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EXcellence in PCOS & Expertise in Reproductive Technology

MODULE 3:
OVARIAN AGING: CHALLENGES AND THERAPEUTIC OPTIONS

Brought to you by

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An Initiative of PCOS Society (India)

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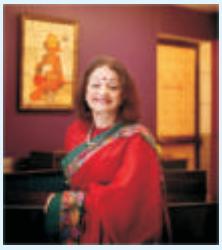
Foreword



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PCOS is quite often associated with infertility, especially in women with irregular periods and hyperandrogenemia. It is one of the most treatable forms of infertility, if ovulation induction is optimal. Unfortunately, many PCOS women, both lean and obese, behave erratically during ovulation induction sometimes leading to ovarian hyperstimulation syndrome which can become a serious iatrogenic complication. Keeping in mind that many PCOS women approach us for management for their infertility issues, it is important for us to understand the principles of management. Which when applied correctly, they can give us great success in making PCOS women pregnant. Of course experience counts, and as we continue treating women, we learn how to tweak our stimulation protocols to avoid complications and give us the best results!

After initiating the Basic Course on Infertility in 2018, we are delighted to introduce to you the Advanced Course called **"EXPERT"- (Excellence in PCOS and Expertise in Reproductive Technology")** a Certificate Course brought to you by the PCOS Society of India, through an unrestricted educational grant by Sun Pharma, Inca Life Sciences.

"EXPERT" will be presented to you in a set of 6 Modules which will update you on various aspects of the management. Infertility in PCOS, from minimal intervention to Assisted Reproduction.

Once you complete the 6 Modules, you could participate in an Online Exam, Assessment and on clearing it, you will be eligible to receive a beautiful certificate from the PCOS Society of India, which you will be extremely proud to display! To own this Certificate, you need to be a member of the PCOS Society, India!

To become a member, please log on to http://www.pcosindia.org/ to download your form and become a Life Member or Patron Member of the PCOS Society of India

If you have any queries, please write to us at thepcossociety@gmail.com

Both Madhuri and myself have worked hard on creating this program and we thank the team at Sun Pharma for their support in making this program a reality !

Enjoy reading.

With warm regards,

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OVARIAN AGING: CHALLENGES AND THERAPEUTIC OPTIONS

Ovarian Aging: An Overview

Introduction to Physiological Ovarian Aging

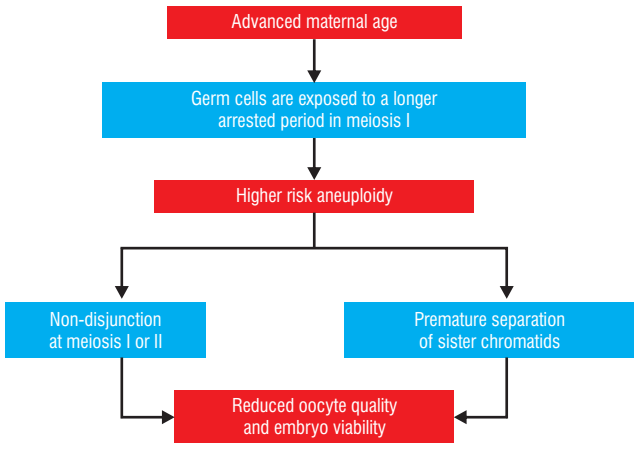
Reproductive function in humans declines with age; however, the decline is more rapid in women. Women abruptly lose fertility at menopause, whereas male fertility declines slowly and progressively after 45—even so, some males are still fertile even after 70 years.[1]

Ovarian aging can be described as the age-related decrease in the follicle numbers of the ovaries that may result in the irregular onset of menstrual cycles and ultimate cessation of menses. Oocyte quality also decreases in parallel, which leads to a gradual decline in fertility until natural sterility occurs.[1] Folliculogenesis continues throughout life: from before birth until menopause. The gradual decline in the size of the antral follicle cohort with age is associated with decreasing levels of anti-Müllerian hormone (AMH) and inhibin B and gradually elevated follicle stimulating hormone (FSH) levels. The maximum germ cells are seen at 20 weeks, when they reach 6 million. At birth, only 2 million are left; and at puberty, there are 300,000 to 400,000—of which only 400–500 will be selected to ovulate, in the entire reproductive span till menopause is achieved when only a few hundred to a thousand remain.[2]

It has also been observed that the decrease in the number of follicles is associated with diminished oocyte quality, which is mainly due to an increase in meiotic non-disjunction, resulting in an increased rate of aneuploidy in the early embryo (Figure 1). There is a change in fertilizability and implantation potential and an increase in the spontaneous abortion rate. Alteration in ovarian endocrine function occurs due to aging of the granulosa and theca cells and impaired microcirculation.[2,3-6]

In general, women aged between 30 and 35 years have a slow but steady decrease in fertility, which is followed by an accelerated decline after 35 years. Ovarian aging among women is variable and not fixed, which is evident from the large variation in age at menopause.[2]

Figure 1: Increased risk of aneuploidy with advanced age.



Folliculogenesis, Oocyte Growth, and Development

A 'folliculogenesis clock' exists that is set by the oocyte. The entire process of folliculogenesis takes 4–6 months: a number of primordial follicles are recruited from the pool of resting follicles for further growth. It has been seen that the pool of primordial follicles recruited for the development increases with age and is related inversely to the density of follicles in the ovary. Oocyte and the surrounding somatic cells undergo a series of changes that eventually result in the formation of a large antral follicle capable of ovulating a mature oocyte. Growth factors and cytokines control primordial follicle development and activation. Regulatory capacity is achieved through the synthesis and secretion of oocyte-specific factors, growth and differentiation factor-9 (GDF-9) and bone morphogenetic protein 15 (BMP-15), through their action on the granulosa cells to modify their proliferation, function, and differentiation. Primordial follicle activation appears to require close communication with somatic cells (granulosa cells) have definite role in releasing oocytes into the growing pool. The trigger for follicular activation is oocyte-generated, and early response must include suppression of FOXO3 activity. During primordial follicle assembly, FOXO3 is imported into the nucleus and re-exported upon primordial follicle activation due to oocyte-specific loss of phosphatase and tensin homolog (PTEN) to induce PI3K activation. Once activated, oocytes orchestrate and coordinate the development of ovarian follicles. The rate of follicle development is controlled by oocytes.[3-6]

During oogenesis, oocytes acquire molecular and cellular properties in a sequential manner that confers meiotic and developmental competence. Following an luteinizing hormone (LH) surge, a competent oocyte can complete meiosis, sustain fertilization and oocyte activation, and organize the transition from maternal transcripts to gene products of embryonic origin. After resumption of meiosis (MI), there is first the segregation of homologous chromosomes from each other MI and then segregation of sister chromatids that are analogous to mitotic division.[3-6]

Although chronological age is the most important predictor of biological ovarian aging, both environmental and genetic factors have an impact on the ovarian response to the FSH. Ovarian aging results in reduced fecundity (the ability to produce offspring) in women who delay childbearing. This probably leads to an increase in the proportion of women ≥35 years of age seeking assisted reproductive technology (ART) treatment.[7]

Transition Period and Human Reproduction: Age-Related Loss of Fertility

- Loss of fertility precedes menopause by ~12 years.
- ~10% of women enter menopause in their early 40s.
- ~1% of women enter the menopause in their 30s, mostly due to starting off with fewer eggs.[3-6]

Cause of Declining Fertility with Ovarian Aging

1. Higher incidence of subtle cycle disorders, such as anovulatory or luteinized unruptured follicle (LUF) cycles
2. Hormonal changes
3. Diminished uterine receptivity
4. Oocyte factor
5. Biochemical loss of pregnancy
6. Increased incidence of spontaneous abortion[8,9]

It is seen that with increasing age, the follicular phase is shorter and the diameter of the dominant follicle before ovulation smaller, which is probably an indication of diminished follicle quality. It was also observed that the dominant follicles identified earlier are probably due to the early growth of follicle as a result of high intermenstrual FSH. It is not clear whether with age, the follicle has accelerated growth or advanced growth.[8,9]

One must remember that the probability of achieving a pregnancy within one year is significantly higher in women <30 years than those in women >35 years. The incidence of pre-implantation or post-implantation loss increases after the age of 35 years.[8,9]

Neuroendocrinology of Ovarian Aging

The pre-ovulatory follicular life cycle can be categorized into 3 successive phases:

a) **Initiation phase:** Occurs from birth to old age and is independent of gonadotropic support

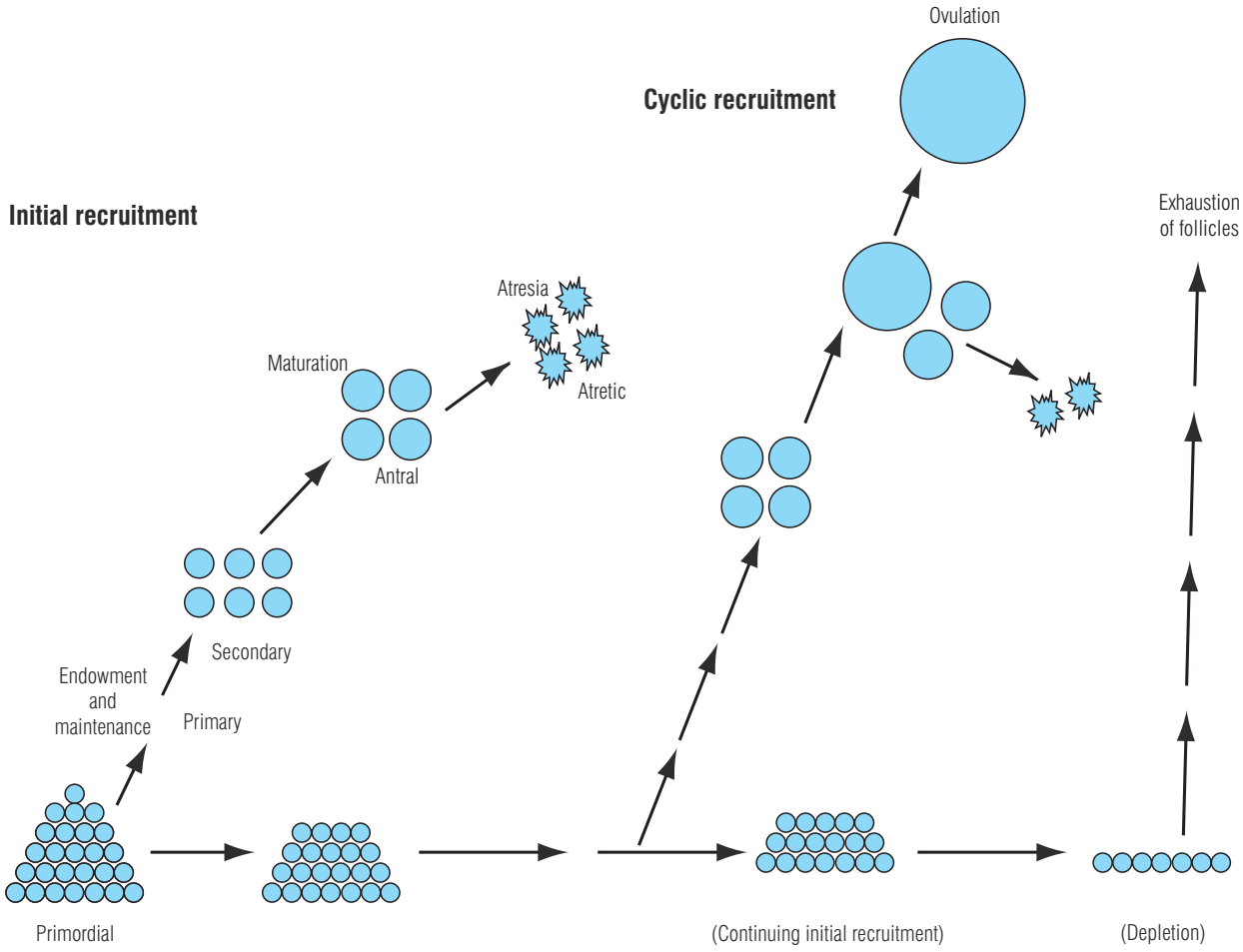
b) **FSH-dependent progression phase:** Requires tonic stimulation by FSH

c) **LH-responsive maturation phase:** Occurs when FSH-induced genes fall under LH control, leading to estrogen secretion and ovulation[7]

The ovaries contain approximately 1–2 million oocytes at birth; however, only a small fraction of viable oocytes is present in the ovaries at the onset of puberty. The oocytes are arrested at the first meiotic prophase before puberty. Upon activation by an ovulation-inducing LH, these oocytes undergo atresia or resume meiosis to form a haploid gamete for fertilization. Almost 99.9% of the oocytes undergo atresia by various apoptotic mechanisms.[7]

The process of folliculogenesis is categorized into 3 stages (Figure 2).

Figure 2: Ovarian follicles formation, maturation, atresia or cyclic recruitment, ovulation, and exhaustion.[2]



Adopted from: Alviggi C, *et al*. 2009;7:101.

Intermediate stage: This stage involves the formation and growth of the antral follicle until it reaches a diameter of approximately 2–4 mm. The antrum is formed by the collation of fluid-filled spaces between the granulosa cells. The formation of the antrum depends on cyclical gonadotropin stimulation. All antral follicles that develop before puberty will become atretic.[7]

Ovulation period: In the ovulation phase, the antral or pre-ovulatory follicle (one developed in each menstrual cycle) increases in diameter from approximately 5 mm at the beginning of the cycle to more than 20 mm by the time of ovulation. This follicle increasingly secretes estrogen and inhibin (INH), the classic biomarkers of pre-ovulatory follicular development. These hormones lead to the mid-cycle discharge. Resumption of oocyte meiotic maturation and ovulation is triggered by LH.[7]

Mechanisms of Ovarian Aging

Follicular Loss and Menopause

Ovarian aging is indicated by the loss of follicles from the ovary, associated with a reduction in oocyte quality. The rate of follicle loss in the ovary follows a biexponential pattern. At approximately 37.5 years of age, the follicle number falls below the critical level of 25,000; from this point onwards, the rate of follicle loss accelerates (almost doubles) until no residual follicular stock remains. Menopause occurs at an average age of 51 years, when approximately 1000 follicles remain in the ovaries.[7,10]

Role of FSH in Termination of Folliculogenesis

During the pre-menopause period, circulating levels of FSH gradually increase with the increased loss of residual follicular stock. The rise in levels of FSH is probably due to reduced secretion of follicular growth and differentiation factors, including INH and anti-Müllerian hormone (AMH). The increase in basal circulating levels of FSH results in inappropriate maturation of granulosa cells of the residual pre-antral follicles. These follicles eventually become atretic due to asynchronous maturation.[2,3]

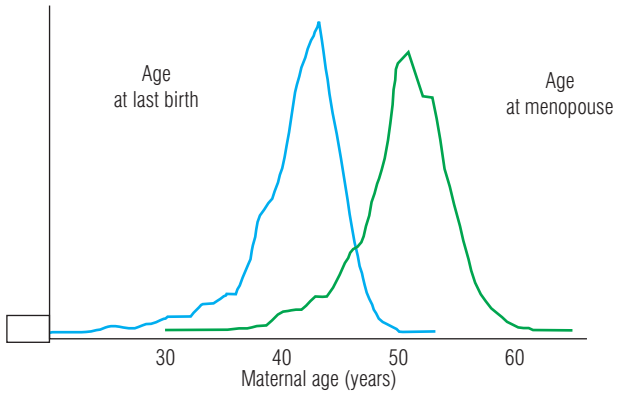
Variability in Ovarian Aging

The process of ovarian aging varies considerably among women and is mainly determined by genetic factors such as initial oocyte stock, the proportion of follicles that undergo atresia, and the rate of initiation of follicular growth. The occurrence of menopause is also vastly different among individuals. Evidential studies report a difference of approximately 10% between the age at which women become sterile and their age at menopause (Figure 3).[2,4]

Variability in Ovarian Aging Reflected by Levels of Gonadotropins

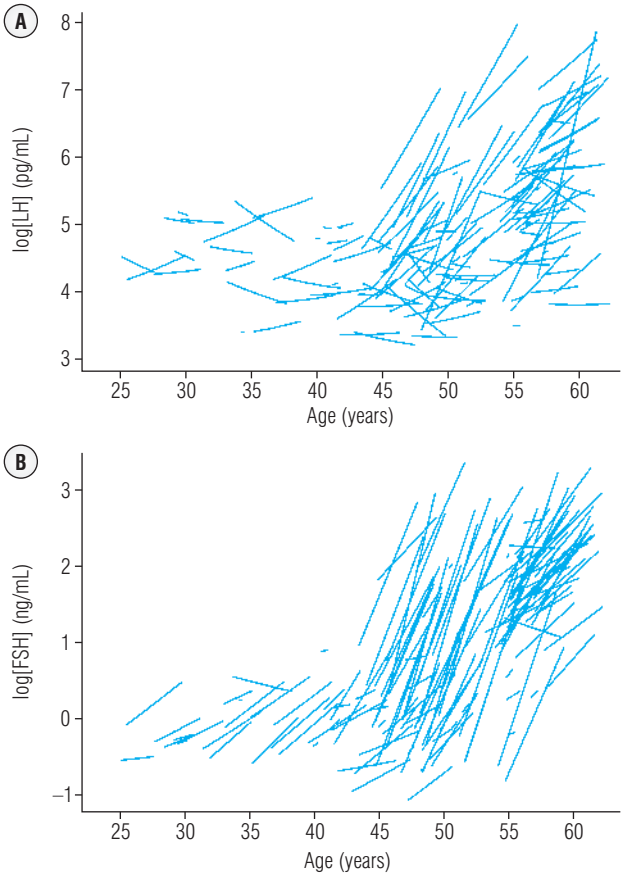
Several studies have shown that the levels of follicular gonadotropins increase significantly during the process of ovarian aging.[11] The FSH levels begin to increase long before the onset of menstrual cycle irregularity, and continue to rise thereafter.[12] Levels of both FSH and LH rise steadily during the peri-menopausal period due to follicle depletion.[11,12]

Figure 3: A comparison of distribution between age at last birth and age at menopause.[9]



A 5-year prospective study conducted by Ferrell *et al*. (2007) investigated age-related changes in LH and FSH in a group of 156 women within an age range of 25–58 years. Both FSH and LH rapidly increased during the later peri-menopausal stages. FSH levels increased from normal to high within a relatively short time frame during the peri-menopausal years (<5 years). Although the most rapid increase in aggregate and individual FSH levels occurred after the age of 45, increasing FSH levels were observed even in young women. Figure 4 illustrates that the serum LH (A) and FSH (B) rise started at different concentrations for each woman.[13]

Figure 4: Fitted individual trajectories of (A) luteinizing hormone and (B) follicle-stimulating hormone.[13]



The findings of Ferrell *et al*.confirmed that both FSH and LH levels increase with age, but that the timing and magnitude of these changes were different for each hormone and varied among individuals.[13]

Summary

- Ovarian aging can be described as the age-related decrease in ovarian follicle numbers associated with a parallel decrease in the oocyte quality that contributes to the gradual decline in fertility and the final occurrence of natural sterility.
- The rate of oocyte decline follows a biphasic pattern, with a distinct acceleration as women progress towards menopause.
- The increased basal circulating levels of FSH due to loss of follicular stock play a key role in the termination of folliculogenesis.
- The process of ovarian aging varies considerably among women and is mainly determined by genetic factors such as initial oocyte stock, the proportion of follicles that undergo atresia, and the rate of initiation of follicular growth.

Genetic Factors Influencing Rate of Ovarian Aging

Genetic variations may influence the rate of ovarian aging by affecting the ovarian response to gonadotropins. This may also result in premature menopause.[2]

Haploinsufficiency of the FSH Receptor Accelerates Oocyte Loss Inducing Early Reproductive Senescence and Biological Aging

Danilovich *et al*. (2002) examined the endocrine and signaling parameters of a genetic variation that might contribute to a decrease in ovulation and reproductive performance in mice. A total of 87 wild-type mice and 92 heterozygous mice with haploinsufficiency of the FSH receptor (FSH-R) were included in the study. Ovarian changes and hormone levels at different ages (3, 7, and 12 months) of FSH-R heterozygous (+/-) mice and wild-type mice were compared.[14]

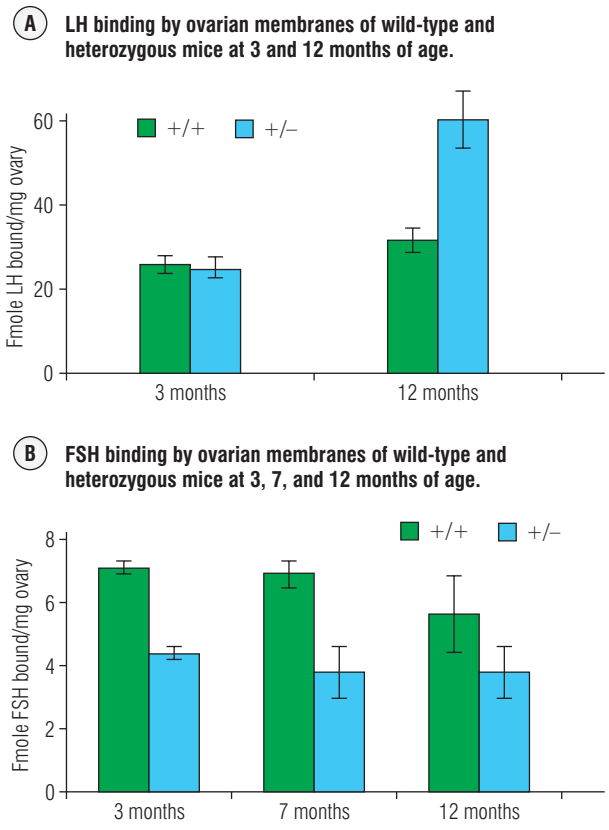
Haploinsufficient FSH-R+/- mice consequently had ovarian insensitivity due to having a reduced number of FSH receptors. FSH-R+/- mice reached reproductive maturity earlier than wild-type mice, but had a smaller litter size compared to their wild-type counterparts. There was no difference in the total number of follicles in the ovaries of 3-month-old FSH-R+/- vs. wild-type mice. However, the ovaries of 7-month-old FSH-R+/- mice contained significantly fewer oocytes than wild-type controls, and levels of serum gonadotropins were higher (Figure 5).[14]

The study findings suggest that the haploinsufficiency of the FSH-R gene may cause premature exhaustion of the gonadotropins, resulting in reproductive failure.[14]

Low Oocyte Mitochondrial DNA Content in Ovarian Insufficiency

Mitochondrial biogenesis plays an important role in oocyte maturation and embryo development. A study conducted by May-Panloup *et al*. (2005) investigated the negative impact of genetically defective mitochondrial biogenesis on oocyte maturity in patients with ovarian dystrophy and ovarian insufficiency. A total of 116 oocytes were obtained from 47 women undergoing IVF procedures. The oocytes were classified into three groups: Group 1 consisted of 39 oocytes collected from 14 patients with a normal ovarian profile; Group 2 consisted of 47 oocytes from 16 patients with ovarian dystrophy; Group 3 consisted of 30 oocytes from 17 patients with ovarian insufficiency. The mitochondrial DNA (mtDNA) content of oocytes from women with a normal ovarian profile was compared with that of oocytes from women with ovarian dystrophy and ovarian insufficiency.[15]

Figure 5: Status of gonadotropin receptors in the ovary.



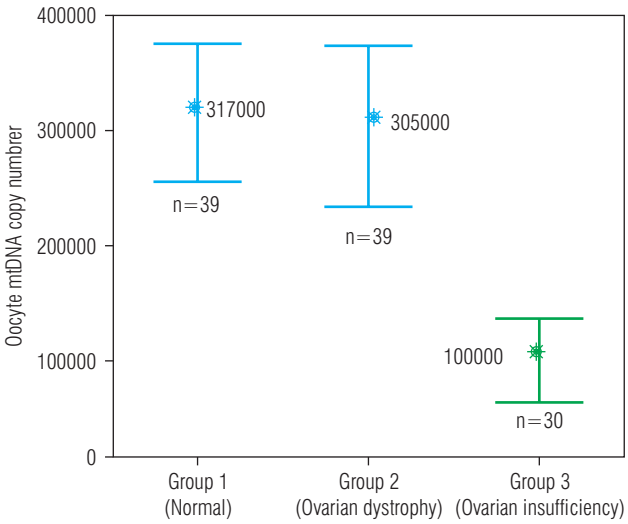
Although the mean mtDNA copy number was not different in the ovarian dystrophy group when compared to the control group, it was significantly lower in the number of oocytes from women with ovarian insufficiency ($100{,}000 \pm 99{,}000$, $p < 0.0001$) (Figure 6).[15]

The study results, therefore, suggest that low mtDNA content is associated with impaired oocyte quality, as observed in women with ovarian insufficiency.[15]

Genetic Deficits of mtDNA Results in Chromosome Anomalies in Human Pre-implantation Embryos

Genetic mutations in the mitochondrial DNA may result in mitochondrial dysfunction and abnormalities in chromatid separation of embryos. Munne *et al*. (1995) investigated the effect of mutant mtDNA on chromosome anomalies in human pre-implantation embryos. A total of

Figure 6: Comparison of mtDNA content in oocytes between the three groups of patients.[15]



524 cleavage-stage human embryos were obtained from IVF procedures and were analyzed simultaneously by fluorescence *in-situ* hybridization using probes for 3 or 5 chromosomes. The embryos were categorized into 3 groups according to morphological and developmental characteristics as arrested embryos, slow and/or fragmented embryos, and normal embryos.[16]

Genetic variations in mtDNA resulted in an age-related increase in oocyte aneuploidy and to a subsequently raised risk of having a fetus with a chromosome abnormality. Aneuploidy increased with maternal age in non-arrested embryos. Dysmorphic embryos had higher rates of polyploidy and diploid mosaicism. Pre-implantation genetic diagnosis successfully detected these abnormalities. The study results, therefore, suggest that in morphologically and developmentally normal human embryos, cleavage-stage aneuploidy significantly increases with maternal age.[16]

Chaotic Mosaicism in Human Pre-implantation Embryos Correlated With a Low Mitochondrial Membrane Potential

The mitochondrial membrane potential decreases linearly with increasing age. Mitochondrial activity strongly influences the inherent developmental potential of individual oocytes. Wilding *et al*. (2003) examined the relationship between intrinsic embryo mitochondrial membrane potential and chromosome constitution of the developing embryo. Living embryos were collected from a total of 52 patients who were undergoing IVF. The mitochondrial membrane potential of the living embryos, followed by chromosomal enumeration, was analyzed with fluorescence *in-situ* hybridization.[17]

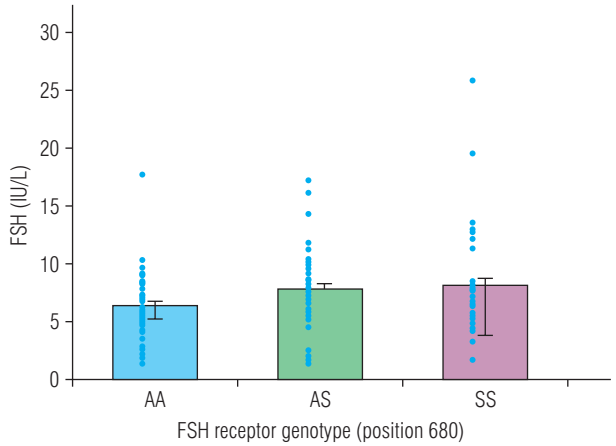
A correlation was observed between low mitochondrial membrane potential and the detection of chaotic mosaicism that influences the developmental fate of embryos. An analysis of oocytes suggested that the correlation was due to the effect of low mitochondrial membrane potential on the morphology of the meiotic apparatus.[17]

Ovarian Response to Follicle-Stimulating Hormone (FSH) Stimulation Depends on FSH Receptor Genotype

The importance of genetic characteristics in determining ovarian cycle and ovarian morphology with respect to the FSH receptor is described in the study by Perez Mayorga *et al*. (2008). The response of two distinct FSH receptor (FSHR) variants, Thr307/Asn680 and Ala307/Ser680, to FSH stimulation was studied in 161 ovulatory women undergoing controlled ovarian stimulation.[18]

The distribution of infertility was 29% for the Asn/Asn, 45% for the Asn/Ser, and 26% for the Ser/Ser FSHR variants. Approximately 20% of the female population showed a significant increase in total menstrual cycle length and time from luteolysis to ovulation compared with control subjects (Asn680/Asn680 wild-type receptor). In addition, despite being normo-ovulatory, women with the Ser680 polymorphism displayed a significantly higher serum FSH level compared with the control population. Consequently, this genotype is associated with a higher ovarian threshold to FSH; and women with the Ser680 polymorphism have been reported to have a lower ovarian response to FSH stimulation during ART (Figure 7).[18]

Figure 7: Basal FSH levels in ovulatory women, grouped according to the FSHR genotype (AA, n=46; AS, n=72; SS, n=43).[18]



The study findings, therefore, suggest that the ovarian response to FSH stimulation depends on the FSHR genotype.[18]

Increased Prevalence of LH Beta-Subunit Variant in Patients With Premature Ovarian Failure

A common variant of the LH gene (Trp8Arg and Ile15Thr of the beta subunit) encodes a protein with altered *in-vitro* and *in-vivo* activity, that may be less effective at supporting FSH-stimulated multi-follicular growth, resulting in a suboptimal ovarian response to standard stimulation. An increased prevalence of the LH gene beta-subunit variant has been reported in Japanese infertility patients with premature ovarian failure. Serum samples were collected from 169 healthy non-pregnant Japanese women, 105 healthy adult Japanese men, and 97 female Japanese infertility patients.[19]

The mean ratio of the variant among infertile patients was significantly lower than among healthy patients ($p<0.01$); the variant occurred more frequently among infertile patients (16.5% vs. 8.3% in healthy patients). Also, the variant was more frequent among patients with ovulatory disorders.[19]

The study concluded that the variant of LH secretion may induce anovulation or delayed ovulation, resulting in infertility.[19]

Association Between C/T Polymorphism in the LRP5 Gene and Circulating FSH in Caucasian Postmenopausal Women

A possible link between low-density lipoprotein receptor-related protein 5-LRP5 genotype and serum levels of gonadotropins including FSH and LH was uncovered by Zofková *et al*. (2007). A C/T (c.4037:A1330V) polymorphism in the LRP5 gene was identified in 165 untreated pre- and postmenopausal women. The distribution of the CC, TC, and TT genotypes of the C/T polymorphism in the whole group was 73.9%, 23.6%, and 2.4%, respectively, which is comparable with other Caucasian populations.[20]

Women with the CT allele combination had markedly higher serum FSH levels as compared to carriers of the CC genotype ($p<0.004$). No differences between these genotypes were found in serum LH levels, as well as in levels of circulating sex-steroids.[20]

This study suggested that although the LRP5 gene is considered to be primarily associated with bone metabolism, gene polymorphisms are associated with a marked variation in circulating FSH levels in normal postmenopausal women.[20]

Table 1: Summary of candidate gene and genome-wide association (GWA) studies for age at menopause[2]

Chromosome	Study	Effect on AMP in years (minor allele)	Gene or gene region
Candidate gene			
12q13	Keevenaar *et al*., 2007 (305)	–2.6	AMHR2
13q13	Tempfer *et al*., 2005 (289) He *et al*., 2009 (311)	+1.5 –1.93	APO-E
2p21-22	Hefler *et al*., 2005 (298) Long *et al*., 2006 (331) Long *et al*., 2006 (331) Long *et al*., 2006 (331) Mitchell *et al*., 2008 (332)	–0.8 –1.0 +1.2 +0.7 +2.6	CYP1B1
6q25	Weel *et al*., 1999 (299)	–1.1	ERα
13q34	van Disseldorp *et al*., 2008 (108)	+0.8	F VII
1q23	van Asselt *et al*., 2003 (290) Tempfer *et al*., 2005 (305)	–3.1 –2.4	F V Leiden
5q21-22	Zhang *et al*., 2007 (333)	+1.58	HDC
1p13	Mitchell *et al*., 2008 (332)	+1.9	HSDB1
GWA studies			
19q13.4	He *et al*., 2009 (311) Stolk *et al*., 2009 (310) He *et al*. 2009 (311) Stolk *et al*., 2009 (310) He *et al*., 2009 (311) Stolk *et al*., 2009 (310) He *et al*., 2009 (311) Stolk *et al*., 2009 (310) He *et al*., 2009 (311) He *et al*., 2009 (311)	–0.49 +0.39 –0.47 –0.38 –0.43 –0.43 –0.40 –0.31 +0.36 +0.33	BRSK1, THEM224 and SUV420H2 BRSK1, THEM224 and SUV420H2 BRSK1,THEM224 and SUV420H2 BRSK1, THEM224 and SUV420H2 BRSK1 HSPBP1, BRSK1
20p12.3	He *et al*., 2009 (311) Stolk *et al*., 2009 (310)	+1.07 +0.50	TRMT6, MCM8 MCM8
5q35.2	He *et al*., 2009 (311) He *et al*., 2009 (311) He *et al*., 2009 (311) He *et al*., 2009 (311) He *et al*., 2009 (311)	+0.39 +0.39 +0.39 +0.36 –0.30	UIMC1 UIMC1 UIMC1, ZNF346 HK3, UIMC1 UNC5A, HK3
13q34	Stolk *et al*., 2009 (310)	+0.52	ARHGEF7
6p24.2	He *et al*., 2009 (311)	+0.29	GCM2, SYCP2L

Association Between Small Gene Variations and Poor Ovarian Response

Hormonal genes, including FSH, FSHR, LH, LHR, CYP17, and CYP19, primarily affect follicle function, whereas other genes (such as BMP15, GDF9, and GPR3) affect the rate of initial recruitment from the primordial follicle pool into growing follicles. Mutations in these genes have occasionally been observed in humans. Small genetic variations in these genes may greatly affect the follicular function and as such dictate variations during their productive lifespan.[2]

Summary of Candidate Gene and Genome-Wide Association Studies for Age at Menopause

Genetic variations are associated with a number of premature ovarian failure (POF) cases. Several studies demonstrated genetic mechanisms involved in women that may result in ovarian aging and POF. Table 1 summarizes candidate gene and genome-wide association (GWA) studies for age at menopause.[2]

Summary

- Genetic variations may influence the rate of ovarian aging by affecting the ovarian response to gonadotropins. They may also result in premature menopause.
- The haploinsufficiency of the FSH-R gene may cause premature exhaustion of the gonadotropins, resulting in reproductive failure.
- A low mtDNA content is associated with impaired oocyte quality, as observed in women with ovarian insufficiency.
- The genetic variant of LH secretion may induce anovulation or delayed ovulation that results in infertility.
- Gene polymorphisms of LRP5 have been associated with marked variations in circulating FSH levels in normal postmenopausal women.

Lifestyle and Environmental Factors Influencing Rate of Ovarian Aging

Age at natural menopause is affected by various social, genetic, environmental, and lifestyle factors, such as smoking, alcohol consumption, nutrition and diet, race, maternal age at menopause, etc.[21]

Relationship Between Smoking and Early Natural Menopause

Tobacco smoking accelerates senescence. Many studies suggest that smoking is a potential risk factor for early menopause. A meta-analysis conducted by Sun *et al*. (2012) evaluated the effect of smoking on age at natural menopause (ANM). A total of 11 studies were included in this meta-analysis, of which 5 were dichotomous studies and 6 continuous studies.[21]

Smoking was found to be significantly associated with early ANM in both dichotomous and continuous studies. The pooled effect was OR=0.74 (95% CI, 0.60–0.91, $p<0.01$) for the dichotomous studies and OR= –1.12 (95% CI, –1.80 to –0.44, $p=0.04$) for the continuous studies (Figure 8).[15]

The study results suggest that smoking is a significant independent risk factor for early ANM.[21]

Alcohol Consumption and Age of Maternal Menopause

A prospective survey conducted among 1227 women aged 47–51 evaluated the effect of alcohol consumption on ANM. The study participants were divided into 3 groups: pre-menopausal (regular menstruation); irregular menstruation; and post-menopausal (absence of menstrual cycle for at least 6 months).[22]

There was a significant univariate association between menopausal status and alcohol consumption ($p=0.005$). Alcohol consumption was significantly correlated with estradiol levels ($r=0.61$, $p=0.02$). These results suggest that moderate consumption of alcohol is associated with delayed menopause.[22]

Disruption of Oogenesis Following Exposure to Bisphenol A

Evidence suggests that a progressive link exists between exposure to synthetic chemicals (that mimic the actions of endogenous hormones) and risks to ovarian function. Short-term exposure to environmentally relevant doses of bisphenol A, an estrogenic chemical, has been associated with a variety of reproductive effects in females, including alterations in mammary gland organization, mammalian oogenesis, brain development, and estrous cyclicity.[23]

Susiarjo *et al*. (2007) investigated the estrogenic effect of bisphenol A at an even earlier stage of oocyte development, i.e. at the onset of meiosis, in the fetal ovaries of pregnant mice.[23]

Oocytes that were exposed to bisphenol A displayed gross aberrations in the meiotic prophase, including synaptic defects and increased levels of recombination. In mature females, these aberrations translated to an increase in aneuploid eggs and embryos. The chemical also resulted in the disruption of ERβ, an estrogen receptor.[23]

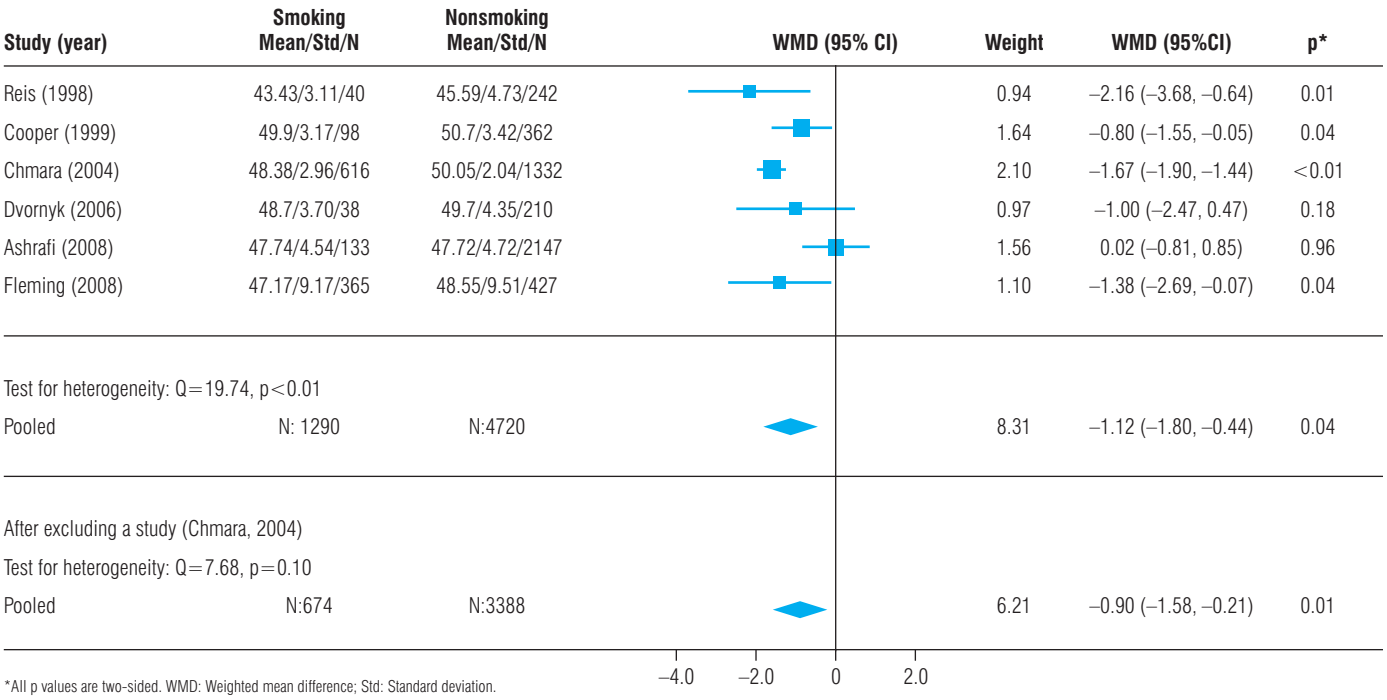
These pre-clinical study findings suggested that bisphenol A influences early meiotic events and affects fetal development in pregnant mice by interfering with the actions of ERβ in the early oocyte.[23]

Toxic Effects of Chemotherapy and Radiotherapy on Female Reproduction

One of the devastating consequences of cancer therapy is ovarian damage. Chemotherapy and radiotherapy may lead to diminished fertility potential and lower post-treatment birth rates in female cancer survivors.[24]

The possible mechanisms of damage include follicular apoptosis and cortical fibrosis. Increased activation of follicles results in accelerated atresia and, eventually, a premature exhaustion of the primordial follicle reserve. The extent of damage depends on the magnitude of anti-cancer therapy, the patient's age, and the chemotherapeutic agent and drug regimen used.[24]

Figure 8: Summary of studies included in the meta-analysis, which showed continuous outcomes.



*All p values are two-sided. WMD: Weighted mean difference; Std: Standard deviation.

Summary

- Age at natural menopause is affected by various social, genetic, environmental, and lifestyle factors, such as smoking, alcohol consumption, nutrition and diet, race, and maternal age at menopause.
- Smoking and alcohol comsumption are associated with early menopuse and delayed reproductive development.
- Short-term exposure to chemicals such as bisphenol A is associated with a variety of reproductive effects in females, including alterations in mammary gland organization, mammalian oogenesis, brain development, and estrous cyclicity.
- Chemotherapy and radiotherapy may lead to diminished fertility potential and lower post-treatment birth rates in female cancer survivors.

Effect of Coexisting Pathology on Ovarian Sensitivity to Gonadotropins

Effects on Endometriosis on Oocyte Production

Endometriosis can be described as a disease that occurs when endometrial tissue, which usually grows as the lining of the uterus (womb), is found in other parts of the body, such as in the ovaries, fallopian tubes, and the pelvis. Symptoms include dysmenorrhea (painful periods), dyspareunia (painful intercourse), and pelvic pain.[25] Approximately 3%–10% of women in the reproductive age group and 25%–35% of infertile women have endometriosis. Endometriosis of the ovaries is known to interfere with fertility. Studies have demonstrated that a higher proportion of infertile women (38.5%) are diagnosed with endometriosis compared with fertile women (5.2%). Moreover, fecundity rates in women with endometriosis tend to be lower compared to normal women.[26]

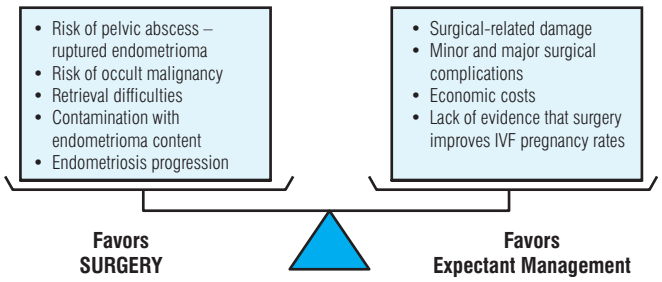
Endometriosis has been implicated as a factor in disordered follicle growth, ovulatory dysfunction, and failure of embryo development. The disease impairs tubal function or gamete quality by causing adhesions that block tubal motility and pickup of the egg. In addition, endometriosis is associated with an increase in levels of prostaglandins (such as 6-keto-prostaglandin F_{1a}), which could, in turn, affect tubal motility or folliculogenesis and corpus luteum function.[26]

Impact of Endometriosis Treatment Prior to IVF Cycles

Ovarian endometrioma removal surgery (ovarian cystectomy) appears to increase the chances of spontaneous conception in most infertile women. However, caution should be taken while performing laparoscopic excision of ovarian endometriomas in all infertile women prior to IVF. Endometrioma-related injury and surgery-mediated damage are risks associated with endometriosis surgery. Ovarian cystectomy is associated with quantitative damage to the ovarian reserve. It may result in reduced ovarian responsiveness to gonadotropins.[27,28]

It is therefore recommended to proceed directly to IVF to avoid potential complications associated with endometriosis surgery and to limit treatment cost. Surgery should be envisaged only in the presence of large cysts, or to treat concomitant pain symptoms that are refractory to medical treatments; or when malignancy cannot reliably be ruled out. Figure 9 lists several factors that should be taken into consideration while performing ovarian cystectomy.[27,28]

Figure 9: Pros and cons of surgical treatment of endometrioma surgery before IVF–ICSI cycles.[28]



Summary

- Endometriosis of the ovaries is known to interfere with fertility. Endometriosis has been implicated in disordered follicle growth, ovulatory dysfunction, and failure of embryo development.
- The disease impairs tubal function or gamete quality by causing adhesions that block tubal motility and pickup of the egg.
- In addition, endometriosis is associated with an increase in levels of prostaglandins (such as 6-keto-prostaglandin F1a), which could, in turn, affect tubal motility or folliculogenesis and corpus luteum function.
- Ovarian endometrioma removal surgery (ovarian cystectomy) appears to increase the chances of spontaneous conception in most infertile women.
- Endometrioma-related injury and surgery-mediated damage are risk associated with endometriosis surgery. Ovarian cystectomy is associated with a quantitative damage to the ovarian reserve. It may result in reduced ovarian responsiveness to gonadotropins.

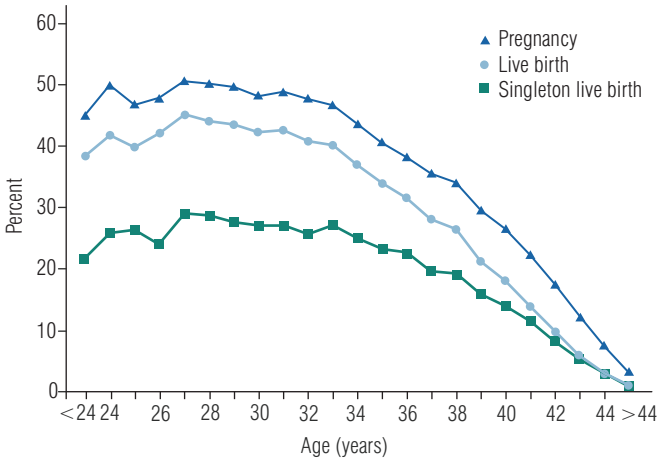
Impact of Chronological Versus Biological Age on ART Outcomes

Poor Ovarian Response to Stimulation is More Common Among Women Aged ≥35 Years

Increasing age is associated with a decline in female fecundity. The decrease occurs dramatically after the age of 35 years, thereby making childbearing difficult for many women. The ovaries become less sensitive to FSH with increasing age. Studies suggest that the rate of ovarian oocyte depletion increases after the age of 37 years. Older women have decreased reserves of healthy oocytes in their ovarian pool. Women who postpone childbearing until their late 30s or early 40s, therefore, frequently face problems in achieving a pregnancy. Successful treatment of these patients remains a major challenge in ART programs. With increasing age, the ovarian response to stimulation also deteriorates, requiring large doses of gonadotropins during ART. It has also been demonstrated that women of advanced maternal age may have oocytes that are compromised by a significant reduction in the amount of mitochondrial DNA in their cytoplasm.[29]

Data from the United States (Centers for Disease Control 2008 report on ART success rates) clearly demonstrate that the potential for embryo implantation and successful delivery of a live birth decreases rapidly in women >35 years (Figure 10).[23] The report also highlighted how the increased incidence of pregnancy loss is related to increased maternal age: less than 12% among women aged <35 years, 29.8% among women aged 35–40 years, and 57% among women aged >44 years.[29]

Figure 10: ART success rates in the United States.[29]



Over the age of 35 years there is an acceleration in the decline in pregnancy rates following ART procedures.[29]

The Probability of Embryo Implantation and Successful Live Birth After IVF Also Declines Progressively in Women Over the Age of 35 Years

The most important independent factors related to the ability of embryo implantation in an IVF procedure are female age and embryo morphology. The best way to describe the relation with female age is a biphasic model with a discontinuity at approximately 37 years of age. Van Kooij *et al.* (1996) investigated the relationship between implantation rates per embryo after replacement in IVF-ET and female age. In this retrospective study, a total of 2320 consecutive treatment cycles of IVF-ET were analyzed for implantation rates (as defined by the number of gestational sacs per embryo replaced).[30]

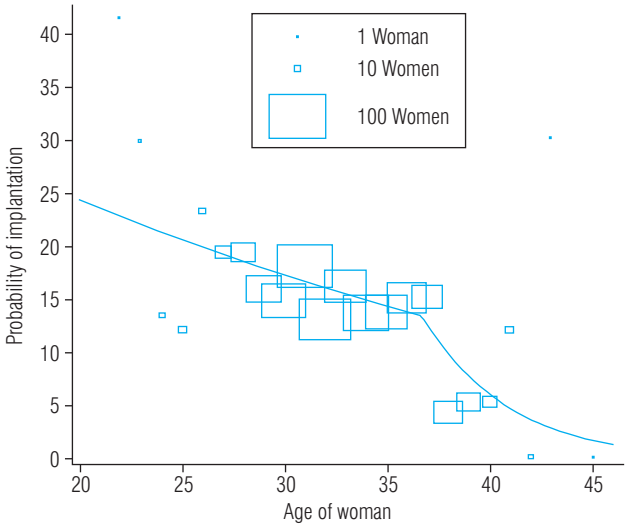
A linear model in a multivariate analysis described that there is an approximately 7% decrease in the implantation rate with age. A biphasic model was also used, resulting in a yearly decrease of >20% in the implantation rate after 37 years of age (Figure 11).[30]

The results suggest that a woman's age is a major factor influencing the number of embryos to be replaced during IVF-ET.[30]

Biological Ovarian Age More Important for the Prognosis of Fertility Treatment Than Chronological Ovarian Age

The biological aging of ovaries can occur independently of the chronological age. Biological aging is indicated by a rise in basal serum

Figure 11: The implantation rate per age group is indicated by blocks, the size of the blocks representing the number of observations.[30]



FSH concentrations and is associated with declining oocyte quality, as indicated by reduced implantation ability. A study conducted among 1019 infertile but ovulating women who underwent IVF treatment demonstrated that the number of oocytes retrieved in the procedure and the embryos available for transfer declined with increasing biological age. The ability to implant fertilized oocytes was inversely related to increasing age and was independent of FSH concentration.[12]

The study findings suggest that biological aging can affect oocyte quality and fecundity, which is independent of the chronological age.[12]

Biological Ovarian Age and Ovarian Response

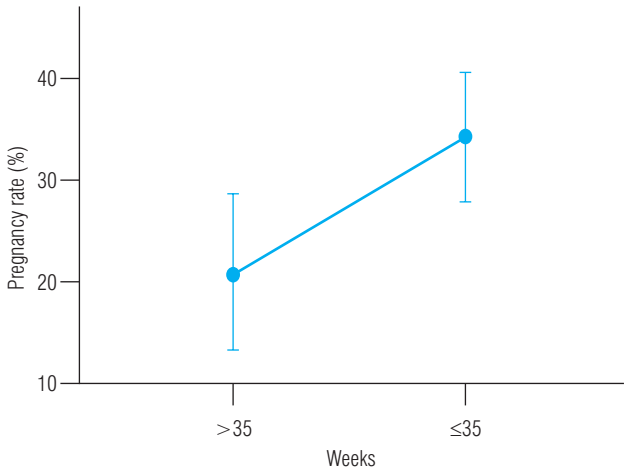
Ovarian aging is associated with poor oocyte response. Early ovarian depletion is likely to be reflected by fewer retrieved oocytes after gonadotropin stimulation during IVF treatment. Indeed, a small case-control study conducted by de Boer *et al*. (2002) observed that the retrieval of a low number of oocytes during the first IVF treatment was associated with a greater risk of early menopause in women <46 years of age.[31]

Biological Ovarian Age and Pregnancy Rates

Sharif *et al*. (1998) investigated the relative effect of ovarian aging on predicting the ovarian response to gonadotropin stimulation, normal fertilization rate, and pregnancy rate in IVF treatment. A total of 344 women undergoing their first IVF cycle were included in the study.[31]

Both increasing basal FSH and age were found to be significantly associated with increased total gonadotropin dose, as well as fewer oocytes collected and a lower pregnancy rate. Logistic regression analysis showed that age, but not basal FSH, was independently associated with the pregnancy rate (Figure 12). The study, therefore, suggested that ovarian age is a strong predictor of the pregnancy rate.[31]

Figure 12: Pregnancy rate related to age groups.[26]



Summary

- The potential for embryo implantation and successful delivery of a live birth decreases rapidly in women >35 years.
- The increased incidence of pregnancy loss is related to increased maternal age: less than 12% among women <35 years, 29.8% among women aged 35–40 years, and 57% among women aged >44 years.
- A woman's age is a major factor influencing the number of embryos that need to be replaced during IVF-ET.
- Early ovarian aging is likely to be reflected by fewer retrieved oocytes after gonadotropin stimulation in IVF treatment.
- Ovarian age is also a stronger predictor of the pregnancy rate.

Assessment of Biological Age Using Biomarkers: Accuracy and Challenges

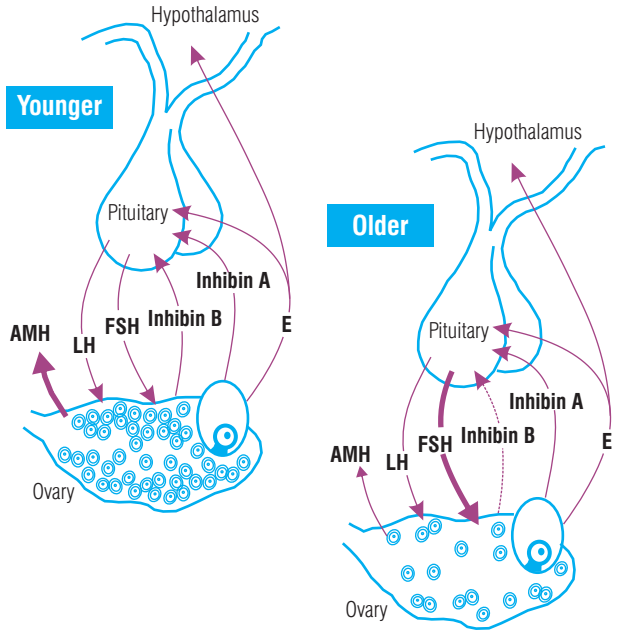
Clinical Testing for Ovarian Reserve: Rationale

Clinical tests currently available to predict the ovarian aging mainly focus on ovarian reserve. All ovarian reserve tests (ORTs) are in relation to follicle cohort size (Figure 13). Antral follicle count and transvaginal sonography are used to measure follicular numbers. The endocrine markers AMH and inhibin B are direct markers of follicular quantity. Basal FSH acts as an indirect marker because pituitary FSH release changes with altered feedback from inhibin B and estradiol. Endocrine challenge tests may add to baseline tests by magnifying the endocrine capacity of the follicle cohort.[2]

The utility of ORT can be evaluated based on 3 criteria:

a) **Accuracy of the test:** This indicates to what extent the test correctly predicts the outcome of interest, generally expressed as the area under the receiver operator characteristic (ROC) curve.

Figure 13: Schematic illustration of the changes in ovarian follicle reserve with increasing female age and the effect of these quantitative changes upon several ovarian and hypothalamo-pituitary endocrine factors.[2]



Adopted from: Broekmans FJ, *et al*. 2009:477.

b) **Clinical value of the test:** It assessed by taking into account how an abnormal ORT would change the management of the infertile couple in an effective way.

c) **Proportion of false-positive tests:** This leads to erroneous management decisions.[2]

Ovarian Reserve Tests

Ovarian reserve tests provide an indirect estimate of a woman's diminishing ovarian reserve or remaining follicular pool. The decline rate of ovarian reserve varies among women, making it a challenge to estimate ORT. Hence, various biochemical and histopathological markers (tests) have been evolved in the past few years for the assessment of ovarian reserve.[32]

a) **Antral follicle count (AFC):** It is defined as the number of follicles smaller than 10 mm in diameter in the early follicular phase. It is considered to have the best discriminating potential for a poor ovarian response, compared to the other tests.

b) **Basal FSH:** An increase in blood FSH levels occurs due to follicle depletion. In females with regular cycling, very high FSH levels may predict a poor response; thus, basal FSH can be useful in the screening of a small infertile group.

c) **Basal estradiol:** An early rise in blood estradiol levels is a consequence of advanced follicular development and early selection of a dominant follicle, observed in cycling females with increased FSH levels.

d) **Inhibin B:** The inhibin B level is used in conjunction with serum FSH and estradiol to assess ovarian function. A decline in inhibin B concentrations in the early follicular phase may be observed before an increase in the FSH level.

e) **Gonadotropin-releasing hormone agonist stimulation test:** It is used for assessment of serum estradiol on days 2–3 of the cycle following subcutaneous application of GnRH agonist 100 μg (e.g. triptorelin). The response of E2 to GnRH agonist is an indirect indicator of ovarian reserve.

f) **Exogenous follicle-stimulating hormone ovarian reserve test:** This test involves the measurement of basal FSH and estradiol following the administration of 300 IU FSH on the 3rd day of the menstrual cycle.

g) **Clomiphene citrate challenge test (CCCT):** It is a provocative test that effectively reflects the quantity and quality of the recruited oocytes; however, its predictive value is low. The test is also expensive and time-consuming.

h) **Ovarian biopsy:** The distribution of follicles is not uniform within the ovary; therefore, the biopsy is not able to represent the true follicular density. Therefore, an ovarian biopsy is rarely necessary, and it is not recommended as an ORT.

i) **Genetic markers of ovarian reserve:** Predicting single-nucleotide polymorphisms (SNPs) in gonadotropins and their receptor genes, BMP-15, GDF9, FMR1, MCM8, and the other candidate genes identifies females with a genetic predisposition to early ovarian aging.[33]

Accuracy of Baseline Tests: Antral Follicle Count and Anti-Müllerian Hormone Level

In several systematic reviews of the existing literature, the predictive performance of baseline tests, either as a single test or in combination, has been analyzed.

- Klinkert *et al*. (2005) suggested that AFC is a better marker than age and basal FSH for distinguishing between older patients with good and poor pregnancy prospects after IVF. A total of 221 women were included in this study. Patients with a normal AFC not only had a significantly higher normal response rate, but they also had significantly better pregnancy rates. The AFC correlated negatively with chronological age.[34]

- Nahum *et al*. (2001) suggested that AFC was a better predictor of IVF outcomes than age or FSH. A total of 272 consecutive IVF cycles of 224 consecutive patients enrolled in treatment cycles for IVF were included in the study. Controlling for patient age and basal follicle stimulated hormone, the pregnancy rate was significantly higher in the group with antral follicle >6 compared to that in the group with antral follicle ≤6.[35]

- Fanchin *et al*. (2003) suggested that anti-Müllerian hormone may reflect ovarian follicular status better than usual hormone markers. A total of 75 infertile women were included in the study. Serum anti-Müllerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH, and LH on day 3.[36]

Accuracy of Clomiphene Citrate Challenge Test

The clomiphene citrate challenge test (CCCT) may be a better predictor of treatment outcomes because it unmasks diminished ovarian reserve in patients who might not be detected by basal FSH screening. This is especially true in cases in which the basal FSH is normal; however, the clomiphene-induced rise in endogenous FSH is not accurately suppressed by rising levels of estradiol and inhibin B.[2]

A systematic review conducted by Hendriks *et al*. (2006) demonstrated that pooling of tests to obtain summary receiver operator characteristic (ROC) curves is not possible because of heterogeneity among individual studies. When basal FSH was compared with CCCT, it appeared that the latter had no clear added value in the prediction of poor ovarian response or pregnancy after IVF.[37]

Another meta-analysis conducted by Jain *et al*. (2009) demonstrated that basal FSH and CCCT are similar in predicting the ability to achieve a clinical pregnancy in women undergoing infertility treatment. With either test, a normal result is not useful, but an abnormal result virtually confirms that pregnancy will not occur with treatment.[38]

Summary

- Clinical tests currently available to predict the ovarian aging mainly focus on ovarian reserve.
- All ORTs are in relation to follicle cohort size.
- Ovarian reserve tests provide an indirect estimate of a female's diminishing ovarian reserve or remaining follicular pool.

Therapeutic Options for Management of Ovarian Aging

Do Conventional ART Protocols Yield Favorable Results? What are the Additional Modifications Required in These Protocols to Improve Patient Outcomes?

Assisted reproductive technology has been used for many years to treat women with early reproductive aging. However, the outcomes of ART are not particularly advantageous.[39]

Due to a long latent period and a lack of uniform criteria, screening for early ovarian aging (EOA) has been a worrisome process. With conventional ART protocols, women with EOA get depressing results. Additional modifications to the process—such as ovarian stimulation protocols adjusted for decreased ovarian reserve; additional medications to control contributing factors (e.g. autoimmune abnormalities, which sometimes present in patients with EOA); and ovarian priming with oral dehydroepiandrosterone (DHEA)—are desirable for increasing the conception rate of ART among these women.[39]

Proper assessment and detection of ovarian aging and employment of current or development of predictors of ovarian reserve at an appropriate time may aid in making ART protocols successful in early pregnancy achievements or fertility preservation in women at risk.[39]

LH Supplementation

Humaidan *et al*. (2004) evaluated the effect of recombinant human LH (r-hLH) supplementation on ovarian response and pregnancy in a prospective randomized study including 231 cycles. The study results suggested that supplementation with r-hLH benefits treatment outcomes among women above 35 years of age and among the subgroup of women exhibiting LH concentrations above 1.99 IU/L on stimulation day 8.[40]

De Placido *et al*. (2006) compared the effect of the GnRH agonist in association with recombinant LH (rec-LH) administration with the standard GnRH-a-short protocol in 133 women at risk for a poor ovarian response. When compared with standard GnRH-a-short protocol, this strategy led to a significant improvement in oocyte quality and maturation, which in turn resulted in a significant increase in the mean number of mature oocytes.[41]

Growth Hormone (GH) Supplementation

Tesarik *et al*. (2005), in a prospective randomized study, evaluated the usefulness of GH administration in women >40 years undergoing ovarian stimulation for ART. The results suggested that administration of GH during ovarian stimulation alleviates the age-related decrease in assisted reproduction treatment efficiency. This effect appears to be mainly due to an improvement in oocyte developmental potential, but GH action on the uterus cannot be excluded.[42]

Another study conducted by Kyrou *et al*. (2009) suggested that addition of GH, as well as performing embryo transfer, appears to improve the probability of pregnancy.[43]

Effects of Long-Term Melatonin Treatment on Ovarian Aging

Tamura *et al*. (2017) examined whether long-term melatonin treatment would delay ovarian aging in mice. The study included female ICR mice (10 weeks old) that were given melatonin-containing water (100 μg/mL; melatonin) or water only until 43 weeks of age. The number of ovulated oocytes was greater among melatonin-treated mice than among control animals. The decreased fertilization rate and blastocyst rate during aging also were higher among the melatonin-treated mice than among the controls, as were the numbers of primordial, primary, and antral follicles. The study indicated that melatonin delays ovarian aging by multiple mechanisms.[44]

Summary

- Assisted reproductive technology has been employed for many years to treat women with early reproductive aging. However, the outcomes of ART are not particularly advantageous.
- Additional modifications in the process—such as ovarian stimulation protocols adjusted for decreased ovarian reserve; additional medications to control contributing factors (e.g. autoimmune abnormalities, which sometimes present in patients with EOA); and ovarian priming with oral DHEA—are desirable for increasing the conception rate of ART.

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