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8 **Reproductive genomics of the mouse:
implications for human fertility and infertility**

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30 **Key Words:** **fertility**, gametogenesis, genetics, genomics, mutagenesis, quantitative trait, infertility

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34 **Summary Statement:** This Primer of the reproductive genomics of the mouse covers single-gene and
quantitative fertility traits affecting gametogenesis and gamete function, and discusses new genetic
resources and their translational applications.

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44 Abstract

46 Genetic analyses of mammalian gametogenesis and fertility have the potential to inform about two
48 important and interrelated clinical areas: infertility and contraception. Here, we address the genetics
50 and genomics underlying gamete formation, productivity, and function in the context of reproductive
52 success in mammalian systems, primarily mouse and human. While much is known about the specific
54 genes and proteins required for meiotic processes and sperm function, we know relatively little about
56 other gametic determinants of overall fertility, such as regulation of gamete numbers, duration of
58 gamete production, and gamete selection and function in fertilization. Because fertility is not a binary
60 trait, attention is now appropriately focused on the oligogenic, quantitative aspects of reproduction.
62 Multiparent mouse populations, created by complex crossing strategies, exhibit genetic diversity
64 similar to human populations, and will be valuable resources for genetic discovery, helping to
66 overcome current limitations to our knowledge of mammalian reproductive genetics. Finally, we
68 discuss how what we know about the genomics of reproduction can ultimately be brought to the clinic,
70 informing our concepts of human fertility and infertility and improving assisted reproductive
72 technologies.

Box: Advocating developmental biology

62 This article is part of Development's Advocacy collection – a series of review articles that make
64 compelling arguments for the field's importance. The series is split into two: one set of articles
66 addresses the question 'What has developmental biology ever done for us?' We want to illustrate how
68 discoveries in developmental biology have had a wider scientific and societal impact, and thus both
70 celebrate our field's history and argue for its continuing place as a core biological discipline. In a
72 complementary set of articles, we asked authors to explore 'What are the big open questions in the
field?' Together, the articles will provide a collection of case studies that look back on the field's
achievements and forwards to its potential, a resource for students, educators, advocates and
researchers alike. To see the full collection as it grows, go to:
<https://journals.biologists.com/dev/collection/59/Advocacy>.

74 Introduction

76 Gametogenesis and gamete function, which together encompass the developmental processes of
oogenesis and spermatogenesis, and gamete interactions in fertilization, are major determinants of
overall fertility and frequently impaired in cases of infertility or marginal fertility. For these reasons,
78 these processes have been the predominant focus of genetic studies of mammalian reproduction. In
mammals, the process of spermatogenesis is relatively accessible for experimental manipulation
80 because many of the events occur in post-natal and adult individuals (Griswold, 2016). In contrast,
important stages of mammalian oogenesis are restricted to fetal development (Bolcun-Filas and
82 Handel, 2018). Nonetheless, the defining events of gametogenesis are conserved in males and females,
albeit with sexual dimorphism in timing and regulation (**Fig. 1**). The key processes of gametogenesis
84 common to the sexes include the mitotic proliferation and specification of spermatogonia or oogonia
(Matzuk and Lamb, 2008), the intricate chromatin dynamics of meiotic prophase and the two meiotic
86 divisions (Bolcun-Filas and Handel, 2018), cytological differentiation of the germ cells, and specification
of oocyte and sperm structures integral to the gamete recognition processes of fertilization (Lu and
88 Ikawa, 2022) (**Fig. 1**). However, there is marked sexual dimorphism in the products of the meiotic
division, with four haploid spermatids in the case of the male, but unequal divisions in the female to
90 produce one haploid oocyte and small polar bodies (**Fig. 1**). Although not covered in detail in this
review, throughout gametogenesis there is cross communication between germ cells and their
92 surrounding somatic cells – the granulosa cells of the ovarian follicle (Su et al., 2009) and the Sertoli
cells of the testis seminiferous epithelium (Griswold, 2018). Thus genes that regulate gametogenesis
94 and gamete function could be expressed in either or both germ cells and gonadal somatic cells.

The past 2-3 decades of ‘infertility gene’ discoveries have provided an impressive parts list for
96 these aspects of gamete production and reproductive success. Nonetheless, neither fertility nor
gamete production are true binary traits and we frequently observe gradations of fertility (sub-fertility,
98 age-related decline in fertility, etc.) in both humans and model organisms, such as the mouse.
Moreover, impairment of fertility can occur at any of several levels of function, such as gametogenesis
100 (i.e. the ability to produce gametes at all), gamete morphology and function (e.g. sperm motility), and
fertilization (i.e. successful interaction between gametes). Here we provide an overview of methods
102 and progress toward unraveling genetic control of these processes. Although epigenetic effects no

doubt play roles in gametogenesis, they are beyond the scope of this article, but reviewed elsewhere (Ben Maamar et al., 2021). We conclude that our current gene discovery paradigms, which have mostly focused on simple Mendelian inheritance (Schimenti and Handel, 2018), have provided important but limited information relevant to understanding gametogenesis and gamete function as complex traits. Mouse genetic diversity models can lead to better understanding of how individual genes, each with perhaps small effects, interact and work together for reproductive success, providing a more holistic view of the genomics of reproduction. Finally, we discuss how the genetic and genomic insights into gametogenesis and gamete function derived from the laboratory mouse can help us understand human infertilities and contribute to assisted reproductive technologies (ARTs), such as *in vitro* fertilization (IVF).

The low-hanging fruit: single-gene reproductive phenotypes

At the outset, it is helpful to consider how we have discovered single-gene Mendelian reproductive phenotypes. Many discoveries can be traced back to sharp eyes and diligence in the mouse room to detect spontaneous mutations in genes affecting observable traits (**Fig. 2**). Interesting early examples include research on coat-color traits, many of which were pleiotropic (where two or more seemingly unrelated phenotypes arise from a single locus). Because a gene affecting stem cell proliferation can affect both pigment cells contributing to coat color and germline stem cells, studying coat color led to unexpected insights on the regulation of spermatogonial proliferation. These traits included the dominant White spotting and coat-color dilution traits that we now know are caused by mutant alleles of the *Kit* gene and *Kitl* genes, encoding the KIT tyrosine kinase receptor and its ligand, KITL (Sette et al., 2000). Genes, such as *Kit* and *Kitl*, with many mutant alleles (forming allelic series) affecting different regions of the protein allow the analysis of specific protein domains, important for dissecting protein function (Sette et al., 2000). Although many of the initially discovered Mendelian reproductive phenotypes (Handel, 1987) were not pursued because they were difficult to map, they are now more tractable through whole-exome sequencing and similar techniques (Fairfield et al., 2015), stimulating renewed interest and extending to the diagnosis of human reproductive phenotypes (Ghieh et al., 2022).

Although these serendipitously discovered reproductive phenotypes have been informative, deliberate mutagenesis has been a more direct and productive approach. The biased ‘reverse’ genetics approach of mutagenesis targeted to specific (known) genes (**Fig. 2**) has been greatly enhanced by the relative ease of CRISPR editing (reviewed in Gurumurthy and Lloyd, 2019; Singh and Schimenti, 2015). The unbiased ‘forward’ genetics strategy of chemical mutagenesis (**Fig. 2**), most frequently with ENU (N-ethyl-N-nitrosourea), includes screening for specific reproductive phenotypes with follow-up to identify the mutated causative genes (Schimenti and Handel, 2018). Productive insights into the genetic regulation of gametogenesis have been reaped from both approaches.

Insights from targeted mutagenesis

With the advent of transcriptome sequencing and efficient gene editing technologies, deliberate targeting of genes known to be uniquely and/or highly expressed in gonads has been especially informative. Targeted mutation of such genes has identified new gene functions essential for reproductive success, for example, genes encoding factors that function in sperm travel through the female reproductive tract (Larasati et al., 2020) and in sperm-oocyte interaction (Noda et al., 2020). By focusing on single proteins highly or uniquely expressed in the reproductive tract, such studies provide new knowledge and show great promise in identifying potential contraceptive targets (Fujihara et al., 2019; Lu and Ikawa, 2022; Robertson et al., 2020).

These biased approaches of targeting known genes in known reproductive pathways have also provided information about what may not be effective contraceptive targets. For example, some evolutionarily conserved genes that are expressed in the reproductive tract have been demonstrated by mutagenesis analysis to be nonessential for reproductive success (Miyata et al., 2016). Moreover, some human mutations that are predicted to be deleterious have not resulted in infertility phenotypes when modeled in the mouse (Singh and Schimenti, 2015). There are several possible explanations for such genetic mutations that affect fertility in humans but not in mice (or vice versa). The phenotypic differences might be due to species specific differences in relevant reproductive processes (e.g., So et al., 2022). Also such findings could suggest inadequacy of predictive algorithms for estimating the conservation of protein function (Ding and Schimenti, 2021). Finally, species differences in genetic complexity (e.g., gene regulatory networks) might be resolved with better models (discussed below).

Despite these puzzling findings, biased and targeted mutagenesis strategies can now be made even more comprehensive and fruitful with longer gene lists emerging from high-throughput and computational methods for analyzing transcriptomes of reproductive tissues at a single-cell level (Robertson et al., 2020).

New gene discovery through unbiased chemical mutagenesis

Unlike gene targeting, chemical mutagenesis is unbiased (no a priori knowledge of which genes will be mutated) and thus phenotype-driven (**Fig. 2**). Since the early studies of Bill and Lee Russell at the Oak Ridge National Laboratory, ENU has been a mutagen of choice for mice (Justice et al., 1999, **Fig. 2**). Several successful ENU mutagenesis programs in the late 1990s and early 2000s identified reproductive phenotypes and the underlying causal genes (Kennedy et al., 2005; Schimenti and Handel, 2018; Weiss et al., 2012). Interestingly, most of the infertility phenotypes discovered in these screens affected males but not females. Moreover, as commonly is the case with new discoveries, the identification of causal genes frequently led to new questions and avenues to explore.

One illustrative example was the '*repro8*' mutation, which produced a phenotype of arrest of spermatogenesis at the end of the meiotic phase, similar to human 'maturation arrest' syndromes. Positional cloning identified the causative gene mutation as a single base-pair change in *Eif4g3*, encoding a ubiquitously expressed protein translation initiation factor (Sun et al., 2010). Why mutation of this ubiquitous protein should seemingly impact only male reproduction is not clear. Even more curiously, given that protein translation generally occurs in the cytoplasm, the EIF4G3 protein is expressed in the nuclei of spermatocytes, localized to a specialized heterochromatin domain, the XY body (Hu et al., 2018; Sun et al., 2010).

Another puzzling example was a single-base-change mutation affecting a splice site in the *Brwd1* gene, encoding a dual bromo-domain protein thought to epigenetically regulate transcription. The *Brwd1* splicing mutation caused both male and female infertility, but the arrest points in female and male gametogenesis were quite different (Philipps et al., 2008). Follow-up studies demonstrated pleiotropic roles for BRWD1 in oocyte chromosome stability and the oocyte-embryo transition, as well as regulation of post-meiotic spermiogenic transcription in male gametogenesis (Pattabiraman et al., 2015; Philipps et al., 2008). Clearly, both the phenotypes and the genes discovered in these and other

examples of unbiased reproductive gene discovery revealed new and unexpected gene regulatory interactions crucial to successful gametogenesis.

Deleterious gene-gene interactions: the oligogenic basis of infertility

Epistatic mutations affecting gametogenesis

Although we have learned much from single-gene causes of infertility, interactions among alleles at two or more loci can also result in deleterious fertility and sub-fertility phenotypes. Such allelic interactions can be informative about human male infertility or subfertility phenotypes. For example, multiple malformations of the flagellum (MMAF) syndromes (Martinez et al., 2022) encompass multiple abnormal phenotypes of sperm flagella morphology. Four genes (*Cfap43*, *Cfap44*, *Armc2* and *Ccdc146*) were demonstrated to contribute to MMAF by genetic knockout in mice, and these four mouse models were used to produce multiple different compound heterozygotes. The results indicated allelic interactions, with increased deterioration of sperm morphology and motility produced by increasing numbers of heterozygous mutations among the four genes (Martinez et al., 2022). These results strongly suggested that oligogenic determination of these complex gametic morphological phenotypes can contribute to overall fertility. Although mechanisms are not yet understood, these findings implicate the importance of proteins potentially acting at different cellular levels and/or in multiprotein complexes.

In many organisms, including the mouse, such negative epistatic interactions are particularly apparent in the context of hybridization between divergent taxa. During the independent evolutionary trajectories of isolated populations or subspecies, distinct combinations of fitness-associated variants can emerge and become common in each population. Hybridization between isolated populations or subspecies provides the opportunity for such diverged alleles to be brought together in a single genome (**Fig. 2**); in such cases these new allelic combinations may manifest as multi-locus genetic incompatibilities that reduce fertility parameters compared to either parent population. These so-called Dobzhansky-Muller incompatibilities (DMIs) are theorized to play a major contributing role to the genesis of new species. One example of a DMI is seen in hybrids between two closely related house mouse subspecies, *M. m. domesticus* and *M. m. musculus*. Male hybrids sired by *M. m. musculus* females and *M. m. musculus* males are typically sterile or subfertile. Gene mapping led to the

218 identification of one of the genetic partners in this interaction: the gene encoding a histone
methyltransferase that defines the positions of genomic recombination events through DNA sequence-
220 specific binding guided by its zinc-finger domain, *Prdm9* (Grey et al., 2018; Mihola et al., 2009; Paigen
and Petkov, 2018). Genetic analysis of the sub-fertile offspring also implicated interaction of *Prdm9*
222 with a still unknown X-linked locus, *Hstx2* (Forejt et al., 2021). It is hypothesized that females are
safeguarded from infertility in this system due to the heterozygous, diploid state of the chr X locus, in
224 contrast to the hemizygous *M. m. musculus* derived X chromosome in *M. m. musculus* x *M. m.*
domesticus male hybrids.

226 Although considerable research attention has focused on unraveling the complex molecular
mechanisms by which *Prdm9* disrupts fertility in intersubspecific house mouse hybrids (Baker et al.,
228 2014; Brick et al., 2012; Imai et al., 2020; Powers et al., 2016), the extent to which this model may be
informative for mechanisms of human infertility is unclear. PRDM9-associated infertility is background
230 and species-dependent; some mammalian genomes lack the *Prdm9* gene (Mihola et al., 2019; Powers
et al., 2020) and there are reports of a fertile woman with mutant *PRDM9* (Narasimhan et al., 2016).
232 Further, the scale of divergence between house mouse subspecies vastly exceeds that between
evolutionarily young human populations. DMIs causing sterility are not likely segregating between
234 populations, but negative interactions among alleles may well contribute to the genetic landscape of
human infertility (Cutter, 2012; Martinez et al., 2022).

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Epistatic mutations affecting fertilization

238 Fertilization provides a second milieu for the manifestation of protein interactions associated with
infertility. However, unlike canonical cases of negative epistasis, the process of fertilization involves
240 interactions between proteins produced by two independent genomes – that of the sperm and oocyte
– rather than a single genome. For example, IZUMO1 is expressed on the mouse sperm membrane
242 surface, where it must interact with a cognate receptor on the oocyte, JUNO, to enable sperm-egg
adhesion, one of the first crucial steps in fertilization (Bianchi et al., 2014; Wassarman, 2014). Similarly,
244 zonadhesion (ZAN) is expressed on the surface of the sperm acrosome, where it must interact with an
unknown partner expressed in the female to achieve species-specific binding to the zona pellucida (Lea
246 et al., 2001). Intriguingly, many of the proteins expressed on the sperm and oocyte surfaces are rapidly

evolving and highly polymorphic (Gasper and Swanson, 2006; Swanson and Vacquier, 2002; Swanson et al., 2001; Turner and Hoekstra, 2008). While the rapid accumulation of new variants may serve to prevent fertilization between species, such variants may also create gamete incompatibilities among individuals (Springate and Frasier, 2017). Such incompatible sperm-egg receptor-ligand pairs may account for instances of unexplained fertilization failure in IVF cycles and partner-dependent infertility in humans.

Quantitative traits, multiparent populations and surveys of diversity

While conceptually simple and often experimentally convenient to categorize fertility as a binary trait (i.e. an individual is fertile or infertile), this scheme artificially dichotomizes trait variations that lie along a continuum. Many cases of infertility may be traced to mutations in one or a few genes, but fertility is itself a complex trait influenced by variants in numerous coordinately regulated and interacting genes. Indeed, approximately 90% of all protein-coding genes are expressed in the mouse testis (Schultz et al., 2003), and more than 25% of all protein-coding genes are expressed in the mouse ovary (Wang et al., 2020), underscoring the complexity of the resultant biological networks required for spermatogenesis and oogenesis, respectively. Moreover, variants in genes affecting fertility can have strong fitness effects and are often under strong selection (Gardner et al., 2022). An important consideration in this context is that most of the successful gene-discovery studies cited above have been based on investigations of inbred strains of mice exhibiting homozygosity at most genetic loci. Inbred mice are experimentally advantageous, allowing repetition of observations over periods of time; however, they do not model the genetic diversity of human populations. Below, we highlight insights into the quantitative aspects of gamete production and fertility that have emerged from studies of genetically diverse mouse populations. These include not only the many different and unique inbred strains (**Fig. 2**), but also the combinations that are created by interbreeding among inbred strains to form so-called “multiparent” populations, derived from complex breeding schemes that have input of several different genetic backgrounds (**Figs. 2 and 3**). Such mouse populations more accurately model the genetic complexity apparent among human individuals.

Recombinant-inbred strains

276 Recombinant inbred (RI) strains capture high levels of genetic diversity while maintaining the
experimental advantages of inbred strains (**Fig 2**). They are generated by intercrossing fully inbred
278 strains, followed by at least 20 generations of subsequent inbreeding, creating homozygous and
reproducible sets of genetically distinct but closely related strains (Peirce et al., 2004). Because
280 infertility is complex with multiple etiologies, crossing strains with different evolutionary backgrounds
can yield new multi-locus gene combinations and enable the discovery of variants associated with
282 reproductive capacity (Flurkey et al., 2007; Langhammer et al., 2014; Schwahn et al., 2018). To date,
recombinant-inbred lines in model species have been used to study sperm quality and morphology
284 (Golas et al., 2003; Golas et al., 2010; Krzanowska et al., 1995; Shukri et al., 1988) and oocyte
maturation (Polański, 1997), but their full potential for reproductive genomics discovery remains
286 unrealized.

The Collaborative Cross (CC) mouse population is a panel of RI strains derived from eight
288 founder strains, including three wild-derived strains (Chesler et al., 2008; Churchill et al., 2004;
Churchill et al., 2012) (**Fig. 2 and 3**). These strains represent three subspecies and capture nearly 90%
290 of the total genetic variation and diversity observed in *Mus musculus* (Roberts et al., 2007; Saul et al.,
2019; Yang et al., 2011). The CC strains are valuable to reproductive biologists because there is
292 considerable variation in reproductive traits across the founder strains (Odet et al., 2015) and, due to
the recombination of alleles from distinct strain backgrounds, these lines can display variation and
294 traits not found in the parental inbred strains via recombination of founder alleles into new, multi-
locus genotypes (Chesler, 2014; Wahlsten et al., 2003). Unexpectedly, nearly 95% of CC lines became
296 extinct during the inbreeding process (**Fig. 3**). This is markedly higher than other less diverse RI
populations (Shorter et al., 2017) and opens avenues for identifying gene variants and incompatibilities
298 that affect gametogenic success. For example, multiple variants influencing reproductive success and
sperm quality have been successfully detected in these strains (Philip et al., 2011; Shorter et al., 2017;
300 Shorter et al., 2019). Continued maintenance of the CC strains (e.g. at the Jackson Laboratory) provides
further opportunity to identify variability in reproductive success and subfertility phenotypes.
302 Historical high-resolution breeding data allow for the determination of strain-level reproductive
metrics that are relevant to normal and clinical human reproductive histories, including lifetime fertility
304 and reproductive aging, variability in litter sizes, inter-litter intervals, and relationships between

reproductive success and behavioral, molecular and morphological traits. For example, a subset of these data was used to document pervasive sex-ratio distortion in the CC lines, a phenomenon intricately associated with infertility across species (Haines et al., 2021).

Outbred mouse models of fertility

In contrast to the reduced infertility of some inbred strains, outbred individuals have the potential to reveal genetic variants at the productive end of the fertility spectrum. Mice from several outbred populations are commercially available and have been widely employed in experimental studies (Chia et al., 2005), including investigations of environmental impacts on mammalian fertility (e.g. Cabaton et al., 2011; Hannon et al., 2015; Niermann et al., 2015). However, few studies have profiled the genetic determinants of fertility in outbred mammalian systems, in large part due to the complexities of their unidentified genetic background. Among currently available outbred mouse populations, the most diverse and genetically well-defined is the Diversity Outbred (DO) population (**Fig. 3**). DO mice were derived from early generation CC strains and have been maintained by pseudo-random intercrossing through generations to produce outbred individuals (Churchill et al., 2012; Svenson et al., 2012). Unlike RI strains, the DO mating approaches avoid mating closely related individuals to produce outbred, highly recombinant populations that more closely model human diversity (Chesler, 2014). Each DO individual is unique and amenable to high-resolution genotype analysis using dense, commercially available SNP arrays (Morgan et al., 2015). The high genetic diversity of DO individuals, and a mosaic of heterozygous and homozygous alleles across individuals, allow for high-powered, extremely precise mapping of complex traits as well as assessing the influence of allele states. Furthermore, whereas inbreeding funnels reduce the influence of selection on deleterious alleles, outbreeding can stabilize and buffer deleterious alleles, offering alternative insight into multigenic features of fertility.

Due to hybrid vigor, DO mice have high fertility, with average first litter sizes between 7 and 9 pups (Churchill et al., 2012). The high fertility of outbred mouse populations (Langhammer et al., 2014) is in marked contrast to the prevalence of sub- or infertile phenotypes obtained by the more traditional approaches discussed above and in reviews of mutations affecting humans (Jamsai and O'Bryan, 2011; Matzuk and Lamb, 2008). Therefore, examining high-fertility models, such as the DO, has the potential to provide unique insights into the complex dynamics of gametogenesis and provide

334 targets for improving or extending human reproductive lifespans or treating infertility. For example,
the DO mice were used to map genetic determinants of testis weight, a trait with a significant genetic
336 component that substantially affects reproductive success (Le Roy et al., 2001; Yuan et al., 2018),
leading to the discovery of five high-probability candidate genes, all with human orthologs. The success
338 of this study demonstrates the potential utility of the DO for investigating other fertility-related
phenotypes to identify causal genes and gene networks.

340 **What have we learned and how can we apply it?**

342 Both studying single-gene effects on gamete production and function as well as evaluating the
quantitative aspects of fertility in diversity populations are valuable for unraveling genetic control of
344 these processes and show promise of translation to the biology of human fertility and infertility and
beyond. Indeed, single-gene analyses have given us an impressive list of gametogenesis genes and
346 potential clinical targets. However, it is also clear that many genes, some probably with small effects,
cooperate in complex and overlapping pathways and the totality of this ‘reproductive interactome’ is
348 not yet fully appreciated. But the successes have been significant, and we address below several of the
most translationally relevant areas of these endeavors.

350 ***Potential contraceptive targets and important mechanisms have been identified***

352 The search for gametogenesis genes is founded on curiosity about basic biology but also the
need for contraceptive targets. Both goals are being realized more or less simultaneously. One early
354 example is the mouse protein BRDT, a tissue-restricted protein found in developing spermatocytes and
spermatids and demonstrated by gene targeting to be essential for chromatin remodeling. Pharmaco-
356 structural analyses led to the development of a small-molecule inhibitor that had effective
contraceptive action in mice (Matzuk et al., 2012), providing proof of concept. As detailed above, this
358 approach of strategic targeting of genes known to be expressed in the reproductive tract has enriched
our knowledge of the development of sperm structures and their roles in fertilization. For example,
360 one study identified multiple genes encoding proteins that regulate sperm travel through the oviduct,
a process ripe for contraceptive targeting (Fujihara et al., 2019). Newly validated sperm tail proteins
362 expressed post-meiotically and in epididymal sperm in both mouse and human (Touré et al., 2021)

might be particularly amenable to contraceptive targeting, particularly as new methods for rapid
screening of druggability of protein variants become more common (Modukuri et al., 2022).

Mechanisms of gametogenic pathways are being elucidated by gene mutations even when
contraceptive targets are not revealed. In this context, meiosis is not only the defining event of
gametogenesis but its sequential steps form the most thoroughly understood process of mammalian
gamete differentiation (Li et al., 2020). Meiotic prophase of male germ cells, which is accessible for
experimental analysis in the adult, is particularly well understood, but informative sex-based
differences are emerging (Bolcun-Filas and Handel, 2018). Importantly, advances clarifying mechanisms
of meiotic recombination have relevance not only for understanding gametogenesis, but also have far-
reaching implications for mechanisms of DNA repair and basic aspects of chromatin biology in somatic
cells (Alavattam et al., 2021).

Events surrounding fertilization are being revealed

The requirements for the various events immediately surrounding mammalian fertilization have been
historically difficult to determine, which is crucial gap because millions of couples turn to IVF for a
variety of reasons (an estimated 1% of all births being from offspring conceived by IVF). Related ARTs
include intracytoplasmic sperm injection (ICSI), procedures for injection of immature sperm cells, and
in vitro recombination of maternal and paternal, and mitochondrial, genomes. These procedures are
important not only for mitigating infertility, but for maintenance and distribution of genetic resources
(e.g. inbred mouse strains, mutation recovery, etc.), technologies that stand to benefit from what can
be learned about genetic variability and mechanisms underpinning the fertilization process. In
fertilization following natural mating, key events include epididymal maturation of sperm, sperm
capacitation and transport through the female reproductive system, sperm penetration of the cumulus
complex, sperm-zona and sperm-egg recognition, sperm-egg fusion, and transport of the zygote to the
uterus. As complex as this process is, ARTs involving fertilization *in vitro* are arguably even more
fraught, because the developmental stage of donor sperm may not be well-defined and the natural
barriers that normally act to select ‘good’ sperm are absent. Targeted gene approaches have been
helpful in addressing some of the open questions. As discussed above, we now know that IZUMO
(Inoue et al., 2005) and TMEM95 (Lamas-Toranzo et al., 2020; Noda et al., 2020) are essential sperm

392 proteins. In addition, JUNO (Bianchi et al., 2014; Jean et al., 2019) is an essential egg protein in mice
and humans for sperm-egg fusion, while ZAN (Tardif et al., 2010) is involved in the species-specificity of
394 sperm-zona recognition. This knowledge might possibly lead to contraceptive approaches and could
provide diagnostics for genetic incompatibilities, thereby ultimately improving application of various
396 ARTs. However, evidence that there may be selection among interacting gametes *in vivo* (Nadeau,
2017) suggests that there is far more yet to learn. Finally, both natural fertilization and IVF require
398 robust production of oocytes in response to hormonal stimuli, modifications to the sperm surface
during their transport through the male and female reproductive tracts, and acquisition of competence
400 to support normal development. Because inbred strains of mice differ significantly in numbers of
ovulated oocytes and overall IVF success, multiparent populations are proving to be facile resources to
402 address issues such as super ovulatory responses, IVF success, and zygotic development.

404 ***Proving causality of human reproductive gene variants is difficult***

These successes have not immediately translated to better understanding of human reproductive
406 genetics, where, because we cannot apply mutagenesis strategies, we must rely on discovery of
infertility variants in the clinic. Given that such genetic variants cause infertility, they are rare in the
408 population and require large case-control populations for statistical validation, although more and
more information is coming from whole-exome and whole-genome sequencing (Houston et al., 2021).
410 The strategies mentioned above have led to an ever-expanding list of human gene variants associated
with but not yet proven to be involved in the genetic regulation of gamete production and function,
412 particularly in cases of male infertility (Houston et al., 2021). For example, there is now detailed
knowledge of the ultrastructure and list of proteins involved in the assembly of the human sperm tail,
414 with examples of mutant phenotypes (Touré et al., 2021). Nonetheless, for most putative human
infertility variants, the challenge of validating causality remains (Ding and Schimenti, 2021). As
416 mentioned, CRISPR modeling in the laboratory mouse of some human variants predicted to be
deleterious has, in some cases, yielded no evidence for infertility (Tran and Schimenti, 2019; Tran et al.,
418 2019). Even when there is some correspondence between human and mouse phenotypes for a gene,
evidence can suggest species-specific differences in function (Hu et al., 2019; So et al., 2022). Thus, the
420 ability to predict phenotypes or explain causality by cross-species comparisons is imperfect, and

caution is required for clinical inferences.

Future perspectives

Despite the remarkable advances and insights into mechanisms of gamete production and function, there are still many aspects of gametogenesis that are poorly understood. For example, the number of oocytes and follicles present in the ovary at birth and the continued maintenance of that ovarian reserve are crucial for female reproductive life span, and are fundamental issues in the face of cell-damaging treatments for cancer, particularly pediatric cancers, because the ovarian reserve maintains ovarian function and the hormonal environment crucial for health and wellbeing (Woodard and Bolcun-Filas, 2016). As the age at reproduction continues to advance to later in life in societies across the globe, understanding the sources of ovarian aging will empower women to make informed decisions about their reproductive health and optimize strategies for family planning. On the male side of this issue, genetic determinants of sperm number and reproductive life span are largely not known, but work treating fertility as a quantitative trait may be a promising route to candidate regulators (Hsu et al., 2010). The adoption of multiparent populations for the study of such quantitative aspects of fertility is still in its infancy. However, early work is quite promising and suggests that resources such as the DO and CC harbor significant segregating, heritable variation influencing reproductive performance (Morgan and Welsh, 2015; Saul et al., 2019; Threadgill and Churchill, 2012). Multiparent populations can be used to estimate genetic effects (heritability), gene-by-environment interactions, correlations between reproductive traits, and will enable the discovery of novel candidates through the high statistical power and high mapping precision.

We know that gametogenesis and fertility are significantly influenced by environmental conditions, contaminants and exposures (Hruska et al., 2000; Ma et al., 2019; Woodruff et al., 2010) in both males (Mima et al., 2018; Skakkebaek et al., 2016; Wong and Cheng, 2011) and females (Mendola et al., 2008). Genetics likely mediates many of these responses through gene-by-environment interactions, and inbred mouse diversity panels, including RI strains, can reveal gene effects under various environmental conditions (Williams et al., 2001).

Gaining recent attention, considerable epidemiological evidence suggests that in humans, impaired fertility can be associated with later-in-life detrimental health consequences or co-

450 morbidities, including cancer (Cedars et al., 2017; Chen et al., 2022; Choy and Eisenberg, 2018). Thus,
fertility status could be an early and actionable biomarker to mitigate the risk of future adverse health.
452 However, human findings on fertility status and overall health have been confounded by high genetic
diversity in populations, which poses a crucial limitation to translating these emerging relationships
454 into clinical prognostics.

The future is exciting, in spite of the challenges remaining in the study of the genetics of
456 mammalian gametogenesis. With facile and rapid CRISPR editing technologies and precise populations
of mice incorporating genetic diversity equivalent to that of humans, the tools and resources are
458 available to uncover the molecules, mechanisms and ‘reproductive interactome’ that determine
gamete production and function. Rapid testing using *ex vivo* and *in vitro* cellular systems, as well as *in*
460 *vitro* derivation of gamete-like cells and germ-cell transplantation techniques, will facilitate bringing
the new knowledge to the clinic for ARTs and to couples for fertility preservation and as
462 contraceptives.

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476 Figure Legends

478 **Figure 1. Schematic of female (left panel) and male (right panel) gametogenesis and fertilization**
480 **(bottom panel).** Illustrated are the post-mitotic events of gametogenesis, beginning with the pre-

meiotic oocyte and spermatocyte, each shown with two pairs of chromosomes, blue and orange (for simplicity, the obligatory recombination events on each chromosome are not illustrated here). The reductional division of Meiosis I is followed by the equational division of Meiosis II, with sexual dimorphism in both timing and products. Fertilization involves species-specific recognition of the zona pellucida followed by sperm-egg fusion, which is temporally associated with completion of the Meiosis II division of the oocyte.

Figure 2. Genetic strategies in mouse fertility research. The **left panel** illustrates the single-gene strategies discussed in the main text. These include exploiting spontaneous mutant phenotypes (**a.**, where the black extremities represent a visible morphological phenotype); targeted mutagenesis strategies, including CRISPR editing (**b**) (middle); and unbiased random ENU mutagenesis (**c**) (bottom). The **right panel** illustrates strategies that take advantage of genetically diverse strains of mice to detect genes controlling reproductive parameters. As discussed in main text, the standard inbred strains (**a**) exhibit considerable diversity in quantitative parameters of gamete production and function. These strains can be interbred to form F1 hybrids (**b**), which often can identify dominant traits. Further intercrossing of hybrids (**c**) leads to bi-parental recombinant inbred strains (left), and the multiparent strains with representation of several input genomes (right), e.g., the Collaborative Cross inbred strains illustrated in more detail in **Fig. 3**.

Figure 3. Schematic of production of genetically diverse Collaborative Cross (CC) and Diversity Outbred (DO) strains. (Top) Homozygous genotypes of the eight progenitor strains are with standardized color codes for each strain in the upper left. All combinations of mating are performed, each one leading to an inbreeding ‘funnel’ (middle) to produce inbred CC strains (bottom). During the inbreeding process, roughly 95% of CC strains became extinct (see main text for details). The horizontal bands on the chromosomes represent regions from the founder strains that are in new combinations due to recombination events during the breeding process. CC strains have been further subjected to randomized outbreeding, continued over generations, which is producing the DO individuals (right) that have many more new gene and allele combinations and thus model human individuals in the extent of genetic diversity.

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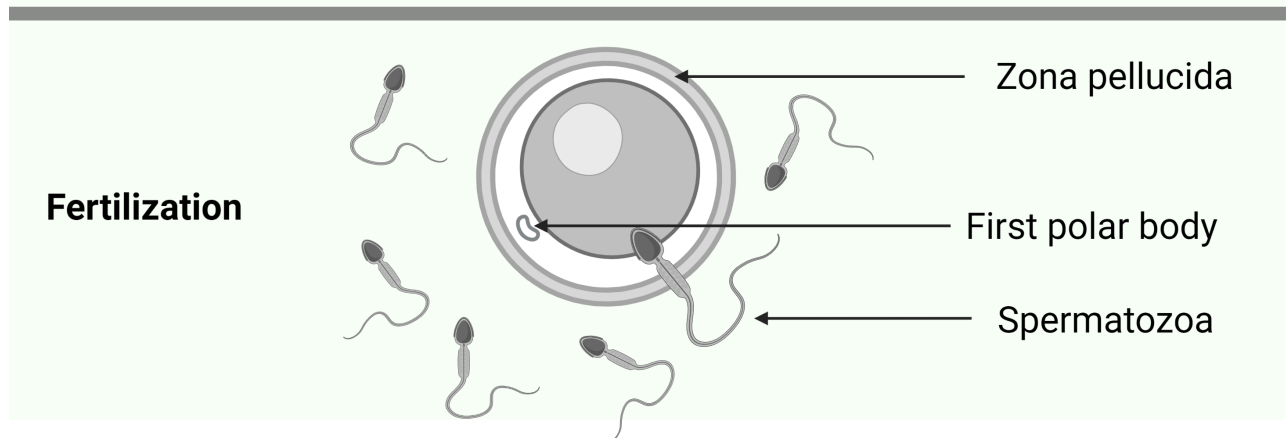
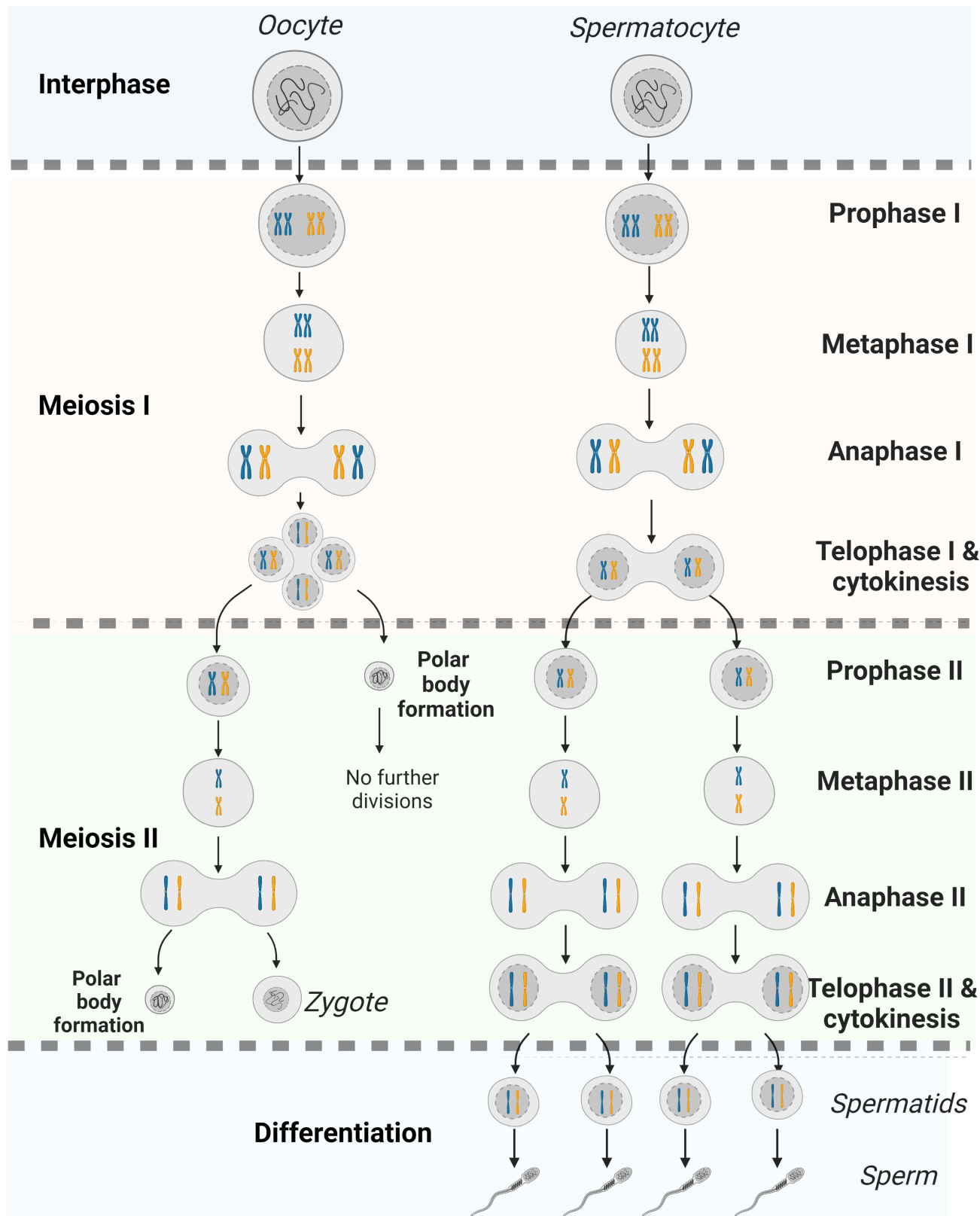
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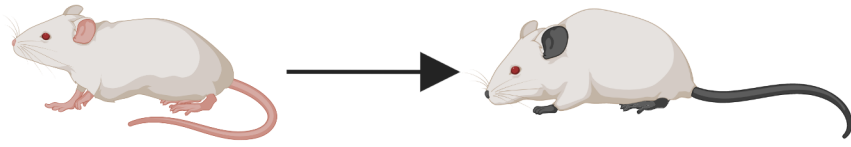
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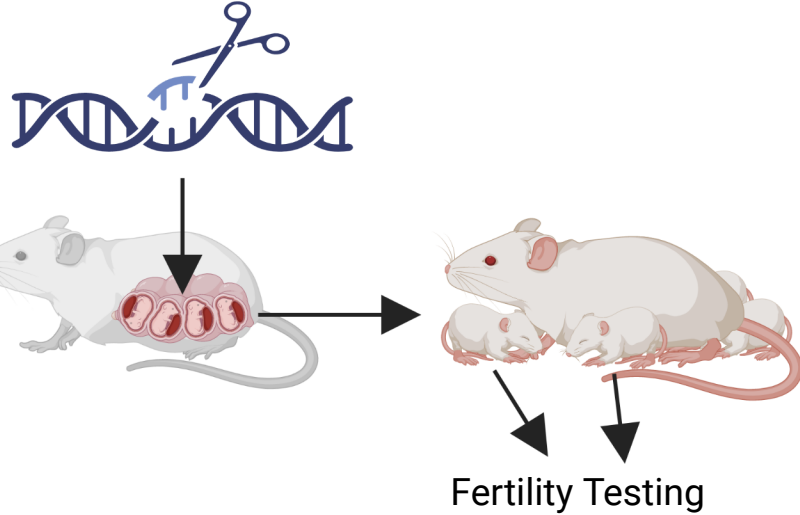


Single-Gene Strategies

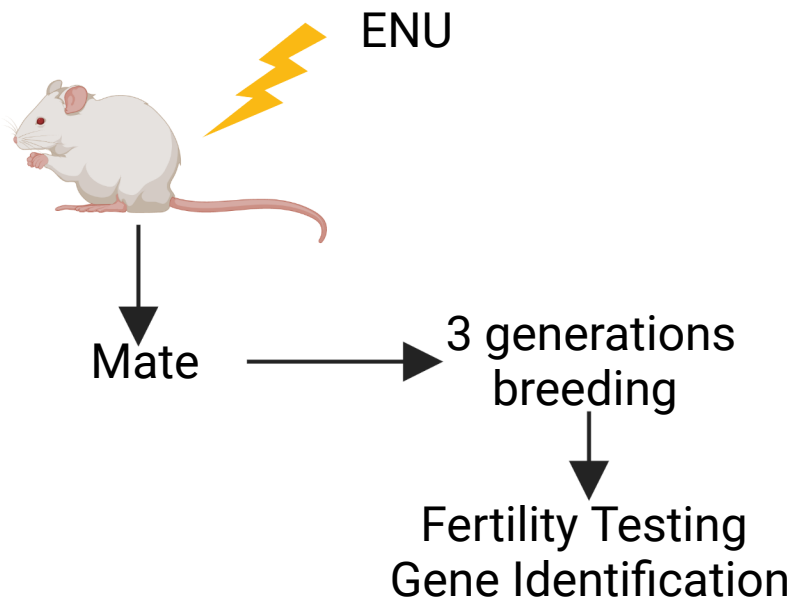
a. Spontaneous Mutants



b. Targeted Mutagenesis

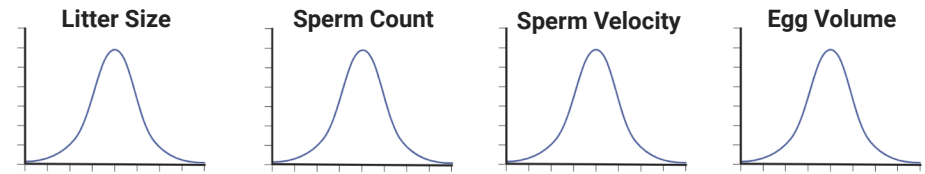


c. Random Mutagenesis

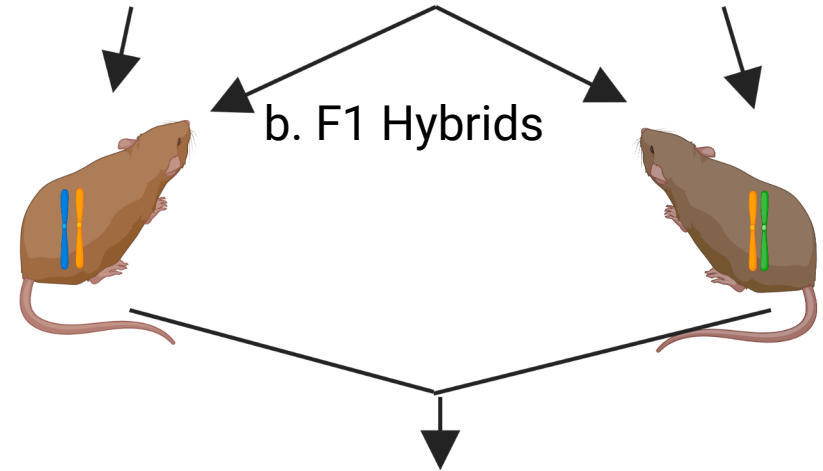


Multigene Strategies

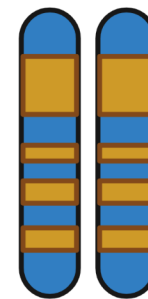
a. Diverse Inbred Strains



b. F1 Hybrids



c. Recombinant Inbred Strains



Biparental
(ex. BxD)



Multiparent
(ex. CC)

