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Review

From Ovarian Development to Folliculogenesis: Essential Networks Sustaining the Ovarian Reserve

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Abstract

In the last four decades, transgenic and knockout mouse models have helped to understand the mechanisms of mammalian sex determination, germ cell development, and adult gonad functions. We have gained crucial insights into molecular factors and pathways of the cells generating either the supporting gonadal cells or germ cells of both sexes. In this review, we highlighted some of the main gene networks and regulatory mechanisms involved in the plasticity of sex-determining pathways that help to establish a functional ovary that can nurture the follicles to generate a good ovarian reserve, both in quantity and quality. Although this level of plasticity is still found in the fully differentiated gonads, errors like mutations or epigenetic modifications impact ovarian development and, later, folliculogenesis, resulting in infertility. To highlight some adverse reproductive outcomes associated with perturbations at the molecular and cellular levels in human folliculogenesis, two examples, i.e., Polycystic Ovary Syndrome (PCOS) and Premature Ovarian Insufficiency (POI), were selected and briefly discussed in this review.

Keywords

Embryonic ovary; folliculogenesis; PCOS; POI; ovarian reserve; fertility; infertility

1. Introduction

The development of the gonadal and reproductive tract (uterus and vagina in females, and seminal vesicles, prostate glands, and penis in males) starts around E10.5 in mice, and they are almost completely developed at birth. In mammals, the genotype first determines the sex in the genetic sex phase. The sex chromosome combination, particularly the presence of a Y chromosome, triggers a male pattern of development while its absence promotes female development. Second, networks of genes and hormones regulate the advancement of sex determination and gonad differentiation, known as the gonadal sex phase. In this review, we focused on the molecular mechanisms of ovarian sex determination and on understanding mutual cross-talks between central molecules in sex development which might impact fertility later in adult life. An error during these developmental steps in females may lead to defective gonads, affecting the differentiation and/or function of the gonads and the development, differentiation, and maturity of the germ cells. This might affect the ovarian reserve and lead to the diagnosis of infertility later in life. Metabolic traits are another key factor, and they may trigger infertility, which can affect the normal development and function of the ovaries as well. The most common human ovarian pathologies that cause infertility are Polycystic Ovary Syndrome (PCOS) and Premature Ovarian Insufficiency (POI), and both pathologies have been briefly discussed at the end of the review. Such a review cannot be comprehensive due to space constraints and I focused on the commitment of the female cell lineage and avoided discussing the male cell lineage.

2. Female Pattern of Gonadal Development

2.1 Bipotential Gonad

From a single primordium, the gonad is initially bipotential and can develop either into a testis or an ovary, demonstrating the plasticity of the undifferentiated gonad. When the differentiation of the primordium is perturbed by either mutation or *in vitro* manipulations, the process of development of the cells toward a male or female pattern is disrupted, the development of the gonad is compromised, and consequently, the gender of the embryo is modified or altered [1-3].

The gonads originate from the mesonephros close to the nephrogenic cords and generate the genital ridges, which are composed of somatic cells derived from the coelomic epithelium and mesonephros, and composed of germ cells after they migrate from the allantois [4]. Until E11.5, the mesonephros of the future gonad cannot be distinguished in female (XX) or male (XY) embryos, and the gonads remain undifferentiated [5]. The differentiation depends on environmental and genetic cues that direct the development of the gonad toward an ovary or a testis pattern.

Five important transcription factors are implicated in the growth and maintenance of the bipotential gonad. During the genital ridge formation, these factors create an intricate signaling network that regulates the growth of the coelomic epithelium (Figure 1A). These factors include GATA-binding protein 4 (GATA4), Empty spiracles homolog 2 (EMX2), Wilms tumor 1 (WT1), Lim homeobox protein 9 (LHX9), and Steroidogenic factor-1 (SF1/NR5A1) [3, 6]. Inactivation of these genes leads to advanced deterioration of the developing differentiated somatic cell lineages (Sertoli

cells in males and granulosa cells in females) and disturbs the mouse gonad phenotypes. Similar phenotypes are observed in both sexes, which suggests that these transcription factors can either function together or are involved in a hierarchical pathway to initiate and preserve the bipotential gonad toward the differentiation step.

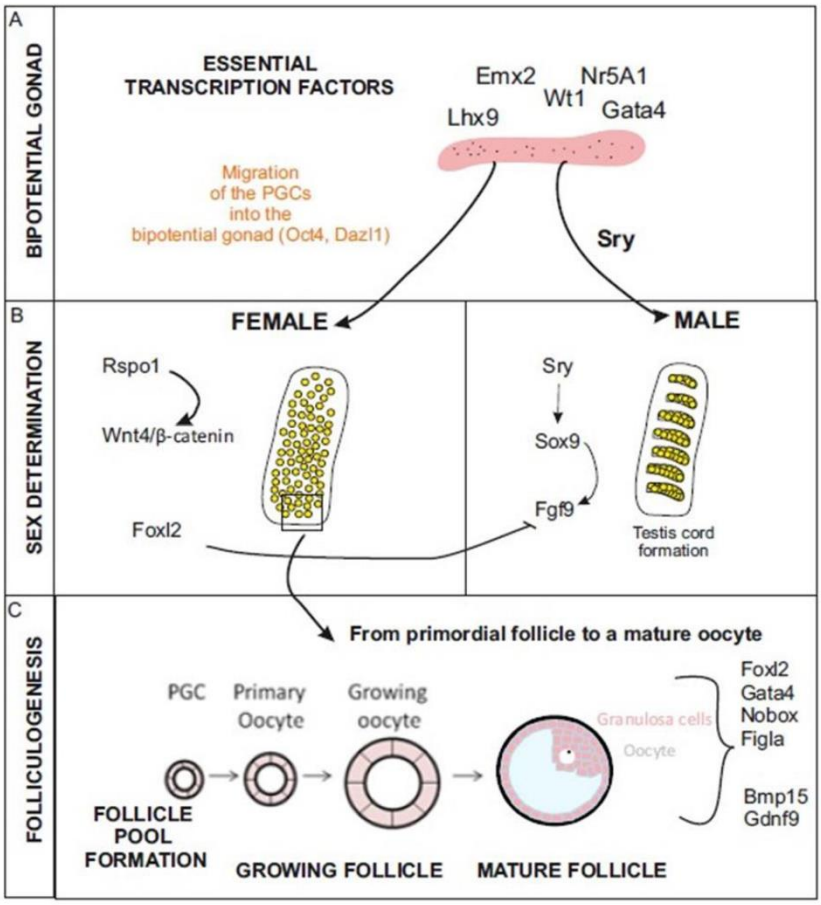

Figure 1 The main developmental events in ovarian organogenesis. A) The five main transcription factors for establishing the bipotential gonad. Following localization in the gonad, germ cells multiply and undergo meiosis in the ovary stopping in prophase I. B) Important signaling pathways in sex differentiation with the highlights of the molecular interactions involved in female and male sex differentiation. C) Formation of the follicle pool, based on the PGCs, which will form the ovarian reserve. The primary follicles are activated to grow and generate a mature oocyte ready to ovulate or, if not ovulated, to become an atretic oocyte.

Deletion of GATA4, EMX2, and NR5A1 transcription factors leads to the absence of gonads. GATA4 is a conserved member of the GATA transcription family and is mostly found in the developing, supporting cell lines in the undifferentiated genital ridge of both male and female mouse embryos at E11.5 [7]. When *Gata4* is deleted specifically in the undifferentiated gonad, the mice fail to initiate the development of the gonad, and the proliferation of the monolayer of initial cells forming the genital ridge is impaired [8]. EMX2, the paired-like homeobox gene, is found in the primordia of the urogenital system in both male and female bipotential gonads [9]. *Emx2*-null mice completely lack the urogenital system, including the kidneys, ureters, genital ridge, and genital

tracts, with a disruption in the formation of the ureteric bud [10]. Furthermore, in the *Emx2* knockout mice, the epithelized somatic cells are reduced in number and are less polarized than in the wild-type mice. This affects the regulation of the tight junction assembly during their migration to form a bipotential gonad due to an increase in cell apoptosis [11]. In the case of NR5A1, which encodes an orphan nuclear receptor, the *NR5A1* knockout mice exhibit adrenal and gonadal agenesis and have female internal genitalia in male and female mice [12]. In humans, most of the observations of the mouse phenotype have been confirmed, such as primary adrenal failure, relatively severe gonadal dysgenesis, and impaired Müllerian structures, due to heterozygous missense mutations in *NR5A1* in 46, XY individuals [13]. The transcription factors WT1, LHX9, and GATA4 bind to the promoter of *Nr5A1* to regulate its expression [14].

The last two transcription factors are involved in the male to female sex reversal due to defects in the formation of the bipotential gonads. WT1 encodes a zinc finger transcription factor which has several alternative splice isoforms. *Wt1* knockout mice present gonadal dysgenesis because of apoptosis of the genital ridge. This phenotype was confirmed through observations in humans. XY patients with mutations in the germline WT1 variant have a male-to-female sex reversal and present gonadal dysgenesis. This confirmed the results observed in the mouse genotype [15, 16]. LHX9 is a member of the LIM homeobox domain gene family and is initially expressed on the surface of the urogenital ridge. *Lhx9* XY knockout mice show a partial male to female sex reversal as the Müllerian duct is kept and the Wolffian duct, which is typical for the developing male embryo, degenerates. Lack of testosterone production could explain the partial sex reversal observed in this mouse [17]. This suggested that the development of the bipotential gonad needs several critical genes, and in their absence, failure of the gonadal development is observed, considering that Sertoli or granulosa cells do not differentiate in these mutant mice.

The primordial germ cells (PGCs), derived from pluripotent cells of the epiblast, migrate into the gonads and colonize the bipotential mouse gonads by 10.5 dpc. They divide mitotically for 2-3 days; their number increases from/50-100 at the beginning of the migration to/25,000 at/13.5 dpc in the mouse [18]. The pluripotent genes POU-Type Homeodomain-Containing DNA-Binding Protein (*Pou5f1* known as *Oct4*) and Nanog Homeobox (*Nanog*) are crucial for the maintenance of PGCs during their expansion [19]. Additionally, some of the PGCs undergo apoptosis to eliminate those which failed to migrate correctly to the genital ridges or divided improperly. The PGCs, colonizing the bipotential gonads, can follow either spermatogenesis or oogenesis. This induction to choose one specific male or female pathway is triggered by the environment and the expression of specific germ cell genes such as *Deleted in azoospermia-like* (*Dazl*) and *DEAD-box helicase 4* (*Ddx4*). DAZL stimulates PGCs to respond to male-specific or female-specific gonadal signals [20].

2.2 Sex Determination

Primary sex determination is established by the sex chromosomes, which activate different genetic programs depending on the sex. As the gonads are composed of multiple cell types (germ cells, supporting cells, endothelial cells, stromal cells, and steroidogenic cells), such sub-fractions need to be well-investigated to characterize the sex determination regulatory networks. In mice, single-cell transcriptomics has elucidated the genetic networks that differentiate the different sub-types of gonadal cells [21, 22]. The results confirmed that sexually dimorphic genetic programs are dynamic and are reinforced by epigenomic modulations that impact transcription and methylation

profiles of the gonadal sub-populations [23, 24]. Errors in DNA methylation can affect the expression of the regulatory genes, which may lead to the development of infertility that is only diagnosed after puberty when young females express primary anestrus (i.e., failure to reach puberty).

The control of primary sex determination triggers the identity of the gonad and maintains the identity of the differentiated adult supporting cells (Figure 1B). The control is complex and regulates active genetic pathways in male and female embryonic gonads. In males, the SRY-related high mobility group (HMG) box (SRY), a conserved HMG DNA-binding domain located in the Y chromosome, is the master regulator of mammalian sex determination. When *Sry* is not activated, then by default, the ovary-determining gene regulatory network is triggered, which is the "default" pathway. In males, the gonadal cells differentiate in a nonsynchronous manner as *Sry* expression starts by E10.5-E11.0 from the center of the gonads toward the poles, and this wave ends by E12.5 [25]. SRY targets genes such as *Sox9*, *Fgf9*, and *prostaglandin D*. After *Sox9* is initiated, its expression is maintained in the fetal and adult testis, which indicates that SOX9 can sustain its expression [26].

In ovaries, the default pathway involves the Wnt and Rspo signaling pathways with two key molecules: R-spondin homolog 1 (RSPO1) and Wingless 4 (WNT4). The RSPO1 protein acts on the Wnt-4 signaling pathway. Both knockout mice have a similar phenotype, a sex reversal from female to male. The gonads of *Rspo-1* and *Wnt-4*-deficient mouse embryos contain similar structures, resembling testis cords associated with coelomic vessels developing in the ventral part of the gonad, which is a pattern characteristic of the testis [27, 28]. Both molecules act either directly or indirectly on the cytoplasmic pool of β-catenin.

β-catenin is also localized at the plasma membrane, and its interaction with cadherins forms intercellular junctions that maintain cell and tissue integrity, regulating the intersection between cell-cell junctions. Contrastingly, the cytoplasmic pool of β-catenin participates in the Wnt signaling pathway. WNT4 and RSPO1 establish a complex with a WNT ligand, increasing β-catenin levels in the somatic cells [28-30]. An increase in the level of β-catenin induced by the activation of WNT4 signaling in granulosa cells leads to degradation of transient *Sox9* expression, preventing the expression of *Fgf9* in the somatic cells and the formation of the testis cords [29]. WNT4 and RSPO1 suppress the male sex determination pathway and sustain the pathway for the female germ cell survival [28, 30].

The Forkhead box L2 (FOXL2) transcription factor is another essential molecule for ovarian folliculogenesis and a key regulator of the development of granulosa cells [31]. In humans, heterozygous F0XL2 mutations lead to ovarian disorders such as BPES (Blepharophimosis Ptosis Epicanthus Inversus Syndrome), which is associated with premature ovarian insufficiency (POI). In mice, *Foxl2* null mutations do not affect initial ovary formation [32], indicating that FOXL2 is more important in humans for ovarian development than in mice [33]. Vidal et al. showed that FOXL2 downregulates SOX9 and Inhibinβ-B (INHBB), two key proteins maintaining male identity [34]. After the activation of the Rspo1/Wnt-4/β-catenin and Foxl2 signaling pathways, two female-specific genes, Bmp2 and Follistatin (Fst), are induced [35]. Fst expression is activated by the Wnt-4 signaling pathway, and its expression is maintained by bone morphogenetic protein 2 (BMP2) and FOXL2 [36]. By inducing the Wnt-4/Fst signaling cascade, Inhbb expression is inhibited. The function of FOXL2 in ovarian differentiation is supported by different studies, which have shown that, in mice, deletion of *Foxl2* interrupts the ovarian follicle formation and leads to partial ovary-to-testis sex reversal. The somatic cells in knockout mice start to differentiate into Sertoli cells and form the seminiferous tubules [32, 37]. Such development takes place independently of the activities of WNT and RSPO1

[38], as the complete ablation of *Wnt4* and *Foxl2* leads to complete female to male sex reversal [36]. However, when *Foxl2*$^{-/-}$ *Rspo1*$^{-/-}$ are deleted together in female mouse gonads, the resulting ovaries are more masculinized than XX *Rspo1*$^{-/-}$ female gonads, indicating that Rspo1 and Foxl2 function synergistically to ensure normal differentiation of the ovary [39]. Furthermore, it has been hypothesized that the induction of WNT4, BMP2, and FST signals is required to block the formation of the coelomic blood vessel in the ovary and to sustain germ cell survival [40]. Sex determination is based on the ongoing induced antagonism mechanisms between male and female gene networks that are essential for the differentiation of the supporting gonadal cells.

2.3 Formation of Primordial Germ Cells

After the colonization of the gonad (Figure 1A), *Deleted in azoospermia-like* (DAZL1), encoding an RNA-binding protein is necessary to maintain the developmental potential of the germline and determine the fate of germ cells. In mouse embryos lacking DAZL1, PGCs migrate to the gonads but maintain the expression of a network of pluripotency factors and retain the ability to give rise to pluripotent cell lines until at least E15.5, independent of the gender of the embryo, resulting in teratoma formation [41]. During normal ovarian differentiation, PGCs form clusters called germ cell nests or cysts, which originate from one founder cell of the group and are interconnected by intercellular bridges. This is crucial for the foundation of oocyte development [42]. By 13.5 dpc, female germ cells enter meiosis to become primordial oocytes and get arrested at the prophase of meiosis I. This step is regulated by the expression of retinoic acid (RA), which is known as the meiotic inducer. Without RA, the female PGCs fail to initiate meiosis [43].

2.4 Folliculogenesis and Ovulation

Folliculogenesis comprises two major steps: the initial follicular recruitment and the cyclic recruitment [20] (Figure 1C). The first step characterizes the ongoing activation of the primordial follicles, which are the ovarian reserve of pre-antral follicles. This step occurs just after birth and takes place throughout the female reproductive life until senescence. It is independent of FSH and LH and is referred to as the gonadotropin-independent phase. It leads to the multiplication of granulosa cells to form several layers around the oocyte regulated by intraovarian factors. The second step involves cyclic recruitment and is defined as the growth of the preantral follicle to the early antral follicle, where the oocyte acquires FSH receptors [20]. This is crucial for nurturing and maturing the oocyte properly. The receptors of the FSH ligand are in the granulosa cells of the follicles, and their level of expression increases while the follicles mature from preantral to antral follicles. FSH prevents apoptosis of granulosa cells and follicular atresia [20]. The acquisition of FSH dependence in the preantral follicles is stimulated by the theca cells that sustain gonadotropins.

The acquisition of FSH dependence is known as the gonadotropin-responsive phase, and it only occurs after puberty. At the end of this step, a transition from FSH to LH dependence is necessary to prepare for ovulation. This is known as the gonadotropin-dependent phase [20]. The LH ligand receptors are expressed in theca cells (steroidogenic cells) and mural granulosa cells in the antral follicle until ovulation; when the oocyte is ovulated, LH receptors are observed only in the theca cells and the corpus luteum. For the correct progression of the follicles toward ovulation, the acquisition of sufficient numbers of LH receptors is very important [44]. In response to FSH, the granulosa cells synthesize large amounts of estradiol controlling oocyte meiotic resumption,

especially in the dominant follicles [45]. Two theories were proposed to explain the selection of the dominant follicle in humans. The first one is based on the level of estradiol (E2) and Inhibin-B secreted by the granulosa cells of the early antral follicles. E2 and Inhibin-B regulate FSH by reducing its circulating level, and only the follicle with the highest FSHR in granulosa cells can survive and continue to develop as the dominant follicle. The follicles with low levels of FSHR undergo atresia [45]. The second theory is based on follicle selection by the acquisition of LHR in the mural granulosa cells. The first follicle that acquires LH dependence can survive and mature as a dominant follicle [46].

The synchronized assembly of mature follicles in the ovary is associated with an increase in the levels of circulating estradiol, which ultimately peaks and acts on the hypothalamic-pituitary axis to elicit the secretion of LH. LH receptors on preovulatory follicles allow the follicles to respond to the LH surge selectively. Before ovulation, the LH surge triggers the oocyte in the dominant follicle to complete meiosis and generate a haploid gamete ready for ovulation [47]. After ovulation, the luteogenesis process starts; the remaining granulosa cells of the follicle become the corpus luteum and start secreting progesterone to sustain any potential pregnancy.

Selected oocytes mature and become haploid during ovulation to generate a meiotic oocyte II (M2) by going through meiosis II and getting arrested in metaphase II. Several growth factors [BMP15, growth differentiation factor 9 (GDF9)] in the oocytes, along with transcription factors (FOXL2 and GATA4) in the granulosa cells, are triggered to recruit several follicles to form a cohort [48]. In humans, from this cohort, only a single follicle is selected to ovulate an oocyte, whereas, in mice, several follicles are selected for ovulation. GDF9 is essential for the last stage of follicular growth from pre-antral to antral follicles by inducing the production of androgen in the theca cells as it is involved in their differentiation. In preantral follicles, the maximum level of expression of FSHR occurs in the granulosa cells, and the GDF9 signaling pathway regulates androgen synthesis in the theca cells [49].

BMP15 plays a key role in the regulation of fertility in mono-ovulatory mammals compared to polyovulatory animals such as rodents. *Bmp15* knockout mice undergo normal folliculogenesis with subfertility, whereas, in humans, *BMP15* homozygous deletion leads to infertility. In humans, it exhibits an early blockage in folliculogenesis due to the suppression of the sensitivity of granulosa cells to FSH in pre-antral follicles. In humans, 20 mutations have been identified in the *BMP15* gene, which is associated with POI and/or polycystic ovary syndrome (PCOS). Half of these mutations are related to the disruption of BMP15 synthesis and protein production [50].

In mature ovaries, Foxl2 is expressed in granulosa cells of primary and secondary follicles when the follicles mature. Studies on *Foxl2* knockout mice (in which no primary follicles were formed) showed that granulosa cells differentiated under the influence of FOXL2 by changing their shape from squamous to cuboidal granulosa cells [51]. FOXL2 represses the steroidogenic acute regulatory gene (Star), which regulates the transcription of two key genes (aromatase P450scc and cyclin D2), involved in the proliferation, differentiation, and production of steroids in the granulosa cells [51, 52]. FOXL2 has been implicated in the development of POI.

3. Two Examples of Reduced Fertility in Human

If the balance between testis-determining and ovary-determining pathways is disturbed, the spatial and temporal expression patterns of specific female or male genes depending on the sex of

the embryo are altered, and consequently, the fate of the supporting adult cell lineages become disturbed, which affects the functions of the gonads. In the case of ovaries, folliculogenesis is affected, and consequently, the quality and quantity of the ovarian reserve might decrease, resulting in infertility. These conditions not only cause fertility problems but also increase the risk of other health issues in women.

3.1 Polycystic Ovary Syndrome (PCOS)

Polycystic ovary syndrome (PCOS) affects 10-20% of women worldwide and represents 30% of the cause of infertility [53, 54]. The syndrome is also an important trigger for metabolic disorders such as type 2 diabetes, reduced fertility, pregnancy complications, and some psychiatric disorders [54]. Studies have shown that it results from the combination of several factors (impact of genetic and epigenetic, intrauterine, and environmental factors) leading to the development of PCOS. However, the inheritance of PCOS is unclear.

Several GWAS metadata indicates mutations in genes affecting neuroendocrine, metabolic, and reproductive dysfunction (*ERBB4*, *DENN*, *FSHB*, *YAP1*) [55-57], which partially explains the phenotype. Based on these data, several signaling pathways were linked to PCOS, e.g., the ERBB, WNT, PI3K-Akt, and androgen signaling pathways [57-59]. These pathways are active in embryogenesis during the development of the somatic lineages and germ cells. Additionally, some of them, such as ERBB4, are crucial for the proper development and maturation of the follicles [59].

A recent analysis of cohorts demonstrated that PCOS might be inherited and can be transmitted from mother to daughter [60, 61]. These results support the hypothesis that PCOS might originate during fetal life due to prenatal exposure to hormones (primarily steroid hormones) in the uterus. Critical exposure during gestation might permanently reprogram physiological or morphological characteristics of the fetal female gonads and potentially masculinize some other organs like the brain, which might increase the susceptibility to developing the disease in adulthood [62]. Thus, women with PCOS have a higher amount of circulating AMH in the blood and are hyperandrogenic [53]. AMH plays a role in promoting the growth and survival of the pre-antral follicle toward its maturation [63]. Tata *et al.* demonstrated that in mice, in late gonadal development, an excess of AMH alters GnRH receptor signaling in the brain of the female embryos and blocks aromatase expression in the placenta which reduces the estrogen level in the embryos. AMH treatment also affects the circulating testosterone in the female offspring of treated mice (mother) and increases the anogenital distance of the female offspring, reduces the number of corpus lutea in the ovaries, and alters the estrous cycle compared to the offspring of non-treated mice. Perinatal gonadal steroids influence the sexual differentiation of the embryonic brain, and higher androgen levels lead these female embryos to exhibit a masculinized brain [64]. These results indicate the mechanism of transmission from the mother to the first generation of daughters.

A study provided more evidence by analyzing three consecutive generations of female mice. Dihydrotestosterone was injected daily in pregnant mice (mothers) between E16.5 and E18.5 of gestation. The daughters of F1 to F3 presented PCOS-like reproductive and metabolic phenotypes. The three generations of female offspring in the androgenized lineage exhibited a longer anogenital distance than the non-androgenized female offspring, with an increase in body fat. These results suggest a transgenerational effect due to prenatal androgen exposure affecting reproductive and metabolic traits. When the daughter MII single oocytes were sequenced, the transcriptomic profile

revealed enriched genes related to glucose homeostasis, steroid hormone signaling pathways, and mitochondrial activity [65]. These results indicated that high androgen levels during gestation adversely affect genes regulating metabolism during the development of the future female offspring and might be related to transgenerational transmission, considering that the first observed trait in women with PCOS is metabolic dysfunction.

To further confirm the importance of the transmission between the mother and daughters, studies on altered gene expression regarding epigenetics (DNA methylation) were conducted. DNA methylation is a key regulator of gene expression and affects the chromatin state by activating or inhibiting transcription and involving small RNAs. Aberrant DNA methylation might contribute to the etiology of PCOS. Studies on methylation in the granulosa cells of women with PCOS revealed an altered DNA methylation profile of granulosa genes that are crucial for folliculogenesis [66]. This was further confirmed by an experiment with the established animal model where AMH was injected at the end of pregnancy in the mother [64]. The female offspring in the third generation (F3) had the same PCOS-like traits as those in the F1 generation (difference in anogenital distance, corpora lutea, and estrous cycle). As the phenotype was established across generations, the DNA methylation pattern of the ovarian tissue in the F3 female offspring was analyzed and correlated with the transcriptomic data of the same tissue. The results showed that the DNA methylation patterns were altered and correlated with higher expression of genes related to ovarian function and metabolism (regulation of insulin). To determine if this mechanism occurs in non-ovarian tissues as well, the hypothalamus was assessed. The authors found a similar trend of DNA methylation in the hypothalamus as in the ovarian tissues, suggesting that AMH exposure leads to alterations in ovarian and metabolic gene expression in the third generation [67]. These results suggested that the causes of PCOS etiology originate from complex networks involving metabolism, endocrine, and neuroendocrine systems controlling intricate pathways which regulate ovarian function.

As PCOS symptoms often include insulin resistance, the treatment and prevention of insulin resistance might improve the symptoms of PCOS, thus increasing fertility and minimizing other health issues such as cardiovascular risks. One of the frequently administered pharmacological treatments to regulate diabetes is Metformin, which helps to improve insulin resistance and reduce weight, considering that individuals with PCOS are often obese or overweight. Studies that were performed to assess the effects of the combination of metformin and lifestyle interventions (exercise and diet) found an improvement in the outcome of more regular ovulation, and the chances of conceiving increased considerably [68, 69].

3.2 Premature Ovarian Insufficiency (POI)

Premature Ovarian Insufficiency (POI) is characterized by an acceleration of the impairment of ovarian function along with a deficiency in ovarian sex hormones due to the loss of the ovarian follicles. Patients become infertile, estrogen-deficient, and enter menopause. POI is characterized by a decrease in the number of follicles constituting the ovarian follicle pool, which represents less than 1% of the total number of follicles [70]. POI is divided into two categories: induced premature ovarian insufficiency due to cancer treatments (chemotherapy) and spontaneous premature ovarian insufficiency. Both categories of POI affect 0.3% to 1.1% of reproductive-age women who experience menopause prematurely [71]. POI is associated, in the long term, with an increase in the risk of cardiovascular disorders and osteoporosis and with some degree of cognitive deterioration.

Additionally, POI is associated with earlier mortality [71]. The risk of developing POI is high during cancer treatment. Various high-dose chemotherapy regimens and combined chemotherapy and radiation therapy increase this risk when the treatment is administered before or after puberty in young women [72].

About 20 -25% of the POI cases are due to genetic causes that influence the size of ovarian follicles and the rate of follicular atresia and might determine the age of menopause. POI genetic aberrations are mostly associated with the X-chromosome, but other mutations on genes that are known to play a role in folliculogenesis and ovarian function are also associated. These mutations were localized on genes related to ovarian transcription factors FOXL2, NR5A1, newborn ovary homeobox (NOBOX), factor in germline alpha (FIGLA), and growth factors for folliculogenesis BMP 15, GDF-9, and INHA. These genes have been described above and play an essential role in sustaining the granulosa cell line and the maturation of the oocyte during folliculogenesis [73]. To identify potential genetic variants of POI, such as Spermatogenesis and oogenesis-specific basic helix-loop-helix 1 (SOHLH1), FHSR, BMP15, and MutS protein homolog 4 and 5 (MSH), other approaches based on GWAS have been carried out to highlight other genetic associations. These candidate genes regulate the cell cycle, meiosis, or are markers related to the DNA repair machinery [73]. To further understand the regulation of these variants, recent approaches using next-generation sequencing led to the identification of some regulators of the causative genes, such as long non-coding RNA (lncRNA) [74]. The role of lncRNA *HCP5* in POI was elucidated. In granulosa cells, lncRNA *HCP5* regulates *MSH5* expression, which is involved in DNA damage repair in double-strand breaks (DSB) during meiosis I and II. The lncRNA *HSP5* recruits YB1 from the cytoplasm into the nucleus, where YB1 binds to the *MSH5* promoter. In POI, lncRNA HCP5 is absent, and the recruitment of YB1 at the MSH5 promoter does not occur. Thus, MSH5 is not expressed, and any error during DSB is not repaired, leading to the death of the oocytes [75].

4. Future Perspectives

Genes that regulate the determination and differentiation of sex have been discovered for four decades, but more evidence is required to understand their complex network regulation. To further determine their function, next-generation sequencing can help to understand these intricate and dynamic networks. Furthermore, how the epigenome modulates transcription and methylation of these networks in a controlled manner might be better understood, which might provide insights into the unknown genetic etiologies associated with PCOS and POI.

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Author Contributions

The author did all the research work of this study.

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Competing Interests

The author has declared that no competing interests exist.

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