Scholars Journal of Applied Medical Sciences (SJAMS)
Abbreviated Key Title: Sch. J. App. Med. Sci.
ISSN 2320-6691 (Online)
ISSN 2347-954X (Print)

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Endocrinology

Review of Premature Ovarian Failure over the years – Geneticist's Perspective

Priyankka Damani-Singh, Dr. Usha Dave*
MILS International India, Goregaon West, Mumbai, India

Review Article

***Corresponding author**
Dr. Usha Dave

Article History
Received: 29.11.2017
Accepted: 12.12.2017
Published: 30.12.2017

DOI:
10.36347/sjams.2017.v05i12.030

Abstract: Premature ovarian failure/ insufficiency (POF/ POI) or premature menopause is commonly referred to the development of amenorrhea caused by the cessation of ovarian function before 40 years of age. In clinical practice, diagnosis of menstrual irregularity is confirmed by biochemical markers like follicle stimulating hormone (FSH), estradiol (E_2), inhibin B or anti-mullerian hormone (AMH). The etiological causes of POI are highly heterogeneous but most causes of POI are idiopathic. The symptoms can vary from patient to patient and could occur spontaneously or gradually develop over the years. Here we review the various aspects of premature ovarian failure from the geneticist perspective. With advances in genomic technologies various reproductive options are now available for women suffering from POI. Genetic counselling of such women not only helps them make an informed choice but also helps them to cope with feelings of anxiety, guilt or altered feelings of self-worth. Our correlative studies on fragile x mental retardation 1 (FMR1) mutation in Indian women for POI revealed that infertile Indian women do not comprise a high-risk population to be screened for FMR1 mutation. However, diagnosing POF early would help in early intervention as well as provide for potential targets for therapeutic intervention. The use of a multidisciplinary approach would be significant in such a situation.

Keywords: Premature ovarian failure/ insufficiency (POF/ POI), anti-mullerian hormone (AMH), fragile x mental retardation 1 (FMR1) mutation, idiopathic, genetic counselling.

INTRODUCTION

Menstruation with ovulation is a spontaneous, regular onset, predictable in duration and amount of flow. Any deviation in this set pattern of menstrual flow is associated with some bodily abnormality which could even be a disease of the outflow tract or the failure of normal follicular maturation with consequent anovulation which could be transient or chronic. The ovaries are the female gonads that produce the eggs/ ova. Ovaries are intraperitoneal structures that play an important role in reproduction and secrete the hormones estrogen and progesterone. The basic functional unit of the ovary is the follicle and the process of folliculogenesis (Figure-1) begins at fetal life till follicle senescence. There are many reasons and factors that affect, influence and limit the number of eggs (ovarian reserve) stored in a woman's ovaries. The factors could be of genetic nature or environmental or even of a combination of both. The average age of menopause in Western population of women is approximately 51 years and in case of Indian women it seems to be 47.5 years [1]. Premature ovarian failure/ insufficiency (POF/ POI) or premature menopause is commonly referred to the development of amenorrhea caused by the cessation of ovarian function before 40 years of age. The diagnosis is based on elevated follicle secreting hormone (FSH) levels in menopausal range (usually above 40 IU/ L) detected on at least two occasions a few weeks apart [2].

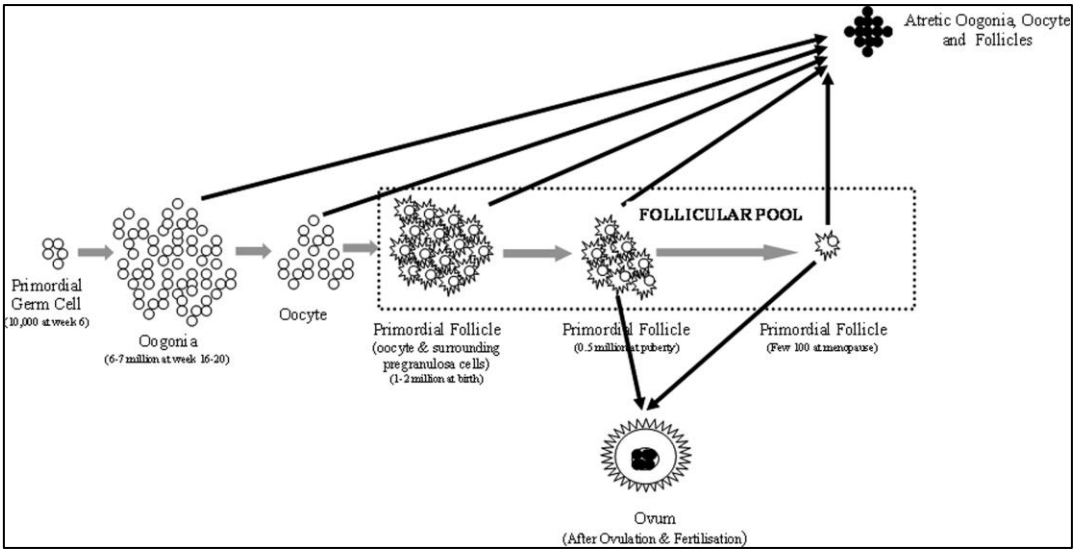

Fig-1: Schematic illustration of the evolution of primordial follicle (Source: Arora and Polson, 2011) [13].

Premature ovarian Failure or Insufficiency - POF or POI

Over the years various studies have been carried out to understand premature ovarian failure. It is now clear that premature ovarian failure is an inadequate term to account for the spectrum of ovarian insufficiency it defines [3]. It is now recommended that primary ovarian insufficiency be used as a scientifically accurate term which better describes the disorder and an increased understanding of the clinical spectrum of POI will promote research to uncover further etiologies so that better therapeutic recommendations can be developed [3].

Oocyte reserve and POI

Very often loss of ovarian function in POI is entangled with low ovarian reserve but these two are separate entities representing different patients with different management needs. Ovarian reserve encompasses both quantity and quality of primordial follicles and is a condition in which the ovary loses its normal reproductive potential [4]. A vast majority of the eggs within the ovary of a woman die steadily, until they are depleted at menopause. At birth approximately 1 million eggs [5] are present and by the time of puberty, only about 300,000 remain and of these 300 – 400 will be ovulated during a woman's reproductive lifetime [6]. The eggs continue to degenerate during pregnancy, with the use of birth control pills and in the absence or presence of regular menstrual cycle. However, an early depletion in the number of eggs leads to premature menopause. Various studies have now enabled us to understand that endocrine changes occur at a cellular level perhaps in response to the depletion of follicles more than a decade before its time. Around 37 years of age an accelerated loss of follicles from the ovaries is noted [7].

Aetiology of POF

Ovarian insufficiency in most cases occurs because of an anticipated depletion of primordial follicular pool and can be caused due to amenorrhea – primary or secondary nature also. Two types of consequences occur because of POI. One is premature hypoestrogenism which in turn causes premature aging of several tissues, targets of estrogen action and thus increases the risk of osteoporosis, cardiovascular diseases or neurodegenerative diseases while the second cause is infertility. But women who have this condition have only a 5 - 10% chance to conceive without fertility treatments. The etiological causes that may activate such mechanisms are highly heterogeneous and include chromosomal, genetic, autoimmune, metabolic, infectious, and iatrogenic factors [8]. Most of the causes of POI are idiopathic; but 20 – 25% of the idiopathic cases are now known to have a strong genetic component (Table-1). Different causes are as follows [9] –

- Iatrogenic origin (surgery, chemotherapy, radiations)
- Autoimmune, including polyglandular autoimmune syndrome, as well as autoimmune polyendocrinopathycandidiasis-ectodermal dystrophy (APECED) due to mutations in AIRE gene
- Infections (e.g. herpes zoster, cytomegalovirus)

Chromosome X defects

1. Turner syndrome
2. Fragile X syndrome (FMR1 gene premutation). Among the women who suffer from menopause prior to 40 years of age, commonly referred to as POF, 2% who do not have a family history of POF and 14% of those with a family history of POF carry a fragile X premutation [10]. Many recent studies have shown that the FMR1 premutation carriers have an increased risk of POF.

- Monogenic defects

Syndromic defects

1. Congenital disorders of glycosylation (CDG, formerly named carbohydrate-deficient glycoprotein syndromes) (recessive)
2. Galactosemia (recessive)
3. Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) (female-limited, dominant)
4. Pseudohypoparathyroidism (PHP) type Ia (parental imprinting : maternal inheritance)

Isolated defects

1. Follicle stimulating hormone (FSH) receptor mutations (FSHR), (recessive)
2. Luteinizing hormone (LH) receptor mutations (LHR), (recessive)
3. FOXL2 (transcription factor involved in BPES) mutations (female-limited defect, dominant)
4. Bone morphogenetic protein 15 (BMP15) mutations (female-limited defect, heterozygous mutation)

- Idiopathic

Table-1: POF candidate genes and their functions in relation to POF pathogenesis [24]

S.No.	Gene	Chromosomal location	Gene function
1	FMR1	Xq27.3	Oocyte development and maturation
2	NR5A1	9q33.3	Ovarian steroidogenesis
3	NOBOX	7q25	Early folliculogenesis
4	FIGLA	2p12	Regulation of zona pellucida genes
5	FOXL2	3q23	Granulosa cell differentiation and follicle development
6	SOHLH 1/2	13q13.3	Early folliculogenesis
7	BMP-15	Xp11.2	Follicular maturation
8	GDF-9	5q23.2	Follicular maturation
9	INHA	2q33-36	Folliculogenesis regulation through FSH inhibition
10	FSHR	2p21	Follicular growth and development, ovarian steroidogenesis
11	LHR	2p21	Follicle maturation, ovarian steroidogenesis and ovulation
12	ESR1	6q25.1	Follicle growth and maturation

Abbreviations: FSH – Follicle-stimulating hormone; POF – Premature ovarian failure

Clinical Presentation

The symptoms can vary from patient to patient and could occur spontaneously or gradually develop over the years. Symptoms typical of menopause sometimes preceded by menstrual cycle changes are seen in women with POI. Symptoms may be transient or intermittent and the severity may be variable thus reflecting the fluctuations in ovarian activity which occurs during the spontaneous onset of POI [3]. The most severe forms of hypergonadotropic ovarian failure present with absent pubertal development and primary amenorrhea [9]. Amenorrhea is seen to be an uncommon presentation in reproductive medicine and polycystic ovary syndrome, hypothalamic amenorrhea, ovarian failure and hyperprolactinemia are seen to be the four most common causes of amenorrhea [11]. Post pubertal ovarian failure represents a majority of the cases [12] and is characterized by secondary amenorrhea which is the result of premature folliculogenesis depletion or arrested folliculogenesis. Although women with POI may present with typical symptoms of estrogen deficiency like vasomotor symptoms, the clinical presentation of POI patients is seen to be variable and several misunderstandings regarding the symptoms of POI exist [4]. In certain cases the cause of POI can be iatrogenic including chemotherapy, radiotherapy or pelvic surgery in the past and hence clinical history of such patients is always required. A history of obstetric catastrophe, severe bleeding, dilation and curettage or infection indicates a uterine cause (Asherman syndrome) [13]. In certain cases the cause of POI is genetic and hence a physical examination including height, weight and body mass index is essential to be carried out to recognize any dysmorphic features present [13].

Diagnosis & Management

Baseline tests like measurement of urine human chorionic gonadotropin levels to rule out pregnancy, serum prolactin and thyroxine levels to rule out hyperprolactinemia and thyroid dysfunction respectively should be done. But the measure of direct

marker like anti-mullerian hormone (AMH) is of particular importance as measurement of serum AMH levels follow reduction in follicular number over time in healthy women and fall to very low levels prior to menopause [4]. But low AMH can also be found in women with regular cycles and low ovarian reserve and hence the assay used by most studies till date are insufficient in this context as AMH levels become undetectable approximately 5 years before menopause [4]. In clinical practice, diagnosis of menstrual irregularity is confirmed by biochemical markers like FSH, estradiol (E_2), inhibin B or AMH [14]. Most commonly performed initial workup includes measurement of serum FSH, karyotype, fragile X carrier screening, serum TSH and dual energy x-ray absorptiometry scan. Ultrasound is also done to check for small ovaries without evidence of growing follicles as well as histological examination of ovarian biopsies are carried out to check for hypoplastic ovaries. Karyotyping and other cytogenetic and molecular based investigations are done to help identify the cause if possible. However, as per ESHRE guidelines [4] there is not enough evidence to include ultrasound as ovarian function may fluctuate in women with POI and follicular activity may not be seen thereby not distinguishing POI from other diagnoses. Also there exists no evidence to include laparoscopy with or without ovarian biopsies. As per the ESHRE guidelines [4] diagnosis of POI is confirmed in women <40 years by combination of 4-6 month period of amenorrhea or oligomenorrhea and 2 serial measurements of elevated FSH taken > 4 weeks apart while measurement of AMH is not sufficiently discriminative for diagnosing POI. No ideal biomarkers for POI diagnosis exist and the existing biomarkers may fluctuate over time.

On diagnosis of POI in women, the main aim of management and treatment in this group can be categorized as [13]:

- Education, counseling and psychological support
- Prevention and treatment of estrogen deficiency symptoms
- Specific fertility management

Early diagnosis of familial POF is useful in predicting the likelihood of premature menopause and hence providing the woman with alternates for making a reproductive choice such as hormone replacement therapy (HRT) and infertility treatment - oocyte donor, adoption. Embryo cryopreservation, ovarian tissue cryopreservation and oocyte cryopreservation hold promise in treating POF in especially women undergoing cancer therapy.

Management, Counseling and Genetic Counseling

For women, POI is a difficult diagnosis to accept and therefore a well-planned and sensitive approach is required to inform the patient. For this a dedicated multidisciplinary clinic or team separate from the routine menopause clinic is useful to provide ample time and information to such patients. A multidisciplinary team consists of appropriate qualified professionals who are trained to meet the needs of such emotionally distraught patients. Counseling provided to women with POI should include an explanation that remission and spontaneous pregnancy can still occur as well as highlight the difference between POI and normal menopause [15]. Such a multidisciplinary team provides information with audio-visual aids. Advice in the specific areas of management of POI includes counseling and emotional support, diet and nutrition supplement advice, hormone replacement therapy and reproductive health care options like contraception and fertility issues [15].

The long term consequences of POI include infertility, psychological distress or depression, decreased sexual and general well-being, autoimmune disorders, osteoporosis, ischemic heart disease and increased risk of mortality and the diagnosis of POI in women can have devastating for patients. Thus, supportive therapy and psychological counseling plays a significant role. Several genetic causes of POI make genetic counseling a recommended option. There is enough convincing evidence worldwide that fragile x mental retardation 1 (FMR1) premutation does relate to ovarian function and loss of fertility by premature menopause. Fragile X is a CGG trinucleotide repeat disorder which when fully expanded (>200 repeats) leads to fragile X syndrome, the most common cause of inherited mental retardation as well as the most common known genetic factor associated with autism. The premutation form of this gene (55-200 repeats) leads to two disorders distinct from fragile X syndrome - fragile X – associated tremor/ ataxia syndrome, abbreviated as FXTAS and fragile x - premature ovarian failure/ insufficiency, abbreviated as FXPOI which affects about 15% of women who are carriers of the premutation [16-19]. Among the women who suffer from menopause prior to 40 years of age, commonly referred to as POF, 2% who do not have a family history of POF and 14% of those with a family history of POF carry a fragile X premutation [10]. A possible explanation of the association between ovarian insufficiency and the premutation state of FMR1 gene is that the transcription from premutated alleles is significantly increased [20]. The premutation allele has been found to co-segregate with POF in families [21]. But in sporadic cases of POF with no history of fragile X syndrome or mental retardation this fact still needs to be investigated. Hence, women having FMR1 premutation need to be informed about risk of having a child with fragile X syndrome which is the second most common cause of intellectual disability after Down syndrome. The identification of mother as an index case should also trigger the need for genetic counseling throughout the pedigree.

Genetic counseling not only helps the carrier women of X-linked chromosomal abnormalities to

make informed reproductive decisions but also helps them further to discuss the diagnosis in terms of risks with other family members. Genetic counseling also helps the woman in dealing and coping with feelings of anxiety, guilt or altered feelings of self-worth that are raised when told about being a carrier.

Genomic Approaches for Diagnosis

The development of genome-wide association studies (GWAS) has bettered our understanding of many complex human diseases, but so far there are only 3 GWAS performed for POI [22]. The first GWAS performed in POF patients was in Korea and it was an indirect GWAS which implies that the word indirect relies on the fact that this study was performed in 2 steps, the first relying on linkage disequilibrium study (LDS) followed by the second step of analysis. The study found an association between POF and two PTHB1 SNPs (rs3884597 and rs6944723) but the role of this gene remains unknown in the ovarian function [22]. The second study was a GWAS performed in a large Dutch family who had 7 patients suffering from POF and this study revealed 3 genomic regions on chromosomes 5, 14 and 18 and haplotype analysis supported only 1 locus on chromosome 5q14.1-q15 [22]. The third GWAS was performed by Knauff *et al.*, [23] on 99 POF patients with 235 unrelated female controls, and one single nucleotide polymorphism (SNP) which approached a genome-wide significance after adjusting for multiple testing was mapped with an intron in the gene ADAMTS19 (a disintegrin-like and metalloprotease with thrombospondin type I motif). This gene needs further investigation as it appears to play a role in normal gonad formation and function. Although there are 3 GWAS studies on POI, replications in independent cohorts need to be performed as well as large cohorts of women with POI so as to find new candidate genes responsible for POI. To the best of our knowledge, such studies are lacking in the Indian women with POI.

Another approach in providing relief to women with POI is ovarian stem cells. Although the presence of germline stem cells is seen to be capable of generating oocytes in mice, the studies carried out remain controversial and is not seen to have any direct clinical applicability [3]. Various highly sensitive and advanced techniques like array comparative genomic hybridization (aCGH) and mass spectrometry are the other exploratory options to identify the cause of POF, if possible.

The next step for trying to identify the relevant mutations of POF in the human genome would be to completely sequence it using next-generation sequencing (NGS) or massively parallel sequencing which is a rapid high-throughput technique but is expensive, especially for Indian patients. To date, four studies using the whole-exome sequencing (WES) technique have been carried out in consanguineous families with inherited POF and pathogenic variants in the stromal antigen 3 (STAG3), HFM1, MCM8 and MCM9 genes were identified [24]. These genes are known to play a role in DNA replication and repair, meiosis and chromosome stability and thus these results support the importance of these pathways in idiopathic POI pathogenesis. Although the exact mechanism of these mutations in the DNA repair pathways and genomic instability contributing to POI is unknown, it can be suggested that the accumulation of DNA damage and chromosomal instability in the ovary could lead to accelerated follicle atresia thereby predisposing women to POI [24]. A study by Fonseca *et al.*, [25] using targeted NGS technique on 70 candidate POF genes demonstrated an association between mutations in the ADAMTS19 and BMP receptor 2 (BMPR2) genes with POF pathogenesis, in 12 unrelated idiopathic POF women. Thus, despite the cost of this technology, the results of these preliminary findings suggest NGS technologies to be powerful new tools useful in identifying novel genetic regions and pathways involved in POF-pathogenesis.

Our studies in Indian subjects

We had done a short study to screen the FMR1 mutation in clinically diagnosed cases of premature ovarian failure in Indian women. We screened 54 POF cases using the fluorescent methylation – specific PCR and Gene Scan analysis assay. Despite the clinical signs and symptoms and diagnosis of primary/ secondary amenorrhea in correlation with hormonal, ultrasound and karyotype results, all the 54 samples screened revealed no FMR1 premutation. Results of this study from an Indian perspective indicate that FMR1 premutations (55-200 repeats) are rare in sporadic cases of POF (1 – 3%), which have no family history of fragile X syndrome or mental retardation. These results are seen to be in accordance with an earlier study carried out by Chatterjee *et al*., [26], in a similar Indian setting. Another study on FMR1 mutation and POI association conducted by us in 20 controls and 20 infertile patients revealed both controls and patients to be within normal range (5-44 repeats; approximately 45-54 repeats being intermediate alleles) [27]. Thus, it can be inferred that infertile Indian women including those with limited ovarian reserve do not comprise a high-risk population requiring routine FMR1 mutation screening. However, larger cohort studies are required to arrive at this conclusion.

CONCLUSION

Although published literature demonstrates the prevalence of genetic alterations in POI patients to be about 20 – 25%, the pathogenic mechanism causing POI still remains unknown in most cases. It is thought to be a heterogeneous disorder having a multifactorial origin and hence a genetic test could prove to be a beneficial diagnostic tool especially in those having a family history of POI. A larger Indian population study is necessary to precisely understand the frequency of

FMR1 premutation in sporadic cases of POI in Indian ethnicity. However, other known genetic mutations studied in POI as well as autoimmune ovarian damage and iatrogenic causes cannot be ruled out as a cause of POI. Early detection and diagnosis of POI would be important for potential therapeutic intervention using a multidisciplinary approach. As of now the mainstay of treatment in women with POI is seen to be appropriate counseling, psychological support and HRT. Though POI is a devastating diagnosis which has significant emotional and clinical long-term consequences, the timely diagnosis, counseling and intervention can alleviate some of these consequences.

REFERENCES

1. Jha U, Gajaraj J, Unni J, Meeta DM, Malik S, Swasti, Jha RM. Clinical Practice of Menopausal Medicine: How and Why? Indian Menopause Consensus Statement; 2008.
2. Conway GS. Premature ovarian failure. Br Med Bull. 2000; 56:643–649.
3. Welt C. Primary ovarian insufficiency: a more accurate term for premature ovarian failure. Clinical Endocrinology. 2008; 68:499-509.
4. Management of women with premature ovarian insufficiency. Guideline of the European Society of Human Reproduction and Embryology (ESHRE). POI Guideline Development Group. 2015 December.
5. Himelstein-Braw R, Byskov A, Peters H, Faber M. Follicular atresia in the infant human ovary. J Reprod Fertil. 1976; 46:55.
6. Block E. Quantitative morphological investigations of the follicular system in women: Variations at different ages. Acta Anat. 1952; 14:108.
7. Bopp B and Seifer D. Age and Reproduction. In Reproductive Endocrinology, Infertility and Genetics. Volume 5: Gynecology and Obstetrics CD-ROM. Lippincott Williams and Wilkins. 2004.
8. Goswami D and Conway GS. Premature ovarian failure. Human Reproduction Update. 2005; 11(4):391–410.
9. Beck-Peccoz P and Persani L. Premature ovarian failure. Orphanet Journal of Rare Diseases. 2006; 1:9.
10. The Fragile X Premutation: A Cause for Premature Ovarian Failure. Emory University School of Medicine. Department of Human Genetics. Division of Medical Genetics. 2006.
11. The Practice Committee of the American Society for Reproductive Medicine, Birmingham, Alabama. Current evaluation of amenorrhea. Fertility and Sterility. 2008; 90(3):S219-25.
12. Santoro N. Mechanisms of premature ovarian failure. Ann Endocrinol. 2003; 64:87-92.
13. Arora P and Polson DW. Diagnosis and management of premature ovarian failure. The Obstetrician & Gynaecologist. 2011; 13:67-72.
14. Nelson LM. Primary Ovarian Insufficiency. N Engl J Med. 2009; 360(6):606–614.
15. Panay N and Kalu E. Management of premature ovarian failure. Best Practice & Research Clinical Obstetrics and Gynaecology. 2009; 129-140.
16. Allingham-Hawkins D, Babul-Hirji R, Chitayat D, Holden J, Yang K, Lee C, Hudson R, Gorwill H, Nolin S, Anne Glicksman, Edmund C. Jenkins, W. Ted Brown, Patricia N. Howard-Peebles, Cindy Becchi, Emilie Cummings, Lee Fallon, Suzanne Seitz, Susan H. Black, Angela M. Vianna-Morgante, Silvia S. Costa, Paulo A. Otto, Regina C. Mingroni-Netto, Anna Murray, J. Webb, F. MacSwinney, N. Dennis, Patricia A. Jacobs, Maria Syrrou, Ioannis Georgiou, Phillipos C. Patsalis, Maria L. Fragile X premutation is a significant risk factor for premature ovarian failure: the International Collaborative POF in Fragile X study—preliminary data. Am J Med Genet. 1999 Apr 2; 83:322–325.
17. Murray A, Ennis S, MacSwiney F, Webb J, Morton NE. Reproductive and menstrual history of females with fragile X expansions. Eur J Hum Genet. 2000 Apr; 8:247–252.
18. Sherman S. Premature ovarian failure in the fragile X syndrome. Am J Med Genet. 2000; 97:189 –194.
19. Sullivan A, Marcus M, Epstein MP, Allen EG, Anido AE, Paquin JJ, Yadav-Shah M, Sherman SL. Association of FMR1 repeat size with ovarian dysfunction. Hum Reprod. 2005 Feb; 20:402–412.
20. Loesch DZ, Bui QM, Huggins RM, Mitchell RJ, Hagerman RJ, Tassone F. Transcript levels of the intermediate size or grey zone fragile X mental retardation 1 alleles are raised, and correlate with the number of CGG repeats. Journal of Medical Genetics. 2007 Mar; 44:200–204.
21. Conway G, Hettiarachchi S, Murray A, Jacobs PA. Fragile X premutations in familial premature ovarian failure. Lancet. 1995 Jul 29; 346:309–310.
22. Christin-Maitre S and Tachdjian G. Genome-wide association study and premature ovarian failure. Annales d'Endocrinologie. 2010 May; 71(3):218-221.
23. Knauff EA, Franke L, van Es MA, van den Berg LH, van der Schouw YT, Laven JS, Lambalk CB, Hoek A, Goverde AJ, Christin-Maitre S, Hsueh AJ, Wijmenga C, Fauser BC. Genome-wide association study in premature ovarian failure patients suggests ADAMTS19 as a possible candidate gene. Human Reproduction. 2009; 24:2372–2378.
24. Chapman C, Cree L and Shelling A. The genetics of premature ovarian failure: current perspectives. International Journal of Women's Health. 2015; 7:799-810.
25. Fonseca DJ, Patiño LC, Suárez YC, de Jesús Rodríguez A, Mateus HE, Jiménez KM, Ortega-Recalde O, Díaz-Yamal I, Laissue P. Next generation sequencing in women affected by nonsyndromic premature ovarian failure displays new potential causative genes and mutations. Fertil Steril. 2015 Jul; 104(1):154-62.

26. Chatterjee S, Maitra A, Kadam S, Patel Z, Gokral J, Meherji P. CGG repeat sizing in the FMR1 gene in Indian women with premature ovarian failure. Reproductive BioMedicine Online. 2009; 19(2):281-286.
27. Saul RA, Tarleton JC. FMR1-related disorders. 2012.