	Males have defective sperm formation in the testes	MMU 6 (85.55 to 85.66 Mb, +strand)	Alstrom syndrome 1 homolog (human)	Li G *et al.* (2007) PLoS Genetics
Pcsk1N222D	Testes show a significant decrease in weight	MMU 13 (75.55 to 75.60 Mb, +strand)	Proprotein convertase subtilisin/kexin type 1	Lloyd DJ *et al.* (2006) Human Molecular Genetics
Pahenu1	Slightly reduced litter size; small drop in number of pups surviving to weaning	MMU 10 (86.956 to 87.01 Mb, +strand)	Phenylalanine hydroxylase	McDonald JD *et al.* (1990) Mar
GhSma1	Female sexual maturation is delayed; the number of litters with no surviving pups is significantly increased; median litter size at weaning	MMU 11 (106116361 to 106117955 Mb, – strand)	Growth hormone	Meyer CW *et al.* (2004) Endocrinology
fit51R	Average testis weight is 0.007 *v.* 0.073 g in controls; entire reproductive tract is underdeveloped and small	UN	Undefined	Mouse Genome Informatics (MGI), Oak Ridge
Grm1wobl	Homozygous mutant mice do not breed	MMU 10 (10.37 to 10.77 Mb, –strand)	Glutamate receptor, metabotropic 1	Mouse Genome Informatics (MGI), The Australian Phenomics Facility at The Australian National University
Aff1Rob	Females fail to carry pups to term; males mated to wild-type females sire small litters	MMU 5 (103.93 to 104.09 Mb, +strand)	AF4/FMR2 family, member 1	Nolan PM *et al.* (2000) Nature Genetics
Evi1Jbo	Heterozygote female undergo repeated spontaneous abortion	MMU 3 (30.140 to 30.33 Mb)	Ecotropic viral integration site 1	Parkinson N *et al.* (2006) PLoS Genetics
Gnrhrhh	Anogenital distance is reduced; genital papilla is reduced in size; internal reproductive tract appears prepubescent; absent corpus luteum; large antral follicles are absent; arrest at the early antral stage; small ovary; small uterus; opening of the vagina is delayed; blocked at the mid-pachytene stages of meiosis; small seminal gland; decreased testis weight; cryptorchism; small penis; small epididymis	MMU 5 (87.25 to 87.27 Mb, –strand)	Gonadotropin releasing hormone receptor	Pask AJ *et al.* (2005) Molecular Endocrinology
Chd7Flo	Females have vulva abnormalities; most females have clitoral hypoplasia	MMU 4 (8.62 to 8.79 Mb, +strand)	Chromodomain helicase DNA binding protein 7	Pau H *et al.* (2004) Otology and Neurotology
Sfrp2C50F	More residual bodies within the seminiferous tubules are present compared to in wild-type mice	MMU3 (83.85 to 83.86 Mb, +strand)	Secreted frizzled-related protein 2	Quwailid MM *et al.* (2004) Mammalian Genome
Herc2jdf2-1R	Male and female infertility	MMU 7 (55.91 to 56.09 Mb, +strand)	Hect (homologous to the E6-AP (UBE3A) carboxyl terminus) domain and RCC1 (CHC1)-like domain (RLD) 2	Rinchik EM *et al.* (1995) Proceedings of the National Academy of Sciences USA
Gja11Jrt	Only a few scattered gap junction plaques are seen in the ovaries compared with wild type and granulosa cells exhibit weak gap junction coupling	MMU 10 (56.06 to 56.08 Mb, +strand)	Gap junction protein, alpha 1	Rossant J (2003) MGI Direct Data Submission
Mbtps1wrt	Females are sterile when bred with homozygous males, and crosses with heterozygous males only give birth to heterozygous pups	MMU 8 (122.39 to 122.44 Mb, –strand)	Membrane-bound transcription factor peptidase, site 1	Rutschmann S *et al.* (2007) MGI Direct Data Submission
EgfrWa5	Vaginal opening delayed until around 11 months of age; no offspring produced by either gender by 3 months of age	MMU 11 (16.65 to 16.81 Mb, +strand)	Epidermal growth factor receptor	Thaung C *et al.* (2002) Human Molecular Genetics

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Table 3 *Continued*

Name	Phenotype	Chromosomal position	Candidate gene/mutation	Reference
tmgc28	Infertility	MMU 15	Undefined	The Tennessee Mouse Genome Consortium (2003) MGI Direct Data Submission
tmgc47	Females have a large anogenital distance	MMU X	Undefined	
baln4	Reduced fertility	UN	Undefined	
esgd12d	Few intact epididymal spermatozoa present; very low epididymal sprem concentration; predominantly sperm heads without tails or with short tails; sterility in males only; very low motility	MMU 16 (22.15 cM)	Undefined	Ward JO *et al.* (2003) Biology of Reproduction
lsgd9a	Spermatogenesis appears effective in a subset of seminiferous tubules; lack epididymal sperm; only round cells present in the epididymus	MMU 2	Undefined	
swm6	Few intact epididymal spermatozoa present	MMU 5	Undefined	
swm2	Nearly aspermic; abnormal hammerhead nuclear shapes and impairment in tail elongation; many sperm lack heads or tails and the remainder have abnormal head and/or tail morphology;	MMU 7	Undefined	
mei11b	GHV breakdown fails	UN	Undefined	
mei7	Sterility in males only; meiosis arrested early in prophase; prophase I nuclei are large and swollen; cell death by mid-prophase I	UN	Undefined	
Ccnb1ip1mei4	Ovarian dysmorphology; seminiferous tubules lack post meiotic cell differentiation; small testis; reduced numbers of oocytes; meiosis arrested at first cell division; sterility in both sexes	MMU 14 (49.71 to 49.72 Mb, −strand)	Cyclin B1 interacting protein 1	
Lrp4mitt	Abnormal mammary buds	MMU2 (91.26 to 91.31 Mb, +strand)	Low-density lipoprotein receptor-related protein 4	Weatherbee SD *et al.* (2006) Development
Nsun7Ste5Jcs1	Low percentage of motile sperm; sperm swim in slow circles, exhibit slow swimming velocities and fail to make forward progress; the mid-piece section of the flagellum is abnormally rigid and fails to contribute to flagellar bending; sperm mitochondrial sheath exhibits abnormal electron density	MMU 5 (66.53 to 66.57 Mb, +strand)	NOL1/NOP2/Sun domain family, member 7	Wilson L *et al.* (2005) Genome Research
KitW-1Bao	Many, but not all males, are sterile; average litter size is half that of background strain	MMU 5 (75.85 to75.93 Mb, +strand)	Kit oncogene	Wu B *et al.* (2003) Chinese Science Bulletin

genes with pleiotropic effects. Consortia tend to submit many mutations with a coarse localization, but with a fine phenotype description. Therefore, while most mutations remain imprecisely mapped, their potential for discovering genes important in mammalian fertility pathways will be very important in the future.

Conclusions

In conclusion, sterility analysis in hybrid animals or hypofertility in subspecies crosses revealed some interesting trails for understanding some molecular bases of infertility. One of the major problems of such genetic studies aiming at identifying genes is that QTL locations are generally far from precise, but could be overcome in some cases using IRCS.

In this context and despite the unquestionable progress in the knowledge brought by mice KO analysis, we make the hypothesis that positional cloning approach will be able to give new insights in the complexity underlying mammalian fertility pathways. Several genes identified in mice have seen their application in human cases of infertility (see, for a review, Furnes and Schimenti, 2007). We expect that starting from the phenotype is an efficient way to focus more closely on genes of interest, especially on factors related with important quantitative phenotypic effects, which will be applicable to target species such as humans or domestic ruminants.

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