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Genetics of human female infertility†

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Abstract

About 10% of women of reproductive age are unable to conceive or carry a pregnancy to term. Female factors alone account for at least 35% of all infertility cases and comprise a wide range of causes affecting ovarian development, maturation of oocytes, and fertilization competence, as well as the potential of a fertilized egg for preimplantation development, implantation, and fetal growth. Genetic abnormalities leading to infertility in females comprise large chromosome abnormalities, submicroscopic chromosome deletion and duplications, and DNA sequence variations in the genes that control numerous biological processes implicated in oogenesis, maintenance of ovarian reserve, hormonal signaling, and anatomical and functional development of female reproductive organs. Despite the great number of genes implicated in reproductive physiology by the study of animal models, only a subset of these genes is associated with human infertility. In this review, we mainly focus on genetic alterations identified in humans and summarize recent knowledge on the molecular pathways of oocyte development and maturation, the crucial role of maternal-effect factors during embryogenesis, and genetic conditions associated with ovarian dysgenesis, primary ovarian insufficiency, early embryonic lethality, and infertility.

Key words: female infertility, follicular development, genetics, oocyte development, preimplantation embryo, premature ovarian failure, X chromosome.

Overview

Infertility is a disease of the reproductive system, encompasses a wide spectrum of conditions that affect the capacity of an individual to reproduce. A diagnosis of female infertility, defined as the inability to establish a clinical pregnancy after 12 months of regular unprotected sexual intercourse with a healthy partner, can also lead to anxiety and depression. Genetic, endocrine, physiological, anatomical, and immunological abnormalities of the reproductive system can affect a woman's likelihood of becoming pregnant and delivering a living child.

The high prevalence of reproductive disorders and infertility, affecting ~10–15% of couples worldwide, is perhaps not surprising as successful reproduction requires the precise regulation of complex processes essential for development of functional gonads and other reproductive organs, sex determination, gametogenesis, neuroendocrine competency, and ability to carry a pregnancy. Oogenesis is a process by which the mammalian egg becomes competent for fertilization and involves complex interaction between the oocyte and somatic cells that surround it, including the interplay of multiple transcriptional regulators. The disruption of these transcriptional

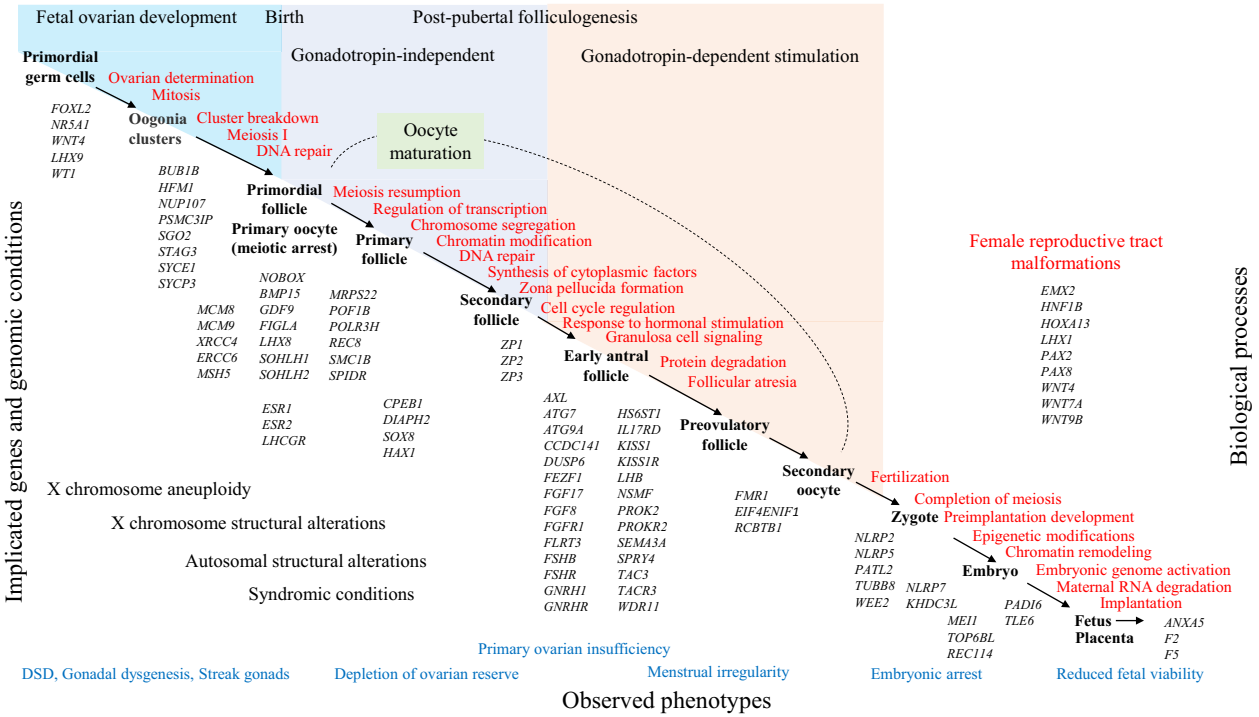

Figure 1. Biological processes and genetic causes implicated in female infertility. Large X chromosome alterations are the frequent cause of ovarian dysgenesis. A less severe phenotype manifesting as primary or secondary amenorrhea as well as reduced fetal viability can be seen in patients with balanced rearrangements, submicroscopic alterations, and single gene defects. Reproductive potential in patients with genomic alterations and syndromic conditions greatly depends on diagnosis, genes affected, and concomitant manifestations. Causative genes are displayed beneath the key biological processes, however can be involved in multiple pathways, affecting several stages of oocyte development.

regulators leads to ovarian dysgenesis or disorders of sex development. Newborn girls' ovaries have, on average, 1–2 million of primordial oocytes, and by the time of puberty, only about 400,000 remain. Individual oocytes are enveloped by somatic cells to form follicles and stay arrested at the diplotene stage of meiosis I (MI) until puberty. Completion of MI and ovulation are triggered by pituitary gonadotropins (Figure 1). The initial endowment of follicles (ovarian reserve) determines a woman's reproductive potential and, subsequently, her reproductive lifespan and age of menopause onset (between the ages of 40 and 58). Diminished ovarian reserve, either due to a low number of follicles at birth or puberty or to a rapid decline in the ovarian follicle pool after puberty, is associated with irregular menstruation, follicle exhaustion, and premature menopause (before age 40).

Multiple genes have been implicated in the production and maturation of germ cells, defects in which can cause accelerated cell apoptosis and follicle atresia, resulting in primary ovarian insufficiency (POI). Follicular atresia is one of the major mechanisms for eliminating germ cells from the ovary. About 1–2% of women are affected by POI, a phenotypically and etiologically heterogeneous condition characterized by primary or secondary amenorrhea, infertility, decreased estrogen production, elevated gonadotropins (follicle stimulating hormone and luteinizing hormone), and increased risk for osteoporosis and cardiovascular disease. POI is a term that is increasingly used and has been adopted to encompass diagnostically similar conditions, including hypergonadotropic hypogonadism, premature ovarian failure, and ovarian dysgenesis. POI is etiologically due to intrinsic problems within the ovary itself, which may include iatrogenic causes, such as surgery or radiation, or disruption of genes that primarily affect ovarian function. In a more severe presentation, POI presents with streak ovaries due to the depletion of germ cells and surrounding somatic, granulosa cells. Other causes of POI include abnormal chromosome segregation, deficiency in DNA repair, insufficient hormonal synthesis or signaling, defects in pathways that affect folliculogenesis, oocyte maturation and/or ovulation.

Functional disruption at the level of the hypothalamus or pituitary glands leads to hypogonadotropic hypogonadism, small ovaries, amenorrhea, and infertility. Infertility can also result from fertilization failure, either due to oocyte maturation arrest and the inability to produce a haploid zygote or to a defective zona pellucida.

Embryo development is initially regulated by maternal transcripts that are synthetized and stored in the cytoplasm during oocyte maturation. The euploid maternal genome and the cytoplasmic components are essential for normal embryonic development. Other pathologies, such as disruption of reproductive tract development, endometriosis, uterine fibroids, polycystic ovary syndrome, or autoimmune factors, may have a negative impact on implantation and pregnancy, leading to infertility or recurrent pregnancy loss.

Numerous genetic studies in animal models have identified thousands of genes that are essential for mammalian reproduction. Only a small proportion (less than 200) of these genes shows strong association with human infertility. Recent applications of whole exome and genome sequencing to families with infertility, have led to unbiased approaches and discovered new genes and novel variants involved in human infertility. These family-based approaches and recent genome-wide association studies have unearthed a small number of human infertility genes, and more discoveries are expected in the near future.

X Chromosome and female infertility

In the normal ovary, the primordial germ cells carry two X chromosomes, one of which is initially inactivated similarly to any other somatic cell. Importantly, the second X chromosome is reactivated prior to meiosis as the presence of two transcriptionally active X chromosomes is essential for oogenesis [1, 2]. In females, both X chromosomes must be active during meiosis to pair efficiently like autosome homologues [3]. Moreover, ~20% of X-linked genes escape inactivation and continue to be expressed from the inactive X chromosome, maintaining the dosage of female-specific transcripts in surrounding somatic cells [1–3].

In humans, chromosomal abnormalities such as monosomy X (Turner syndrome), cytogenetically visible deletions and duplications, and balanced and unbalanced X-autosome rearrangements are associated with an accelerated loss of primordial oocytes during female fetal development, resulting in streak gonads at birth [4, 5]. They account for close to 10% of cases of POI [6]. The mechanism of such massive germ cell loss is not well understood; however, some studies suggest the presence of a surveillance mechanism that may detect and eliminate cells with unpaired or unsynapsed chromatin, such as cells with monosomy X [7, 8]. It is also possible that accelerated germ cell depletion is caused by inadequate signaling from surrounding somatic granulosa cells that also carry a single X chromosome [9]. Failure in either dosage of X-linked genes or the X chromosome structural integrity can lead to POI [10]. Despite great importance of X chromosome in ovarian biology, only few X-linked genes have been implicated in ovarian function. *BMP15*, *FMR1*, and *PGRMC1* genes are located on the human X chromosome and have been linked with ovarian development and POI. Structural X chromosome abnormalities, rather than gene-specific abnormalities, may be the major reason for germ cell loss.

Beside POI, X chromosome abnormalities such as deletions, duplications, inversions, complex rearrangements, X-autosome translocations, and single-gene sequence variants, carried by at least 5–6% of women, can lead to recurrent fetal losses [11, 12]. In females, X chromosome abnormalities may result in early embryonic or fetal lethality, particularly of male conceptuses. To date, ~30% of X-linked genes are associated with a specific phenotype in humans, while pathogenic variants in a significant number of X-linked genes, such as *BCOR, EBP, FLNA, HCCS, IKBKG, MECP2, OFD1, OTC,* and *REP1*, are presumed to be male-lethal [12–14]. In addition, germline mosaicism is another explanation of infertility and recurrent miscarriages of affected fetuses with X-linked or autosomal dominant lethal alterations. Maternal germline mosaicism for pathogenic variants has been reported for male-lethal genes, including *IKBKG* [15], *FLNA* [16], and *MECP2* [17], and is likely an under-recognized condition. Female fetuses can also be affected by male-lethal X-linked disorders in cases of skewed X chromosome inactivation.

Genetic causes of gonadal dysgenesis due to defects in sex determination

Sexual development includes two processes: "sex determination," in which the undifferentiated gonad develops into a testis or ovary based on XY or XX chromosomal complement; and "sex differentiation," which leads to sex-specific hormone production governing anatomical and psychological differences between males and females.

XY gonadal dysgenesis

Phenotypic females with defects in sex determination, also known as Swyer syndrome, commonly present as XY individuals with female-appearing external genitalia and Müllerian structures (uterus and fallopian tubes), but dysgenic and non-functional gonads, lack of spontaneous pubertal development, absent breast development, primary amenorrhea, and infertility [18–20]. Approximately 10–15% of individuals with XY gonadal dysgenesis have deletions or pathogenic sequence variants affecting the *SRY* gene on the Y chromosome. Other causes comprise Y chromosome structural alterations, mosaicism for a cell line with monosomy X, heterozygous deletions, and sequence variants in the *NR5A1* gene, duplications in the Xp21 region involving *NR0B1*, *WNT4* gene duplications, and deletions and loss-of-function pathogenic variants in the *AR, MAP3K1, GATA4, DMRT1, DMRT2, ZNRF3,* and *DHH* genes [21–27]. Disorders of sexual development are also often seen as a manifestation of other syndromic conditions.

XX gonadal dysgenesis

While many genes have been identified as key regulators of the testis developmental pathways, less is known about the mechanisms triggering ovarian development. Most of the current knowledge of key regulators involves genes predominantly expressed in the somatic compartment of the gonad. In XX individuals, the absence of SRY is not a default mechanism for ovarian differentiation and requires the activation of genes associated with female pathway development and suppression of male development factors [28–30]. Our knowledge of the genetic regulators of ovarian developments is based on studying animal models, as well as human families afflicted with ovarian dysgenesis. Animal, mainly mouse studies, imply numerous biological processes that are essential for ovarian determination, differentiation, and maintenance. Ovarian development is orchestrated by several transcription factors, including SOHLH1, SOHLH2, NOBOX, LHX8, FIGLA, GATA4, WNT4, WT1, LHX9, FOXL2, and NR5A1 [31–37], and comprises mitotic proliferation of XX primordial germ cells, their progression into meiosis, and the formation of primordial follicles (Figure 1). Mouse studies show that *Sohlh1* and *Sohlh2* affect differentiation of both male and female gametes, while *Nobox*, *Lhx8*, and *Figla* are gamete-specific genes known to regulate oogenesis, without affecting male germ cell differentiation. In humans, the gonadal development in females and males is identical within first four weeks after conception. In fact, orthologues of above mouse genes are present in the human genome. Heterozygous and biallelic pathogenic variants in *SOHLH1, SOHLH2, NOBOX, FIGLA, WNT4, WT1, FOXL2*, and *NR5A1* genes were found in women with a wide spectrum of reproductive abnormalities, including isolated as well as syndromic conditions associated with complete gonadal dysgenesis and less severe forms of POI [37–40]. Phenotypic expression and severity of impaired gonadogenesis and oogenesis may depend on the type of alteration (loss of function vs. gain of function) and the effects on specific protein domains.

Defects in early oogenesis and chromosome segregation as cause of infertility

Once primordial germ cells cease proliferation, they become oogonia, and enter meiosis circa 12 weeks of human gestation. During the initial stages of meiosis, germ cells undergo a set of highly organized processes that include homologous chromosome pairing,

alignment, synapsis, and crossover [41–43]. The accurate segregation of chromosomes involves multiple processes to establish a physical connection of the homologous chromosomes via centromere interaction and recombination, to ensure correct positioning (chromosome alignment) at the spindle equator at metaphase I and to control a proper meiotic chromosome segregation [44, 45]. During MI prophase, homologous chromosomes pair and undergo recombination, that consists of programmed DNA double-strand breaks (DSB) governed by SPO11 protein, a crossover event resulting in an exchange of large chromosomal segments, and a repair of DNA breaks. In humans, a total number of DSB repair events outnumber the count of crossover events by more than 10-fold. Cell cycle checkpoint responds to incorrectly repaired/unrepaired DNA breaks and to a non-disjunction of chromosomes by inducing cell cycle arrest and activating apoptotic programs.

Programmed cell death is a naturally occurring mechanism to eliminate abnormal, damaged or excess cells at every stage of oogenesis. The fetal ovary contains ~7 million germ cells, only ~400 000 (5%) of which will remain at puberty, and ~400 oocytes (0.005% from the original germ cell pool) will ovulate during a female reproductive lifespan [46–48]. Accelerated apoptosis and disruption in signaling can lead to depletion of the primordial follicle pool, incomplete development of follicles, or failure of normal sexual differentiation, resulting in dysgenic or streak gonads.

Premature loss of germ cells is a common cause of human gonadal dysgenesis, primary amenorrhea, and infertility in human females with defects in genes implicated in germ cell mitosis and meiosis (Table 1) and genes important in chromosome segregation, such as *BUB1B, STAG3, SYCE1, SYCP3, HFM1, PSMC3IP,* and *SGO2,* as well as genes involved in DNA damage repair, such as *MCM8, MCM9, XRCC4, ERCC6,* and *BRCA2* [49–60].

There is compelling evidence that infertile women have a higher incidence of aneuploid oocytes [61, 62]. Rare deleterious alterations in genes required for chromosome synapsis and segregation during meiosis, such as *SYCP3* [49], *STAG3* [50], *HFM1* [51], *SYCE1* [52, 53], *NUP107* [54, 55], *PSMC3IP* [56, 57], *SGO2* [58], *REC8* [59], *SMC1B* [59], and *BUB1B* [60], are the cause of meiotic disturbances, aneuploidy, streak gonads, primary ovarian insufficiency, embryonic arrest, recurrent miscarriage, risk of affected offspring with chromosomal abnormalities, and infertility (Table 1). Meiosis-specific cohesin complexes consist of two structural proteins (SMC1α/SMC1β and SMC3), encoded by *SMC1A* and *SMC1B*, respectively; an α-kleisin protein (RAD21, RAD21L, or REC8); and a stromal antigen protein (STAG1, STAG2, or STAG3) [63]. Pathogenic variants in *REC8, SMC1B*, and *STAG3* are implicated in human infertility [50, 58]. Defects in assembly of the meiotic cohesion and/or synaptonemal complex lead to multi-chromosome aneuploidy during the second meiotic division, resulting in follicle atresia. Such genetic defects have been observed in patients with idiopathic infertility and recurrent miscarriages.

Female meiosis is highly prone to chromosome segregation errors, particularly in older women, resulting in a high proportion of aneuploid zygotes and at least 10% of aneuploid human fetuses [64–66]. The gradual loss of cohesins or cohesin-related proteins such as SGO2, has been implicated in meiotic non-disjunction and age-related aneuploidy and infertility [67]. The *BUB1B* gene encodes a mitotic checkpoint serine/threonine kinase B, a protein that plays an important role in regulation of the spindle-assembly checkpoint. Heterozygous and biallelic alterations in *BUB1B* are rare findings among fetuses and liveborn individuals affected by mosaic variegated aneuploidy [68, 69]. Similarly, chromosome mis-segregations are observed in patients with homozygous pathogenic variants in the *CEP57* gene [70, 71]. Impaired spindle checkpoint function results in aneuploidies involving multiple different chromosomes and is likely to cause oocyte maturation arrest, early embryonic lethality, or miscarriage [60, 72]. The presumed inheritance of these genes is listed in Tables 1 and 2.

DNA repair genes and their role in POI and human female infertility

Many studies implicate DNA repair genes in follicle maturation and quality, reproductive aging, and subsequent age of menopause onset [73–84]. Beside their role in meiosis, many DNA damage response genes also play essential roles in cell cycle control during mitosis. Diversity of DNA repair mechanisms, differential expression in various tissues, existence of the alternative tissue-specific DNA repair pathways, incomplete penetrance, environmental exposures, and other risk factors are the explanations for phenotypic variability in patients with defects in DNA repair genes. Thus, affected individuals may present with a spectrum phenotype manifesting as a syndromic condition or isolated tissue-specific phenotypes. Biallelic defects in DNA repair genes are the cause of syndromic conditions such as Fanconi anemia (*FANCA, FANCB, FANCC, BRCA2, FANCD2, FANCE, FANCF, XRCC9, FANCI, BRIP1, PHF9, PALB2, RAD51C, SLX4, ERCC4, RAD51, BRCA1, UBE2T, XRCC2, MAD2L2, RFWD3*), Werner syndrome (*RECQL2*), Warsaw breakage syndrome (*DDX11*), LIG4 syndrome (*LIG4*), Bloom syndrome (*BLM*), Nijmegen breakage syndrome (*NBN*), ataxia-telangiectasia (*ATM*), trichothiodystrophy 1 (*ERCC2, ERCC3*), and xeroderma pigmentosum (*XPA, XPC, ERCC2, ERCC3, ERCC4*). These syndromes are rare genetic conditions characterized by susceptibility to chromosome breakage and genome instability, triggering permanent cell cycle arrest, and apoptosis of the affected cells. As a result, patients with pathogenic variants present with growth retardation, skin lesions, bone marrow and immune deficiencies, neurodevelopmental problems, endocrine and early gonadal dysfunction, and predisposition to cancers.

There are also a number of patients with biallelic variants in DNA repair genes such as *MCM8, MCM9, XRCC4*, and *MSH5* who present with a non-syndromic primary ovarian insufficiency [78–83]. Homozygous pathogenic variants in the *MCM8* or *MCM9* genes were discovered in women with hypergonadotropic primary amenorrhea, hypothyroidism, growth retardation, and absent or very small ovaries. None of the affected women had cancer at the time of investigation; however, excessive chromosomal breakage was observed in their somatic cells [78, 79]. A single report indicated an affected family with *MCM9* pathogenic variants in association with primary hypergonadotropic hypogonadism with early-onset colorectal cancer [84]. The heterozygous mutation carriers appeared healthy and fertile, although they also had an increased number of chromosome breakages in comparison to wildtype *MCM8* and *MCM9* individuals. Interestingly, population-based genome-wide studies found the strongest association with a non-synonymous single nucleotide polymorphism (rs16991615) in coding exon 9 of the *MCM8* gene and the age of menopause in Caucasian, European, African American [85], and Hispanic women. These results indicate that *MCM8*, and probably other DNA repair genes, regulate the reproductive span from gonadal formation to menopause, and that heterozygous variants may confer risk loci for primary ovarian insufficiency, diminished ovarian reserves, and infertility [85]. Variants in *MCM8*

Table 1. Genes implicated in non-syndromic POI, gonadal dysgenesis, and female infertility.

Gene	Phenotype MIM/[ref]	Phenotype, reproductive outcomes	Genetic defects	Mode of inheritance
ANXA5	#614391	Susceptibility to recurrent pregnancy loss, RPRGL3	Sequence variants in the promoter	AD
ATG7, ATG9A	[199]	Primary ovarian insufficiency	Sequence variants	AD
AXL	[200]	Hypogonadotropic hypogonadism with or without anosmia	Sequence variants	AD
BMP15	#300510	Ovarian dysgenesis, primary amenorrhea, POF4	Sequence variants, deletions	X-linked
BUB1B	#176430	Premature chromatid separation, recurrent pregnancy loss	Sequence variants	AD
CCDC141	[116]	Kallmann syndrome	Sequence variants	AR
CPEB1	[201, 202]	Primary ovarian insufficiency	Deletions	AD
DIAPH2	#300511	Primary or secondary amenorrhea, POF2A	Sequence variants, deletions	X-linked
DUSP6	#615269	Hypogonadotropic hypogonadism HH19 with or without anosmia	Sequence variants	AD
EIF4ENIF1	[115]	Primary ovarian insufficiency	Sequence variants	AD
ERCC6	#616946	Primary ovarian insufficiency, POF11	Sequence variants	AD
ESR1	#615363	Estrogen resistance, primary amenorrhea	Sequence variants	AR
ESR2	#618187	Ovarian dysgenesis, ODG8, primary amenorrhea	Sequence variants	AD
F2	#614390	Susceptibility to recurrent pregnancy loss, RPRGL2	Sequence variants	AD
F5	#614389	Susceptibility to recurrent pregnancy loss, RPRGL1	Sequence variants	AD
FEZF1	#616030	Hypogonadotropic hypogonadism HH22 with or without anosmia	Sequence variants	AR
FGF17	#615270	Hypogonadotropic hypogonadism HH20 with or without anosmia	Sequence variants	AD
FGF8	#612702	Hypogonadotropic hypogonadism HH6 with or without anosmia	Sequence variants	AD
FGFR1	#147950	Hypogonadotropic hypogonadism HH2 with or without anosmia	Sequence variants	AD
FIGLA	#612310	Ovarian insufficiency, POF6	Sequence variants	AD, AR
FLRT3	#615271	Hypogonadotropic hypogonadism HH21 with or without anosmia	Sequence variants	AD
FMR1	#311360#300869	Primary ovarian insufficiency, POF1	Expansion of trinucleotide (CCG)n repeats, duplication	X-linked
FSHB	#229070	Ovarian insufficiency, hypogonadotropic hypogonadism HH24	Sequence variants	AR/AD
FSHR	#233300	Ovarian dysgenesis, primary amenorrhea, ODG1	Sequence variants	AR
GDF9	#618014	Primary ovarian insufficiency, POF14	Sequence variants, duplication	AR, digenic
GNRH1	#614837	Hypogonadotropic hypogonadism HH12 with or without anosmia	Sequence variants	AR
GNRHR	#146110	Hypogonadotropic hypogonadism HH7, primary amenorrhea	Sequence variants	AR
HAX1	[203]	Primary ovarian insufficiency, short stature	Sequence variants	AD
HFM1	#615724	Ovarian dysgenesis, ovarian insufficiency, POF9	Sequence variants	AR
HS6ST1	#614880	Hypogonadotropic hypogonadism HH15 with or without anosmia	Sequence variants	AD
IL17RD	#615267	Hypogonadotropic hypogonadism HH18 with or without anosmia	Sequence variants	AD, AR
KHDC3L	[166]	Preimplantation embryonic lethality	Sequence variants	AR
KISS1	#614842	Hypogonadotropic hypogonadism HH13 with or without anosmia	Sequence variants	AR
KISS1R	#614837	Hypogonadotropic hypogonadism, HH8, primary amenorrhea	Sequence variants	AR
LHB	#228300	Hypogonadotropic hypogonadism, HH23	Sequence variants	AR
LHCGR	#238320	Luteinizing hormone resistance, amenorrhea, and infertility	Sequence variants	AR
LHX8	[99]	Ovarian dysgenesis, ovarian insufficiency	Sequence variants	AD
MCM8	#236700	Ovarian dysgenesis, primary amenorrhea, POF10	Sequence variants	AR
MCM9	#616185	Ovarian dysgenesis, primary amenorrhea, ODG4	Sequence variants	AR
MRPS22	#618117	Ovarian dysgenesis, ODG7, primary amenorrhea	Sequence variants	AR
MSH5	#617442	Primary ovarian insufficiency, POF13	Sequence variants	AR
NLRP2, NLRP5	[160]	Primary infertility, Oocyte maturation defect,	Sequence variants	AR
NOBOX	#611548	Ovarian dysgenesis, primary ovarian insufficiency, POF5	Sequence variants	AD, AR

Table 1 *Continued*

Gene	Phenotype MIM/[ref]	Phenotype, reproductive outcomes	Genetic defects	Mode of inheritance
NR5A1	#612964	Gonadal dysgenesis, ovotesticular DSD, secondary ovarian insufficiency, POF7	Del/dup, Sequence variants	AD
NSMF	#614838	Hypogonadotropic hypogonadism HH9 with or without anosmia	Sequence variants	AD
NUP107	#618078	Ovarian dysgenesis, ODG6, primary amenorrhea	Sequence variants	AR
PADI6	#617234	Preimplantation embryonic lethality, PREMBL12	Sequence variants	AR
PATL2	#617743	Primary infertility, oocyte maturation defect, OOMD4, early embryonic arrest	Sequence variants	AR
POF1B	#300604	Primary ovarian insufficiency, POF2B	Sequence variants	X-linked
POLR3H	[204]	Primary ovarian insufficiency	Sequence variants	AR
PROK2	#610628	Hypogonadotropic hypogonadism HH4 with or without anosmia	Sequence variants	AD
PROKR2	#244200	Hypogonadotropic hypogonadism HH3 with or without anosmia	Sequence variants	AD
PRLR	#615555	Hyperprolactinemia, amenorrhea	Sequence variants	AR
PSMC3IP	#614324	Ovarian dysgenesis, ODG3, primary amenorrhea	Sequence variants	AR
REC8	[99]	Ovarian dysgenesis, ovarian insufficiency	Sequence variants	unknown
SALL4	[205]	Primary ovarian insufficiency	Sequence variants	AD
SEMA3A	#614897	Hypogonadotropic hypogonadism HH16 with or without anosmia	Sequence variants	AD
SGO2	[58]	Primary ovarian insufficiency	Sequence variants	AR
SLC29A3	#602782	Histiocytosis-lymphadenopathy plus syndrome	Sequence variants	AR
SMC1B	[99]	Ovarian dysgenesis, ovarian insufficiency	Sequence variants	unknown
SOHLH1	#617690	Ovarian dysgenesis, ODG5, primary ovarian insufficiency	Sequence variants	AR
SOHLH2	#616066	Primary ovarian insufficiency	Sequence variants	AD
SOX8	[192]	Ovarian dysgenesis, primary or secondary amenorrhea	Sequence variants	AD
SPIDR	[206]	Ovarian dysgenesis	Sequence variants	AR
SPRY4	#615266	Hypogonadotropic hypogonadism HH17 with or without anosmia	Sequence variants	AD
STAG3	#615723	Ovarian dysgenesis, ovarian insufficiency, POF8	Sequence variants	AR
SYCE1	#616947	Ovarian dysgenesis, ovarian insufficiency POF12	Sequence variants	AR
SYCP3	#270960	Susceptibility to recurrent pregnancy loss, RPRGL4	Sequence variants, deletions	AD
TAC3	#614839	Hypogonadotropic hypogonadism HH10 with or without anosmia	Sequence variants	AR
TACR3	#614840	Hypogonadotropic hypogonadism HH11 with or without anosmia	Sequence variants	AR
TLE6	#616814	Preimplantation embryonic lethality, PREMBL1	Sequence variants	AR
TUBB8	#616780	Primary infertility, oocyte maturation defect, OOMD2	Sequence variants (de novo, paternal transmission)	AD, AR
WDR11	#614858	Hypogonadotropic hypogonadism HH14 with or without anosmia	Sequence variants	AD
WEE2	#617996	Primary infertility, oocyte maturation defect, OOMD5	Sequence variants	AR
WNT4	#158330	Müllerian aplasia, hyperandrogenism, primary amenorrhea	Sequence variants	AD
ZP1	#615774	Primary infertility, oocyte maturation defect, OOMD1	Sequence variants	AR
ZP2	[150]	Primary infertility	Sequence variants	AR, digenic
ZP3	#617712	Primary infertility, oocyte maturation defect, OOMD3	Sequence variants (de novo, paternal transmission)	AD

also correlate with antral follicle count [86]. The mechanisms of MCM8 and MCM9 action are currently under intensive study. Both MCM8 and MCM9 are members of a complex that is rapidly recruited at the DNA damage sites to resolve double-strand breaks [87]. During meiosis, programmed double-strand breaks are generated to attain accurate segregation and crossover of homologous chromosomes. To maintain germ cells' genomic integrity and prevent DNA from possible damage, breaks must be repaired via a homologous recombination-mediated mechanism. Inability to efficiently repair chromosomal ends activates cell apoptosis, leading to depletion of germ cells and dramatic loss of ovarian reserves [75, 87]. It is possible that deficiency in MCM8/MCM9 leads to oocyte loss due to unrepaired double-strand DNA breaks.

Abnormal folliculogenesis and female infertility

The number of primordial follicles at birth, their quality, and the rate of germ cell depletion are the factors that define reproductive lifespan in women [88–91]. In some women, oocyte depletion occurs during the fetal stages of oogenesis, endowing them with a relatively

Table 2. Syndromic conditions accompanied by ovarian insufficiency and female infertility.

Gene	Phenotype MIM/[ref]	Phenotype, reproductive outcomes	Genetic defects	Mode of inheritance
AARS2	#615889	Leukoencephalopathy, progressive, with ovarian failure	Sequence variants	AR
AIRE	#340300	Autoimmune polyendocrinopathy syndrome	Sequence variants	AD, AR
ANOS1	#308700	Kallmann syndrome	Sequence variants, deletions	X-linked
AR	#300068	Androgen insensitivity, ovarian dysgenesis, XY females	Sequence variants, deletions	X-linked
ATM	#208900	Ataxia-telangiectasia	Sequence variants, deletions	AR
BRCA2	[73]	Ovarian dysgenesis, microcephaly, cancer predisposition	Sequence variants	AR
CCDC39	#613807	Ciliary dyskinesia, recurrent respiratory infections, infertility	Sequence variants	AR, digenic
CHD7	#214800	CHARGE syndrome, hypogonadotropic hypogonadism, ovarian dysgenesis	Sequence variants, deletions	AD
CLPP	#614129	Perrault syndrome 3, progressive hearing loss, primary ovarian insufficiency	Sequence variants	AR
CYP17A1	#202110	Congenital adrenal hyperplasia due to 17-hydroxylase deficiency	Sequence variants, deletions	AR
CYP21A2	#201910	Congenital adrenal hyperplasia due to 21-hydroxylase deficiency	Sequence variants, deletions	AR
DCAF17	#241080	Hypogonadism, ovarian dysgenesis, partial alopecia, Woodhouse–Sakati syndrome	Sequence variants	AR
DDX11	#613398	Warsaw breakage syndrome	Sequence variants	AR
EIF2B1/B2/B3/B4/B5	#603896	Leukoencephalopathy with vanishing white matter, gonadal dysgenesis, primary or secondary amenorrhea	Sequence variants	AR
ERAL1	#617565	Perrault syndrome 6, progressive hearing loss, primary ovarian insufficiency	Sequence variants	AR
ERCC2	#601675	Trichothiodystrophy 1, brittle, sulfur-deficient hair, ichthyosis, developmental disabilities, decreased fertility, short stature, infections	Sequence variants	AR
ERCC3	#616390	Trichothiodystrophy 2	Sequence variants	AR
FANCA, multiple	#227650	Fanconi anemia	Sequence variants, deletions	AR
FOXL2	#110100	Blepharophimosis with ovarian dysgenesis, POF3	Sequence variants, deletions, duplications, translocations	AD
GALT	#230400	Galactosemia, hypoglycemia, renal tubular dysfunction, muscle hypotonia, hypogonadism	Sequence variants, deletions	AR
H6PD	#604931	Cortisone reductase deficiency 1, hirsutism, oligomenorrhea, infertility	Sequence variants	AR
HARS2	#614926	Perrault syndrome 2, sensorineural deafness, primary amenorrhea, streak gonads	Sequence variants	AR
HSD11B1	#614662	Cortisone reductase deficiency 2, oligomenorrhea, infertility	Sequence variants	AD
HSD17B4	#233400	Perrault syndrome 1, progressive hearing loss, primary ovarian insufficiency	Sequence variants	AR
KHDC3L	#614293	Hydatidiform mole, recurrent pregnancy loss, gestational trophoblastic neoplasia	Sequence variants	AR
LARS2	#615300	Perrault syndrome 4, progressive hearing loss, primary ovarian insufficiency	Sequence variants	AR
LMNA	#151660	Lipodystrophy, familial partial, type 2, abnormal distribution of subcutaneous fat, polycystic ovary syndrome, infertility, spontaneous abortions, fetal death	Sequence variants	AD
LMNA	#212112	Malouf syndrome, cardiomyopathy, skeletal anomalies, amenorrhea	Sequence variants	AD
MEI1	[172]	Hydatidiform mole, recurrent pregnancy loss, gestational trophoblastic neoplasia		
MKKS	#236700	McKusick-Kaufman syndrome, hypogenitalism, hydrometrocolpos	Sequence variants	AR
NBN	#251260	Nijmegen breakage syndrome, primary amenorrhea, microcephaly and immunodeficiency	Sequence variants	AR
NLRP7	#231090	Hydatidiform mole, recurrent pregnancy loss, gestational trophoblastic neoplasia	Sequence variants, deletions	AR
NR0B1	#300018	46,XY sex reversal 2, dosage-sensitive	Duplications	X-linked
NR3C1	#615962	Glucocorticoid resistance, infertility, male-pattern baldness, hirsutism, and menstrual irregularities	Sequence variants	AD

Table 2 *Continued*

Gene	Phenotype MIM/[ref]	Phenotype, reproductive outcomes	Genetic defects	Mode of inheritance
PGM1	#614921	Congenital disorder of glycosylation, cleft lip and bifid uvula, hepatopathy, hypoglycemia, short stature, and exercise intolerance, rhabdomyolysis, dilated cardiomyopathy, and hypogonadotropic hypogonadism	Sequence variants	AR
PMM2	#212065	Ovarian dysgenesis, cerebellar atrophy, mental impairment, pigmentary retinopathy	Sequence variants	AR
PNPLA6	#215470	Boucher-Neuhauser syndrome, pinocerebellar ataxia, hypogonadotropic hypogonadism, visual impairment	Sequence variants	AR
POLG	#157640	Progressive external ophthalmoplegia, amenorrhea	Sequence variants	AD
PPARG	#151660	Lipodystrophy, familial partial, type 3, insulin-resistant diabetes mellitus, infertility	Sequence variants	AD
PROP1	#262600	Combined pituitary hormone deficiency, growth failure	Sequence variants, deletions	AR
RCBTB1	[119]	Retinitis pigmentosa, goiter, primary ovarian insufficiency, intellectual disability	Sequence variants	AR
REC114	[172]	Hydatidiform mole, recurrent pregnancy loss, gestational trophoblastic neoplasia	Sequence variants	AR
RECQL2	#210900	Werner syndrome	Sequence variants	AR
RECQL3	#277700	Bloom syndrome	Sequence variants	AR
RNF216	#212840	Cerebellar ataxia and hypogonadotropic hypogonadism	Sequence variants, digenic	AR
SLC29A3	#602782	Histiocytosis-lymphadenopathy plus syndrome, joint deformities, sensorineural hearing loss, and subsequent development of generalized lymphadenopathy and swellings in the eyelids, hypogonadotropic hypogonadism, amenorrhea	Sequence variants	AR
SOX10	[117]	Kallmann syndrome, deafness	Sequence variants	AD
TOP6BL	[172]	Hydatidiform mole, recurrent pregnancy loss, gestational trophoblastic neoplasia	Sequence variants	AR
TP63	#603543	Limb-mammary syndrome, hand/foot anomalies and hypoplasia/aplasia of the mammary gland, amenorrhea, absence of the uterus and ovaries	Sequence variants	AD
TRIM37	#253250	Mulibrey nanism, gonadal dysgenesis	Sequence variants	AR
TWNK	#616138	Perrault syndrome 5, progressive hearing loss, primary ovarian insufficiency	Sequence variants	AR
WT1	#256370	Nephrotic syndrome, gonadal dysgenesis	Sequence variants	AD
XPA, XPC, ERCC2, ERCC3, ERCC4	#278700	Xeroderma pigmentosum	Sequence variants	AR
XRCC4	#616541	Short stature, microcephaly, and endocrine dysfunction, ataxia	Sequence variants	AR

small pool by the time of puberty. In others, there is accelerated loss of oocytes after the establishment of the primordial follicle pool, and can be due to environmental exposures such as cigarette smoking. Elimination of oocytes is accelerated with maternal age and is related to deterioration of oocyte quality. In fact, about 40–60% of oocytes from women of 40 years are aneuploid [92], while the oocyte quality of younger women with diminished ovarian reserve are unknown.

Diminished ovarian reserve (DOR) is another term used for women with a relatively low antral follicle count on ultrasound examinations (<8) and low AMH levels and affects 10% of women undergoing infertility treatment [93]. DOR is more prevalent than POI but, in biological reality, may very well have similar genetic underpinnings, except that less penetrant mutations may be more frequent in DOR individuals.

Folliculogenesis involves proliferation and differentiation of granulosa cells and maturation of the oocyte. This process includes a series of signaling events between the cells within the follicle. Although the granulosa and theca cells have long been implicated in ovarian function, animal studies have revealed a synergistic effect of GDF9 and BMP15 on granulosa cell differentiation, folliculogenesis, and ovulation [94–97]. *BMP15* and *GDF9*, members of the transforming growth factor-*beta* (TGFβ) superfamily, are expressed in oocytes through folliculogenesis and are involved in a cascade of regulatory processes directing granulosa cell proliferation, steroidogenesis, cumulus expansion, and apoptosis. GDF9 has been implicated in the transition from the primary to the secondary follicle stage [96]. BMP15 stimulates follicle maturation during the gonadotropin-independent phase and regulates granulosa cell sensitivity to follicle-stimulating hormone (FSH). It has been observed that a high level of GDF9 and BMP15 in the follicle is associated with good embryo morphology. Human *GDF9* and *BMP15* gene variants, mostly heterozygous, have been detected in up to 10% of women with hypergonadotropic ovarian failure, POI, primary and secondary amenorrhea, and polycystic ovary syndrome [94, 98, 99]. However, more convincing functional studies are needed to determine the true role of these variants in female infertility.

Multiple oocyte-specific transcription factors, including *FIGLA, NOBOX, LHX8, SOHLH1*, and *SOHLH2*, also control follicular development and play a role in cytoplasmic maturation by

regulating the expression of maternal-effect factors essential for future embryonic activation [28, 30, 100]. Heterozygous and homozygous sequence variants in these transcription factors were found to be associated with ovarian dysgenesis and POI [37, 101–109]. In mice, these genes cause phenotypes in autosomal recessive fashion, and this is likely to be true in humans, but further studies are necessary to determine whether heterozygous variants in these genes can cause pathology.

Pleotropic and syndromic conditions resulting in POI and female infertility

Expansion of CGG repeats in the 5'UTR of the *FMR1* gene is another genetic condition strongly associated with diminished ovarian reserve and primary ovarian insufficiency, as the FMR1 protein is expressed in neurons and granulosa cells. FMR1 is an mRNA-binding protein that also regulates translation. Fragile X intellectual disability syndrome is caused by inactivation of the *FMR1* gene, located on the X chromosome, mainly due to DNA hypermethylation of the promoter and the CGG repeat region when the repeat size exceeds 200. Full mutation alleles arise from premutation alleles with 55–200 CGG repeats that demonstrate a repeat length instability and a tendency to expand from one generation to the next. The prevalence of premutation alleles in the general population is estimated to be 1 per 150–300 females, with the highest incidence, 1 per 100 females, in Colombia and Israel [110, 111].

Interestingly, women with 55–200 CGG repeats (premutation alleles) have elevated mRNA expression and decreased protein level posing a significant risk for premature ovarian aging and infertility, while females with over 200 CGG repeats, full-mutation carriers, do not have any form of ovarian dysfunction. *FMR1* premutation alleles confer risk for adult neurodegenerative disorder in males as well as POI in females. A phenomenon in which a pathogenic variant is the cause of multiple apparently unrelated conditions has been defined as pleiotropy. Such effects can be explained by the failure of a single molecular function involved in multiple biological processes, variably affecting cellular physiology. Premutation carriers were noted to have significantly elevated FSH correlated with increasing CGG repeats and decreased inhibin B/A, progesterone, and antimülerian hormone levels.

The exact mechanism of oocyte toxicity in women with *FMR1* premutation alleles is unknown, although mRNA overexpression may result in RNA/protein gain-of-function disorder affecting follicular maturation, as well as increasing the degree of follicular apoptosis [112–114]. Another gene, eukaryotic translation initiation factor 4E nuclear import factor 1 (*EIF4ENIF1*) has been recently implicated in ovarian germ cell development and primary ovarian insufficiency [115]. Defects in *eIF4ENIF1* may result in decreased mRNA degradation and increased mRNA stability, similar to the mechanism of fragile X primary ovarian insufficiency.

A significant number of syndromic conditions, such Perrault syndrome (*HSD17B4, HARS2, CLPP, LARS2, C10ORF2*), blepharophimosis with ptosis, and epicanthus inversus syndrome (*FOXL2*); leukoencephalopathy with vanishing white matter due to pathogenic variants in the *EIF2B1, EIF2B2, EIF2B4,* and *EIF2B5* genes; galactosemia and carbohydrate-deficient glycoprotein syndromes with pathogenic variants in *GALT* and *PMM2* genes, and many others (Table 2) have ovarian dysgenesis and/or primary ovarian insufficiency among their clinical manifestations [116–122].

Mitochondria and female infertility

Defects in the *POLG* gene, which encodes polymerase gamma, responsible for mitochondrial DNA synthesis, are the cause of progressive external ophthalmoplegia and are also reported in multiple women with POI [123–124]. The association of nuclear and mitochondrial genes responsible for mitochondria function with female oogenesis and fertility is not surprising as the number of mitochondria increases at least 1000-fold in human oocytes [125–126]. Mitochondria are essential for multiple processes during oogenesis, such as ATP production, apoptosis, and calcium homeostasis [127]. Mitochondrial dysfunction can lead to defects in oocyte maturation, impaired spindle assembly and chromosome missegregation, poor pre-implantation development, and implantation failure [124]. Mitochondrial inheritance is exclusively maternal and is considered to be a key determinant of female reproductive aging and infertility [128]. In addition to *POLG*, a number of nuclear genes regulate mitochondrial function, such as the genes responsible for Perrault syndrome (*HARS2, HSD17B4, LARS2*). The clinical significance of other genes involved in mitochondrial DNA maintenance, repair, and response to oxidative stress, such as *RAD51*, *RAD51C*, and *XRCC3*, remains to be established.

Deficiencies in hormonal signaling and response in female infertility

The gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), play a pivotal role in regulation of folliculogenesis, oocyte maturation, ovulation, implantation, and estradiol synthesis [129–130]. Recruitment of primordial follicles is a gonadotropin-independent process, while subsequent follicle growth is gonadotropin-sensitive. Upon binding to the receptors, gonadotropins stimulate oocyte development from early antral stage to maturation and convert androgen to estrogen in granulosa cells. FSH is a pituitary glycoprotein hormone composed of an *alpha* chain (*CGA*) that is similar to other pituitary glycoproteins such as LH, chorionic gonadotropin, and thyroid-stimulating hormone, and a *beta* chain encoded by *FSHB*. Patients with pathogenic variants in the *FSHB* (FSH *beta* subunit) and the *FSHR* (FSH receptor) genes develop follicles up to preantral stages, but further maturation is blocked. Defects in *FSHB* lead to a low production of FSH, resulting in hypogonadotropic hypogonadism [131, 132], whereas changes in *FSHR* cause hypergonadotropic hypogonadism [133–135]. The severity of the phenotype in patients with variations in the *FSHR* gene correlates with the effect of the genetic change in the FSHR function. Pathogenic variants in *FSHR*, specifically the c.566C > T change, are more frequent in a Finnish population [133], but are rare in other ethnic groups [133, 134].

Hypogonadotropic hypogonadism is a rare disorder characterized by the deficiency of gonadotropin-releasing hormone (GnRH) due to its impaired production, secretion, or function. It often manifests as incomplete or absent puberty and infertility. GnRH acts via the GnRH receptor, which affects both synthesis and release of LH and FSH, which in turn, control gonadal maturation, which then feeds back via Inhibin and Activin to complete the hypothalamic–pituitary–gonadal axis [136]. A considerable proportion of patients affected by hypogonadotropic hypogonadism have genetic changes leading to an isolated or a syndromic condition with X-linked, autosomal recessive, and autosomal dominant inheritance. Hypogonadotropic hypogonadism [137] is a genetically heterogeneous condition with both sporadic and familial cases and more than 25

causative genes identified to date (Table 2). The most commonly affected genes are *ANOS1 (KAL1), SOX10, IL17RD, FGFR1, CHD7, PROKR2, GNRHR,* and *TACR3*, while pathogenic variants in *AXL, DMXL2, FEZF1, FGF17, FGF8, GNRH1, HESX1, HS6ST1, KISS1, KISS1R, LEP, LEPR, NR0B1, NSMF, OTUD4, PCSK1, PNPLA6, PROK2, RNF216, SEMA3A, SEMA7A, TAC3,* and *WDR11* are less frequent. Kallmann syndrome is characterized by a defective sense of smell in approximately 50% of patients with hypogonadotropic hypogonadism. The presence of anosmia should alert clinicians for a possible congenital hypogonadotropic hypogonadism and facilitate the search for a definitive molecular diagnosis in affected patients.

Genetics of polycystic ovaries

Development of preovulatory follicles depends greatly on regulation by a number of factors, such as FSH, oocyte-secreted proteins, and insulin-like growth factors [130]. Mature oocytes are surrounded by granulosa and thecal cells, which produce an androgen substrate, a prerequisite for ovarian estrogen biosynthesis. Importantly, hormonal imbalances related to ovarian factors or to systemic hormonal dysregulation is a frequent cause of POI as well as polycystic ovary syndrome (PCOS).

PCOS is the most common form of female infertility, affecting about 10% of women of reproductive age, and is associated with poor conception rates and high (30–50%) pregnancy loss rates. Multiple studies have established a strong correlation between enlarged polycystic ovaries and obesity, hirsutism, and amenorrhea. Hyperplasia of theca and stromal cells, elevated testosterone and LH, and low estradiol and FSH levels have been observed in women with hirsutism and oligomenorrhea [137–140]. Genomic changes in the *FSHR, BMP15,* and *LHCGR* genes as well as noncoding RNAs have been proposed to disturb a fine balance between LH and FSH hormonal stimulation of the thecal and granulosa cell compartments, leading to excessive androstenedione and low estradiol concentration in follicles.

LHCGR is a receptor for both LH and chorionic gonadotropin. *LHCGR* variants have been described in patients with nonsyndromic infertility, cystic ovaries, empty follicles, and primary and secondary amenorrhea [141, 142]. The *LHCGR* c.935G > A polymorphism, promoter hypomethylation, and reduced expression of *LHCGR* were observed in women with PCOS; however, the molecular causes of PCOS remain to be elucidated.

Genome wide association studies (GWAS) studies have been very helpful in identifying novel loci that play important roles in human reproduction. A GWAS among Han Chinese women with PCOS identified eight highly significant loci. Among these are three novel loci that contain LH–choriogonadotropin receptor (*LHCGR*), thyroid adenoma-associated gene (*THADA*), and differentially expressed in normal and neoplastic cells (DENN)–MAPK activating death domain (MADD) containing 1A (*DENND1A*). *THADA* and *DENND1A* loci associations have been replicated in some of the European cohorts [139]. Eight additional loci were identified in a follow up study [143]. Further functional studies are necessary to fully understand the role of these genes in human reproductive pathology.

Other endocrine disturbances

Endocrine disorders such as diabetes, hypothyroidism, conditions affecting the adrenal glands, or hypothalamus are frequently accompanied by delay or lack of development of secondary sexual characteristics, irregular menstrual cycles, or amenorrhea. Females affected by congenital endocrinopathies, such hyperprolactinemia (*PRLR)*, combined pituitary hormone deficiency (*PROP1*); autoimmune polyendocrinopathy syndrome (*AIRE*) frequently present with primary ovarian insufficiency and infertility (Table 2). It is also important to point out that pathogenic variants in DNA damage repair genes can affect endocrine function, and sometimes endocrine dysfunction can be the first presenting sign of a more serious condition such as Fanconi anemia [144].

Genetic causes of fertilization failure

Ovulation in mammals is regulated by a cyclic secretion of LH. Eggs surrounded by the zona pellucida and somatic cumulus cells may be fertilized by a capacitated sperm. Sperm capacitation is a complex process, comprising structural and molecular changes to the acrosome, that happen to the spermatozoa residing within the female reproductive tract [145]. Female-specific factors that affect sperm capacitation and the sperm's ability to recognize and penetrate through the cumulus cell layer and zona pellucida are unknown, but genetic defects in genes responsible for this process are likely to cause fertilization failure and infertility. These patients present with primary infertility despite normal ovarian reserve, regular menstrual cycles, and normal sex hormone levels.

The zona pellucida, an extracellular matrix surrounding the developing oocyte, contains four types of glycoprotein, ZP1, ZP2, ZP3, and ZP4. During oocyte development, the zona pellucida can be first observed in primary follicles as an external coat that physically separates the oocyte from the surrounding granulosa cells. The zona pellucida plays an important role in protection of the growing oocyte, oocyte-follicle cell interactions, and sperm recognition and binding [146]. Right after fertilization, the zona pellucida undergoes changes which prevent polyspermy, support blastocyst development, and preclude premature implantation.

Nucleotide sequence variations in the human zona pellucida genes may cause defects in the formation of zona pellucida and subsequent infertility. An autosomal recessive inheritance has been documented in women with loss-of-function *ZP1* variants and absence of the zona pellucida around their oocytes [147] and in women with pathogenic sequence variants in *ZP2* and dysfunctional zona pellucida [148]. Empty follicle syndrome has been reported to cause infertility in women with heterozygous pathogenic variants in the *ZP3* gene [149]. Oocyte retrieval after an IVF treatment cycle showed a highly disorganized follicle content with absent or degenerated oocytes lacking zonae pellucidae. *ZP3* expression is limited to oocytes; thus, the infertile phenotype is limited to females who have a de novo alterations or inherit pathogenic heterozygous variants from their fathers [148]. Digenic inheritance of two inherited variants in the *ZP2* and *ZP3* genes can also result in oocyte maturation defects and infertility [150].

In humans, ZP2, ZP3, and ZP4 glycoproteins form long filaments connected by ZP1, providing an interactive oocyte-granulosa cell environment. In the absence of a functional zona pellucida, intercellular interaction between the developing oocyte and granulosa cells is interrupted, resulting in oocyte degeneration prior to ovulation. During fertilization, the zona pellucida glycoproteins serve as sperm receptors, providing highly species-specific cell recognition and binding. To date, female-specific alterations leading to inability of sperm binding are unknown, but males with defects in zona pellucida binding proteins may produce dysmorphic spermatozoa with incompetence or reduced ability to penetrate the zona

pellucida. Successful interaction between a single sperm and the zona pellucida as well as the activation of a blocking mechanism that prevents the fusion of additional sperm with the egg is critical for fertilization. Polyspermic fertilization, which leads to the formation of non-viable polyploid embryos, is prevented by the release of zinc ions and an enzyme ovastacin (MIM* 608860) stored in egg cortical granules to immobilize egg-surrounding spermatozoa immediately after fertilization and to cleave the sperm-binding region within ZP2 [151]. Defects in genes implicated in the polyspermy blockage have yet to be discovered in humans.

Maternal effect genes and post-fertilization preimplantation embryonic failure

Identification of human genes that result in early embryonic lethality, before implantation, is challenging. Recent evidence from assisted reproductive technologies and embryo research suggests that idiopathic infertility may be caused by very early forms of embryonic lethality. It is estimated that up to 70% of human embryos produced in IVF are viable, while the remaining embryos arrest during cleavage-stage embryogenesis [152]. After fertilization, the zygotic genome is transcriptionally silent, and the first few cell divisions of the embryo depend on maternally derived cytoplasmic factors accumulated during oocyte maturation. These maternal components include RNA, proteins, subcellular organelles and macromolecules that are encoded by maternal-effect genes [153–155].

Maternal factors play three major roles in preimplantation development: remodeling of condensed maternal and paternal genomes to form the diploid cell upon fertilization; gradual degradation of maternal RNA and proteins; and transcriptional activation of the embryonic genome. Failure in any of these biological processes leads to female infertility. Based on animal models, many genes are involved in early, post-fertilization cleavage events, including chromatin remodeling, epigenetic reprogramming, embryo genome activation, and cell specification.

A few of these genes have been identified in humans very recently, in part due to application of whole exome sequencing to phenotypically well-characterized clinical cases. Women with recurrent unsuccessful attempts of in vitro fertilization combined with intracytoplasmic sperm injection are diagnosed with primary female infertility as the majority of their embryos fail to develop. Biallelic alterations in *PADI6* [156], *PATL2* [157, 158], *TLE6* [159], *NLRP2* [160], *NLRP5* [160], and *WEE2* [161], as well as heterozygous and homozygous pathogenic variants in *TUBB8* [162, 163], were found to be responsible for a spectrum of early embryonic lethality phenotypes, including oocyte maturation arrest, failure of fertilization, early embryonic arrest at the blastomere stage, and implantation failure [164]. The subcortical maternal complex, uniquely expressed in mammalian oocytes is composed of at least four proteins: *OOEP, NLRP5, TLE6*, and *KHDC3L* [165]. Maternal nucleotide variants in the *TLE6* or *NLRP5* gene have been linked to arrested embryo development on Day 3 and failure to form blastocysts [159, 160]. Pathogenic variants in *KHDC3L* are associated with recurrent hydatidiform mole and have also been found in patients with embryos arrested at the morula stage [166].

Loss-of-function variants of *PATL2* were reported in infertile females affected by oocyte germinal vesicle arrest and primary infertility [157, 158]. *PATL2* encodes an RNA-binding protein that acts as a translational repressor and is highly expressed in human germinal vesicle and polar body MI oocytes. The exact role of PATL2 and mechanism leading to oocyte maturation arrest is unknown, although PATL2 deficiency may impact canonical translational repression and further hinder protein synthesis. Defects in *WEE2* result in female infertility due to inability of the oocyte to exit metaphase II [161]. Oocytes from women with loss-of-function pathogenic variants in *WEE2* fail to complete MII exit and form pronuclei, resulting in fertilization failure. The *TUBB8* gene encodes a *beta*-tubulin subunit that is essential for oocyte meiotic spindle assembly. Oocytes of patients with pathogenic variants in *TUBB8* show no spindle formation or disorganized spindle and commonly fail to extrude a polar body. In some instances, the first polar body can be extruded in the absence of a properly assembled spindle; however, those embryos still fail to progress beyond the 4- or 8-cell stage [167].

Post-implantation pathologies: hydatidiform moles

Triploid human pregnancy, a conception that contains an additional set of chromosomes resulting in a total of 69 chromosomes in each cell, is an aberrant condition characterized by early embryonic arrest or first trimester miscarriage. Triploid embryos resulting from fertilization by two spermatozoa are detected in ~1–2% of conceptions [168] and are thought to be caused by inefficient polyspermy blockage by the zona pellucida. Triploidy may also occur from the fertilization of a diploid egg by a haploid sperm (digynic triploidy) or fertilization of a haploid egg by a diploid sperm (diandric triploidy or partial hydatidiform mole). Although rare, recurrent familial cases of triploidy due to a failure to extrude the polar body in meiosis suggest a genetic predisposition [169–171]. Such oocyte maturation defects may lead to an early miscarriage of an unrecognized pregnancy and a diagnosis of primary infertility. Complete hydatidiform mole, a pregnancy formed by the fusion of two sperm or by duplication of a paternal haploid genome in the absence of a maternal counterpart, can be caused by biallelic pathogenic variants in the *MEI1*, *REC114*, or *TOP6BL* genes [172]. These genes are involved in double-strand DNA break formation, and defects can lead to extrusion of chromosomes from oocytes into the first polar body, creating an "empty" egg. Women with such a meiotic abnormality may suffer infertility, recurrent miscarriage, complete hydatidiform mole with excessive trophoblastic proliferation, and failure of embryonic development.

Another cause of a highly recurrent hydatidiform mole with a diploid (normal) set of chromosomes is autosomal recessive inheritance of pathogenic variants in the *NLRP7* and *KHDC3L* genes [173–175]. Interestingly, hydatidiform moles in this condition are not androgenetic but have biparental genomic complements. In such condition, a normal haploid oocyte has been fertilized by a haploid spermatozoon; however, the maternal genome failed to establish a maternally-specific gene expression profile, resulting in trophoblastic proliferation and embryonic failure attributed to abnormal imprinting similar to a phenotype seen in androgenetic molar conceptuses. To date, *NLRP7* biallelic sequence variants, genomic deletions, and complex rearrangements have been observed in affected females [173]. In addition, a heterozygous carrier of a pathogenic variant in the *NLRP7* gene with a history of recurrent miscarriages and abnormal imprinting in two deceased children has been reported [176]. Although women who carry biallelic variants in these genes are healthy, they have a risk of reproductive failure and a possible impact on the fetal imprinting profile and

viability. Other genes involved in establishing a female-specific DNA methylation pattern in mature oocytes remain to be elucidated.

Female infertility due to recurrent miscarriages and fetal lethality

Recurrent miscarriages, defined as two or more consecutive pregnancy losses, may be attributed to maternal, placental or fetal causes of infertility. It has been estimated that up to 60% of all conceptions will abort spontaneously within the first 12 weeks of gestation [66]. Beside fetal aneuploidy, which is responsible for 50–60% of overall fetal losses, many risk factors have been recognized to cause recurrent miscarriages, including anatomical uterine anomalies, polycystic ovary syndrome, antiphospholipid syndrome (up to 15%), endocrinological abnormalities (~10%), unbalanced products of parental balanced translocations (2–4%), X chromosome aberrations, thrombophilic conditions such as heterozygous variants in the coagulation factor II gene (*F2*) and coagulation factor V gene (*F5*), placental insufficiency due to defects in the placental anticoagulant protein annexin A5 (*ANXA5*), as well as other genetic conditions affecting fetal viability [177–180]. Numerous studies have investigated the role of polymorphisms in the methylenetetrahydrofolate reductase gene (*MTHFR*) in the risk of recurrent miscarriages [181]; however, the associations are inconsistent, and *MTHFR* genotyping has no clinical utility at the present. Whole exome and genome sequencing approaches may help in defining the monogenic and complex genetic causes of diminished fetal viability in couples with recurrent miscarriages and stillborn children.

Infertility due to developmental defects in female reproductive organs

Genetic factors play a crucial role in organogenesis of the reproductive system. Beside gonadal dysgenesis, malformation of fallopian tubes and uterus, uterine leiomyoma, or endometriosis, can lead to infertility, miscarriage, premature labor, or fetal death [123, 182]. Abnormalities in the female reproductive tract occur in up to 3.0% of live births in humans and include agenesis, atresia, and abnormal septation of the oviducts (fallopian tubes), uterus, cervix, or vagina, which embryologically arise from the Müllerian duct. Heterozygous sequence variants, deletions, and expansion in the polyadenosine tail of the *HOXA13* gene are well-known causes of hand-foot-genital syndrome characterized by fusion of Müllerian structures and uterine malformations [183, 184]. Rare variants in other Homeobox A family genes (*HOXA10* and *HOXA11*) as well as aberrant expression of HOXA11-AS1 antisense RNA (a long noncoding RNA) have been identified in individuals with sporadic uterine malformations and infertile women with endometriosis [185, 186]. Heterozygous pathogenic variants in *HNF1B, LHX1, WNT4, WNT7A,* and *WNT9B* have been reported in individuals with syndromic and variable uterine malformations, including Mayer-Rokitansky-Küster-Hauser syndrome [187]. Less solid evidence links *PAX2, PAX8*, and *EMX2* genes with female reproductive tract malformations [188].

Concluding remarks and future studies

Mammalian reproduction is regulated by a large number of genes that are essential for differentiation of highly specialized cells, complex intra- and intercellular signaling, and dynamic tissue-specific and organ-specific interactions among hypothalamus, pituitary, and the reproductive tract. Studies conducted on animal models have identified thousands of candidate genes and micro RNAs that might be involved in fertility [189]. Pathogenic alterations in a subset of these genes have been found to cause ovarian dysfunction and infertility in humans. With the advances of exome and genome sequencing and careful phenotyping, substantial progress has been made in the discovery of genes causing isolated and syndromic forms of primary ovarian insufficiency, preimplantation development [153, 190], and germline-limited alterations associated with lethal X-linked and autosomal phenotypes. It is evident that ovarian function depends on the level and timing of transcription of many genes and is controlled by non-coding regulatory DNA sequences [191–193], microRNAs [194–196], and small non-coding RNA molecules [197] that will be the focus of future studies.

For many genes, ovarian dysgenesis and primary ovarian insufficiency are the result of homozygous or biallelic pathogenic variants; however, little is known about the consequences of heterozygous pathogenic variants in these genes. Digenic and oligogenic inheritance in several affected patients points toward a possible polygenic cause of POI and infertility resulting from the cumulative effects of less harmful alterations among genes involved in a highly complex functional interplay. Heterozygous changes in key genes may confer susceptibility loci, while genomic changes in other loci push the effects of any damage above a "threshold." Polygenic inheritance may explain developmental failure in embryos resulting from poor-quality oocytes related to female factors of infertility or incompetence of an embryo or fetus to develop postfertilization due to a biparental polygenic burden. Additional studies, including whole genome sequencing and high-throughput functional assays will continue to enlarge our understanding of the interplay between genomic and reproductive health. Furthermore, a possibility of in vitro gametogenesis from stem cells [198], may offer opportunities to edit pathogenic variants prior to fertilization, and restore fertility in affected individuals.

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