Ovarian Aging

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Contributor: Jan Tesarik

Ovarian aging has a genetic basis that conditions the ovarian activity via a plethora of cell-signaling pathways that control the functions of different types of cells in the ovary. There are various factors that can influence these pathways so as to reduce their efficiency. Oxidative stress, often related to mitochondrial dysfunction, leading to the apoptosis of ovarian cells, can be at the origin of vicious circles in which the primary cause feeds back other abnormalities, resulting in an overall decline in the ovarian activity and in the quantity and quality of oocytes.

Keywords: ovarian aging ; age-related ovarian decay ; premature ovarian insufficiency ; genetics of ovarian aging ; signaling pathways in ovarian aging ; oxidative stress ; mitochondrial function ; mitochondrial therapy ; apoptosis ; melatonin ; growth hormone

1. Introduction

The importance of issues related to ovarian aging has been increasing progressively over the past several decades since, increasingly, more couples in all developed countries choose to postpone parenthood to more advanced female ages [1]. This trend is associated with an increasing rate of aneuploidy in oocytes, causing chromosomal abnormalities in embryos resulting from natural conception, conventional in vitro fertilization (IVF), or intracytoplasmic sperm injection (ICSI) [2][3]. On the other hand, no association has been found between advanced male age and aneuploidy rates in embryos derived from IVF/ICSI attempts using oocytes from young donors [1].

The mechanisms involved in ovarian aging are not completely understood and appear to be multifactorial. A better knowledge of the factors and mechanisms causing age-related or premature ovarian decay is needed to optimize diagnostic tests and tune treatment options so as to reflect the individual condition of each couple.

2. Molecular Mechanisms

The age of menopause is an inheritable trait, and the age at which primary ovarian failure occurs has a strong genetic component [4][5]. However, not all ovarian failures are primary, and different associated pathologies can play a role. Some of them appear to be related to defective DNA repair pathways [6].

2.1. Genetic Basis

Genetic factors can influence ovarian function selectively (primary ovarian insufficiency) or as part of the symptomatology of disorders implicated in other pathologies.

2.1.1. Primary Ovarian Insufficiency

Primary ovarian insufficiency (POI) is the most frequent cause of early menopause, which occurs in about 10% of women before 45 years of age and in 1–2% before 40 years [4], while fertility impairment starts around 20 years before the menopause [7]. Chromosome X structural abnormalities and X-autosome translocations can be at the origin of some cases of POI [6]. As for single-gene perturbations, several genes have been suggested to be implicated in POI (Table 1), some of them located on the X-chromosome and others on autosomes [4][8][9].

Table 1. Overview of the principal nuclear genes with a known role in the protection of the ovaries against aging [1].

Gene	Location	Function
WNT4	1p36.23-p35.1	Female sex determination and differentiation

Gene	Location	Function
FIGLA	2p13.3	Primordial follicle and zona pellucida formation
NOBOX	7q35	Transition from primordial to growing follicles
FOXO3	6q21	Transition from primordial to growing follicles
PTEN	10q23.3	Transition from primordial to growing follicles
FSHR	2p21-p16	Hormone-dependent phase of follicular growth
GPR3	1p36.1-p35	Maintenance of meiotic arrest until the LH surge
MSH4	1p31	DNA mismatch repair during meiotic recombination
MSH5	6p21.3	DNA mismatch repair during meiotic recombination
PGRMC1	Xq22-q24	Apoptosis of ovarian cells
FOXO1	13q14.1	Granulosa cell function
DMC1	22q13.1	Repair of DNA damage during meiotic divisions

[1] See the main text for the full name of each gene. Full review of the genes can be found in references [4][8][9].

The former group includes bone morphogenetic protein 15 (BMP15) (Xp11.2), progesterone receptor membrane component 1 (PGRMC1) (Xq22-q24), androgen receptor (AR) (Xq12), forkhead box O4 (FOXO4) (Xq13.1), premature ovarian failure 1B (POF1B) (Xq21.2), dachshund family transcription factor 2 (DACH2) (Xq21.3), and fragile X mental retardation 1 (FMR1) (Xq27.3).

The genes on autosomes include growth differentiation factor 9 (GDF9) (5q31.1); folliculogenesis-specific bHLH transcription factor (FIGLA) (2p13.3); newborn ovary homeobox gene (NOBOX) (7q35); nuclear receptor subfamily 5, group A, member 1 (NR5A1); steroidogenic factor-1 (SF-1) (9q33); FSH receptor (FSHR) (2p21-p16); TGF beta receptor III (TGFBR3) (1p33-p32); G protein-coupled receptor 3 (GPR3) (1p36.1-p35); wingless-type MMTV integration site family member 4 (WNT4) (1p36.23-p35.1); inhibins: inhibin alpha (INHA) (2q35), inhibin beta A (INHBA) (7p15-p13), inhibin beta B (INHBB) (2cen-q13); POU class 5 homeobox 1 (POU5F1) (6p21.31); MutS homolog 4 (MSH4) (1p31) and MSH5 (6p21.3); forkhead box O3 (FOXO3) (6q21); cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminar domain 2 (CITED2) (6q23.3); spermatogenesis-and oogenesis-specific basic helix-loop-helix transcription factor 1 (SOHLH1) ((9q34.3) and SOHLH2 (13q13.3); phosphatase and tensin homolog (PTEN) (10q23.3); Drosophila nanos homologs 1, 2, and 3: NANOS 1 (10q26.11), NANOS2 (19q13.32), NANOS 3 19p13.13); cyclin-dependent kinase inhibitor 1B (CDKN1B) (12p13.1-p12); anti-Mullerian hormone receptor type II (AMHR2) (12q13); forkhead box O1 (FOXO1) (13q14.1); spalt-like transcription factor 4 (SALL4) (20q13.2); and DNA meiotic recombinase 1 (DMC1) (22q13.1).

However, the implication of some of these genes is suspected rather than confirmed. Those of them with the strongest evidence for playing a role in POI are shown in Table 1.

Most of these genes (Table 1) are known to be somehow related to oogenesis during its different phases, from the fetal period throughout postnatal life until the final phases of meiotic maturation, and to regulate essential events in human oogenesis [4][5]. These include female sex determination and differentiation (*WNT4*), the formation of primordial follicles and the coordinated expression of zona pellucida genes (*FIGLA*), the transition from primordial to growing follicles (*NOBOX, FOXO3, PTEN*), the hormone-dependent phase of follicular growth (*FSHR*), the maintenance of meiotic arrest in antral follicles until the luteinizing hormone (LH) surge (*GPR3*), DNA mismatch repair during meiotic recombination

(*MSH4, MSH5*), the apoptosis of ovarian cells (*PGRMC1*), granulosa cell function (*FOXO1*), and the repair of DNA damage arising from defective meiotic divisions (*DMC1*) [4][6][8][9] (Table 1). However, the incidence of anomalies in some of these genes among women suffering from POI is extremely low, and perturbations of other genes are mostly related to specific ethnic groups [10]. Thus, only a few of these genes, such as *FMR1* premutation, *BMP15*, *GDF9*, and *FSHR*, have been incorporated as diagnostic biomarkers [5][11][12]. More research is needed to use more genes as routine diagnostic tools.

2.1.2. Ovarian Insufficiency due to Mendelian Disorders Implicated in Other Pathologies

Distinct from non-syndromic POI, pleiotropic Mendelian disorders, including fragile X syndrome: familial mental retardation 1 (*FMR1*); (Xq27.3), blepharophimosis-ptosis-epicanthus syndrome (BPES): forkhead box L2 (*FOXL2*) (3q23); galactosemia: galactose-1-phosphate uridyl transferase (*GALT*) (9p13); carbohydrate-deficient glycoprotein syndrome type 1: phosphomannomutase 2 (*PMM2*) (16p13), may manifest POI as part of their phenotypic spectrum [4]. There are some other pleiotropic Mendelian disorders suspected to cause POI, but a definitive confirmation of this association is still lacking.

2.1.3. Gene Mutations Affecting Mitochondrial Function

Genes governing mitochondrial functions may be located in the nucleus or in the mitochondria themselves. Many different nuclear genes affecting mitochondrial function are known nowadays, and according to current experience most gene mutations impairing mitochondrial function are nuclear ones, as reviewed in [4]. Both oocyte and ovarian cell mitochondria are important for the correct folliculogenesis and oocyte maturation [13][14][15][16]. As to the risk of mitochondrial DNA deletions, it was shown to be affected by the global secondary structure of the mitochondrial genome [17].

2.2. Cell-Signaling Pathways

The main cell-signaling pathways involved in physiological (age-related) ovarian failure and POI are those involved in cell protection against oxidative stress. A recent study using single-cell transcriptomic analysis of ovaries from young and aged non-human primates identified seven ovarian cell types with distinct gene-expression signatures, including oocyte and six different somatic cells, and identified the disturbance of antioxidant signaling specific to early-stage oocytes and granulosa cells [9]. The further analysis of cell-type-specific aging-associated transcriptional changes uncovered age-related disturbances of antioxidant signaling specific to early-stage oocytes and granulosa cells [9]. The authors have completed their observations on non-human primates with those on human granulosa cells obtained from follicular fluid samples aspirated from patients undergoing an IVF attempt. Consistent with the results in monkeys, human granulosa cells exhibited the age-related downregulation of the transcription of three genes involved in antioxidative pathways—*IDH1*, *PRDX4*, and *NDUFB10*—and this phenomenon was accompanied by an increase in reactive oxygen species and apoptosis in the granulosa cells [18], indicative of oxidative damage as a crucial factor in ovarian functional decay.

Similar to the primates, an age-related increase in oxidative damage and a decrease in antioxidant gene expression was previously observed in mice [14]. The oxidative damage of mouse granulosa cells was reported to impair the supply of ATP and the mitochondrial gene expression, which are required not only for the proliferation but also the differentiation of granulosa cells during follicular development [13]. Moreover, aging-related mitochondrial (mt) DNA instability also leads to an accumulation of mtDNA mutations in the oocyte, leading to the deterioration of oocyte quality in terms of competence and the risk of transmitting mitochondrial abnormalities to offspring [14]. As mentioned above, the risk of mtDNA damage is also conditioned by epigenetic factors, especially the global secondary structure of the mitochondrial genome; certain patterns of the global secondary structure of the human single-stranded heavy chain of mtDNA make the DNA molecule more prone to deletions than others [17].

Mitochondrial damage leads to the activation of apoptotic pathways in granulosa cells, which, in turn, decreases the expression of aromatase, which is required for the transformation of androgens to estrogens, thus leading to the prevalence of androgens over estrogens within the follicles [18]. Androgens and estrogens present in follicular fluid exert rapid non-genomic effects on maturing human oocytes, affecting oocyte cytoplasmic maturation and postfertilization developmental potential rather than the completion of meiosis [19]. This can explain why age-related or premature disturbances of antioxidative pathways reduce oocyte quality even in cases in which the number of mature oocytes recovered for IVF is not affected.

New data suggest that POI may be of polygenic origin, and that overlap exists between the genetic backgrounds of diminished ovarian reserves and POI [20]. Whole-exome sequencing and bioinformatics analysis may become a useful clinical tool for etiological diagnosis and risk prediction for affected women in the future [20]. In addition, array comparative genomic hybridization or specific next-generation sequencing panels should be considered to identify chromosomal

deletions/duplications under karyotype resolution or other pathogenic variants in specific genes associated with POI. This is particularly important in patients with first-or second-degree relatives also affected with POI, improving their reproductive and genetic counseling [21].

Moreover, even with the same genetic background, with the use of multiple systems biologic approaches to compare developmental stages in the early human embryo with single-cell transcript data from blastomeres, it was shown that blastomeres considered to be totipotent were not transcriptionally equivalent [22].

3. Clinical Management

3.1. Diagnosis

A woman's age is the basic predictor of the degree of ovarian aging. However, ovarian aging can develop prematurely, and specific diagnostic methods are needed to detect this condition. There are two types of ovarian aging manifestations with respect to the ovarian ability to produce oocytes: a quantitative one and a qualitative one. To detect the former, a combination of antral follicle count (AFC), determined by vaginal ultrasound scan at the beginning of the menstrual cycle, and the determination of serum anti-Mullerian hormone (AMH) concentration, which can be performed at any time during the menstrual cycle [23], is used.

By contrast, oocyte quality is not necessarily related to AFC and serum AMH. Premature decay of oocyte quality is mainly related to a low-for-age production of growth hormone (GH) [24]. Due to the pulsatile pattern of GH secretion, insulin-like growth factor-1 (IGF-1), which does reflect the GH secretion pattern, but with a less pronounced pulsatility, was suggested to identify young patients with a premature decay of oocyte quality who could benefit from treatment with GH during ovarian stimulation [25]. The data presented show that the 'GH/IGF-1' age can be more than 20 years higher than the chronological age in some young women, and it was this group of patients who appeared to be more likely to benefit from GH administration during ovarian stimulation, although the authors suggested that larger prospective studies were needed to confirm this assumption [25].

There are only a few clues to detect premature ovarian aging in addition to the above criteria, and further research is warranted to detect more molecular markers that could guide the clinician to propose the best treatment regimen.

3.2. Treatment

In view of the fact that oxidative stress is the main factor involved in ovarian aging, it can be assumed that agents reducing oxidative damage represent the first-line choice. These agents can be direct antioxidants or molecules affecting cell-signaling pathways involved in the antioxidant defense of ovarian cells (Table 2). Some molecules combine both of the above activities.

Table 2. Agents that can be used to treat the consequences of physiological or premature ovarian aging.

Agent	Administration	Mechanisms of Action	References
GH	Subcutaneous	Activation of cell-signaling pathways acting against oxidative stress Possible activation of DNA damage repair	[26][27][28][29][30][31][32]
Melatonin	Oral	Direct antioxidant Indirect antioxidant (signaling pathway modulator) Anti-inflammatory agent Immunomodulator	[33][34][35][36]

Agent	Administration	Mechanisms of Action	References
Coenzyme Q10	Oral	Direct antioxidant	[37][38][39]
Vitamin C	Oral	Direct antioxidant	[40]
Vitamin E	Oral	Direct antioxidant	[40]
Folic acid	Oral	Direct antioxidant	[40]

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