

Premature Ovarian Failure

A brief background to the causes of premature ovarian failure

Introduction

In the United Kingdom the average of age of the menopause is 50 years with one percent of women menstruating after the age of 60 and one percent entering menopause before the age of 40. The age of the natural menopause is closely inherited and it is also affected by environmental factors such as smoking. A menopause before the age of 40 is most commonly taken to be the definition of 'premature ovarian failure' although this definition is arbitrary. Estimates of the prevalence of premature ovarian failure range between 0.3 and 1% and this condition account for approximately 25% of women presenting amenorrhoea.

Normal ovaries and how they work

Women have had two ovaries which are situated in the pelvis either side of the uterus or womb. The ovaries have two functions: to produce eggs and hormones. The ovary comprises four cell types, germ cells, granulosa cells, theca cells and support cells. The integrity of germ cells and granulosa cells is closely interdependent so their survival and failure occur in parallel. In some causes of ovarian the defect is primarily in germ cells, X chromosome abnormalities for instance, while others the granulosa cells are the main target, eg. FSH receptor mutations.

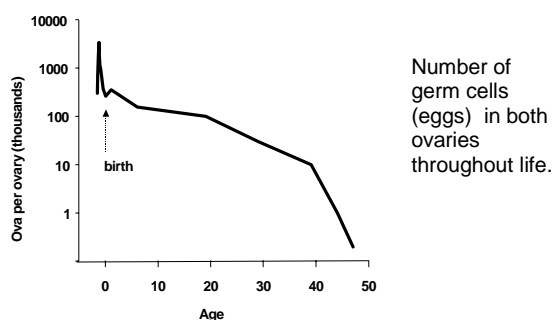
Eggs - how they grow and where they come from

In the normal menstrual cycle, about five follicles start to grow, each containing an egg. One of these follicles becomes 'dominant' and the rest die away. Ovulation occurs after the dominant follicle has grown to approximately 20 millimetres in diameter when it bursts open upon the surface of the ovary. The egg then passes down the fallopian tube where fertilisation occurs. The combined egg and sperm is called an embryo. The embryo completes the passage down the fallopian tube to the uterus where it implants into the lining or *endometrium*. If implantation is successful the embryo grows into can a placenta and foetus and the pregnancy continues. The placenta begins to make the hormone *human chorionic gonadotrophin* or hCG which can be measured in urine. This is the basis of the pregnancy test. If fertilisation or implantation does not occur, then the embryo comes away in the menstrual period about 14 days after ovulation.

All of the eggs that a woman has throughout her life are made before she is born. By the time that the female foetus has been growing for six months about 3.5 million eggs are found in each ovary. Two thirds of these eggs are destroyed in the last three months of foetal life. Most of the remaining 1 million eggs in each ovary are slowly destroyed throughout life until only the 1000 and remain at the average age of the menopause at 50 years. Only a minute proportion of eggs is used in ovulation. If a woman ovulated every month between the ages of 15 and 45, fewer than 500 eggs are used. It is not known how the body chooses which eggs will mature to ovulation and which will be destroyed. Presumably there is some 'quality control' in play.

Ovary hormones - where they come from and what they do

The ovaries make three types of steroid hormone - *testosterone*, *oestrogen* and *progesterone*. Testosterone is made by the cells which surround each follicle - *theca cells*. Some of the testosterone is released into the bloodstream but most of it is handed onto the cells which make up inner lining of each follicle



- *granulosa cells*. Granulosa cells take up testosterone and converted it to oestrogen. As the follicles grow bigger, more and more oestrogen is made and the level of oestrogen in the bloodstream rises. After ovulation the empty follicles turns into the corpus luteum which makes progesterone. Progesterone prepares the lining of the uterus for implantation. Progesterone is only made in the second half of the cycle when it can be measured in the bloodstream to provide proof that ovulation has occurred. Progesterone causes the body temperature to rises by about half a degree and this change can be measured by careful temperature recording as another way to indicate that ovulation has occurred.

The whole reproductive cycle is controlled by two hormones made by the pituitary gland - luteinising hormone (LH) and follicle-stimulating hormone (FSH). LH drives the theca cell to make testosterone and plays a major part in the timing of ovulation. At ovulation the concentration of LH in the blood rises sharply just before the egg is released. This rise in LH can be measured in urine and this is the basis of the ovulation monitoring kits available in chemist shops. FSH stimulates the granulosa cells to multiply and make oestrogen. The hormone FSH is a good marker of the health of the ovary. When the ovary and begins to fail the level of the FSH in the bloodstream rises. A higher FSH measurement is found when the ovaries stop working as in the menopause.

The average length of the menstrual cycle is 28 day but this length commonly varies between 21 and a 35 days. The first day of the menstrual cycle is counted from the first day of a period. The first half of the menstrual cycle is called the '*follicular phase*' because it is during this time that the follicles grow. Ovulation occurs midway through the cycle or more accurately, 14 days before the menstrual period. The second half of the menstrual cycle is called the '*luteal phase*' as it is during this time that the corpus luteum is active in making progesterone.

Terminology

Several terms are applied to different types of ovarian failure. Clearly each of these labels relates to one part of a spectrum of ovarian failure and the bleak out look for fertility improves only slightly with milder forms.

- Amenorrhoea - no periods for over six months. Amenorrhoea can be primary - periods never started on their own - or secondary - periods stopped sometime after puberty having started spontaneously.
- *Gonadal dysgenesis* describes the failure of the ovary to develop but is also loosely applied to all instances when no natural periods ever - primary amenorrhoea - and the failure to develop secondary sexual characteristics.

- *Premature ovarian failure* covers the presentation between menarche and the age of 40. This term has largely taken over from *premature menopause*
- *Resistant ovary syndrome* is another name for partial ovarian failure. It was originally applied to women who appeared to have ovarian failure and normal looking ovaries on biopsy. It was hoped that women with resistant ovaries might have some treatable form of infertility but this did not prove to be the case and most women thought to have 'resistant ovaries' progress to completed menopause quite briskly.
- *Occult ovarian failure* describes lesser form of partial ovarian failure whereby the serum FSH concentration is raised but menstrual cycles persist.
- *Hypergonadotrophic hypogonadism* has been applied to all of the above conditions. It simply means that the gonadotrophins LH and FSH are raised and that oestrogen concentrations are low.

Causes of Premature Ovarian Failure

Gonadal dysgenesis	Turner's syndrome Perrault's syndrome 46XX gonadal dysgenesis 46XY gonadal dysgenesis
Genetic associations	Familial Ovarian Failure Galactosaemia Enzyme defects – P450c17 FRAXA premutations BPES Small X chromosome defects FSH receptor mutations LH receptor mutations
Autoimmune	Autoimmune Polyendocrinopathy Syndrome 1 - APECED Autoimmune Polyendocrinopathy Syndrome 2 Association with various autoimmune diseases Isolated autoimmune ovarian failure
Infection	Viral oophritis
Iatrogenic	Chemotherapy Radiotherapy Pelvic surgery
Idiopathic	? Environmental toxins

Some of the causes of POF above are extremely rare - particularly the genetic causes. For instance only a few women with FSH receptor or LH receptor mutations have ever been reported. In order to give an idea of how common various conditions are, here is a list of the causes of POF in women attending a specialist clinic at the Middlesex Hospital, London.

Diagnosis	n
Idiopathic POF (no cause found)	245
Turner's syndrome	162
Autoimmune polyendocrinopathy type 2	45
Familial POF	36
Galactosaemia	24
FRAXA premutations	7
BPES	3
X chromosome breakpoints	3
APS 1 / APECED	2
Perrault's syndrome	1

Notes on various causes of POF

Gonadal dysgenesis

Women with Turner's syndrome have a variable appearance. The most consistent features are short stature and ovarian dysgenesis. Any defect of an X chromosome may cause Turner's syndrome and the most common karyotype is 45X0 - a complete absence of one X chromosome. The variability of Turner Syndrome is the result of both mosaicism, with only a proportion of cells possessing the abnormal X chromosome, and degrees of genetic loss with deletions of only part of one arm of an X chromosome. Very few women with Turner's syndrome enter puberty spontaneously and even fewer escape primary amenorrhoea. The ovaries appear as small fibrous streaks.

Some women presenting with gonadal dysgenesis have no features of Turner's syndrome and have a normal 46XX karyotype. For the most part the aetiology of 46XX gonadal dysgenesis is unknown. Other women, of identical appearance, are found to have a male genotype - 46XY. Identification of the Y chromosome is important as the abdominal testes in a female body are a risk of cancer later in life. This condition, Swyer's syndrome, can be the result of a deletion or mutation of the SRY gene but in 80% of women the SRY gene is normal and the defect is presumed to be downstream of this initial male determining trigger.

Perrault's syndrome is the combination of congenital deafness, gonadal dysgenesis and short stature and is inherited in an autosomal recessive manner. The genetic defect in Perrault's syndrome is yet to be defined.

Genetic Causes of Ovarian Failure

Familial premature ovarian failure

Between 5 and 30% of women with POF have another affected female relative. The mode of inheritance can be X-linked or autosomal, dominant or recessive and in most instances the causal mutation is unknown.

Galactosaemia

Galactosaemia is caused by mutations of the GALT gene mapped to locus 9p13. The resulting in deficiency of galactose-1-phosphate uridyl transferase results in the presentation of failure to thrive, hypoglycaemia, hepatomegaly and acidosis in the first few days of life. With early diagnosis and strict galactose restricted diet children can healthy although most females do not escape ovarian failure. The mechanism of ovarian failure in galactosaemia is unknown but a toxic effect of galactose or its metabolites has been implicated. In contrast, males with galactosaemia usually have normal gonadal function.

Enzyme defects

Defects in some of the enzymes which are responsible for the conversion of cholesterol to oestrogen cause oestrogen deficiency and hypergonadotrophic amenorrhoea. To this extent the hormone production of the ovary can be considered to have failed even though in many instances the ovaries are enlarged. This family of conditions includes the congenital adrenal hyperplasias and considered here are those which primarily cause oestrogen deficiency. One examples is a deficiency of the enzyme 17-hydroxylase which causes primary amenorrhoea and hypokalaemic hypertension.

Fragile X premutations

The FRAXA fragile site is located in the untranslated exon 1 of the FMR1 gene at Xq27.3. Normally less than 60 trinucleotide (CCG) repeats occur in this exon. A FRAXA premutation is defined as between 60 and 200 repeats at this site and the full mutation is defined as greater than 200 repeats. The full FRAXA mutation is associated with methylation of the FMR1 promoter and silencing of gene transcription with resulting mental impairment most obvious in males. The FRAXA premutation was thought to have no effect on the transcription of the FMR1 gene or translation of its mRNA. Recently, FRAXA premutations have been shown to be associated with premature ovarian

failure particularly in its familial form. The mechanism of this association is unknown.

Blepharophimosis, ptosis and epicanthus inversus syndrome

The BPES locus is on chromosome 3q23 and is transmitted as an autosomal dominant trait. The characteristic appearance of drooping, tethered eyelids is accompanied by ovarian failure in one half of women.

FSH and LH receptor gene mutations

A hand full of women with POF from around the world have been found to have defects in the receptors which sense the reproductive hormones LH and FSH. These gene defects have been found by specialist research teams and are not part of routine investigations.

Complete loss of function of the FSH receptor results in small hypoplastic ovaries while partial loss of function results in secondary amenorrhoea with a normal looking ovarian morphology. Interestingly, men with FSH receptor mutations had only a variable suppression of sperm count and in some cases were fertile.

The hormone, Luteinising Hormone, stimulates the interstitial cell of the ovary to make testosterone which is changed to oestrogen by the granulosa cell. Therefore, without the action of LH there is no oestrogen production and the ovaries appear to have 'failed'. Women who have a defective LH receptor present with primary amenorrhoea with the typical picture of primary ovarian failure.

Autoimmunity and the ovary

Between 10 and 30% of women with POF have a concurrent autoimmune disease the most common of which is hypothyroidism. In addition to the syndromes described below, POF has also been reported in association with myasthenia gravis, systemic lupus erythematosus, rheumatoid arthritis and Crohn's disease.

Autoimmune polyendocrinopathy syndrome (APS) type 1

APS1 is a rare autosomal recessive syndrome comprising mucocutaneous candidiasis, hypoparathyroidism, Addison's disease. Hypothyroidism, IDDM, pernicious anaemia and ovarian failure are additional features of APS1 occurring with variable frequency. Ovarian failure occurs in 60% of affected females while in men, the testis is spared autoimmune attack. This clinical picture of autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy is also known as APECED syndrome. APS1 has no HLA association and is caused by mutations in the AIRE gene which codes for a putative transcription regulator. So far, the variability of the clinical features has not been closely parallel by the genotype.

Autoimmune polyendocrinopathy syndrome (APS) type 2 – Schmidt's syndrome

APS2 classically comprises Addison's disease, hypothyroidism, insulin dependent diabetes mellitus (IDDM) and ovarian failure. The most common accompaniment to ovarian failure is hypothyroidism and this combination probably represents a form APS2. The sequence of glands involved is variable with up to 15 years spanning the first and last. APS2 is associated with the HLA haplotypes HLA-B8, DR3 and DR4. The target antigens were originally recognised by steroid producing cell antibodies but are now known to be P450 cytochromes from the steroidogenic pathway: P450c17, P450c21 and P450scc.

Autoimmunity in isolated ovarian failure

Evidence of autoimmunity in women with isolated POF comes from ovarian histology and the detection of circulating antibodies directed against ovarian antigens. Occasional reports of ovarian histology have noted the presence of a lymphocytic oophritis in women with POF but because of small series the significance of this finding is not known.

Routine anti-ovarian antibodies are rarely positive in women with isolated POF. Antibodies directed against the cytochromes

P450c17, P450c21 and P450scc are typical of APS2, are not in play in isolated POF. Recently, however, the enzyme 3 β hydroxysteroid dehydrogenase has been proposed as a marker for autoimmunity in women with POF. The significance of anti-3 β HSD positivity in terms of pathogenesis is unclear

Viral oophritis

Not infrequently, women with POF recall a notable preceding infection but a pathogenetic link is only particularly strong for mumps oophritis. Between 2 and 8% of women have been reported to have oophritis after outbreaks of mumps infection compared to 25% of males with orchitis. Evidence for destructive oophritis from other pathogens is slight. In immune suppressed states, cytomegalovirus has been implicated.

Chemotherapy / Radiotherapy

With the increasing success of treatment for childhood malignancies more females are surviving to reproductive age. The cytotoxic treatments used in treating leukaemia or Hodgkin's disease in particular cause ovarian failure in approximately 50% females. Alkylating agents and cyclophosphamide are particularly prone to cause gonadal damage and this effect is related to both the dose and the duration of treatment. Cytotoxic induced ovarian failure is notable for occasional spontaneous remission which occurs rather more often for other aetiologies. Women with haematological malignancies who receive total body irradiation (TBI) however, almost universally experience ovarian failure which is rarely reversible.

Pelvic surgery

The life span of ovaries which are preserved during hysterectomy is generally accepted to be reduced. The evidence on which this dogma is based is largely retrospective and anecdotal so it is impossible to estimate the magnitude of this effect.

Idiopathic Ovarian Failure

A large proportion of women with primary ovarian failure have no identifiable cause. It seems likely that occult viral oophritis might account for many of these women. Alternatively, environmental toxins might be invoked. While the effect of toxins on the testicular function has been suggested as a cause of falling sperm counts over this century, there is little comparable epidemiological data in women.

Investigation of POF

The only features of history which are helpful in determining aetiology of ovarian failure are positive a family history, a concurrent autoimmune disorder or stigmata of one of the inherited conditions. In many instances a formal pedigree enquiry is required to determine other female family members who may be affected, particularly if the inheritance is passed through an unaffected male.

Ten to 30% of women with POF already have a concurrent autoimmune disorder the most common of which is hypothyroidism. In the sequence of the most common autoimmune associations found in APSII - ovarian failure can occur at any stage. Thus, a history should include symptoms of Addison's disease, hypothyroidism and diabetes in particular with the rarer association of autoimmune arthritides and inflammatory bowel disease being kept in mind. Further, women with established POF should be monitored for the later appearance of these conditions.

Examination may reveal a clue to an autoimmune background such as vitiligo or signs of rheumatoid arthritis or systemic lupus erythematosus. The congenital associations of blepharophimosis and ptosis with BPES, deafness and short stature with Perrault syndrome and hypertension with CYP17 mutations should be sought - see 'causes of ovarian failure'.

Some women appear to go through self-limiting, brief bouts of ovarian failure and for this reason two measurements of FSH

some weeks apart should be taken before the diagnosis of POF is made. Cytogenetic analysis is essential in women presenting with primary amenorrhoea when chromosomal defects, usually a Turner variant or the presence of a Y chromosome, can be found in 40% of cases. It is rare for women with secondary amenorrhoea to have a chromosomal defect but small deletions of the X chromosome, have been found in women presenting with amenorrhoea as late as 35 years old.

The value of antibody screening is debatable as the sensitivity of the current tests for autoantibodies directed against the ovary is low. Only a few percent of women show positive immunofluorescence against the ovary by standard methods while 20 - 30% are positive for thyroid autoimmunity. While the definition of an autoimmune cause may not alter immediate management, long term follow-up of another component of a polyendocrinopathy is defined in this way.

The place of ovarian biopsy and pelvic ultrasound is restricted to research. Neither investigation affects management. While some investigators have found that the histological appearance of the ovary can predict fertility prospects, it remains the case that women with no ova visible on biopsy have spontaneously conceived making the application of this procedure largely obsolete. Similarly, while pregnancy after the diagnosis of POF is more likely if ovarian apparatus is identifiable on ultrasonography, the event is so rare that the discrimination offered by this test is not routinely useful.

Treatment of Premature ovarian failure

The diagnosis of premature ovarian failure in a young women is a devastating event. One of the most neglected aspects of POF is the long-term psychological scar left by the diagnosis. This is especially true of younger women who experience low self-esteem and depression which cannot be accounted for solely by oestrogen deficiency. Counselling or patients support group should be offered to all women.

Hormone replacement therapy

Most women with primary ovarian failure opt for long term oestrogen replacement therapy in order to prevent symptoms of oestrogen deficiency and osteoporosis. The youngest women may require HRT for nearly 40 years. The degree to which this long term administration of oestrogen prevents cardiovascular disease or increases risk of breast cancer is unknown and we can only extrapolate from studies in older postmenopausal women.

The choice of oestrogen in formulation must be made on individual basis. Remembering that occasional sporadic pregnancy can occur, some women might choose a combined oral contraceptive preparation. Combined oral contraceptives however, provide oestrogen only for three weeks out of four. Also, the combined oral contraceptives supply supra-physiological oestrogen which may increase the risk of thromboembolic events to a greater degree than HRT formulations, especially in women who smoke.

The main choice in oral HRT formulations is between conjugated oestrogens and oestradiol valerate which largely interchangeable. For those women are troubled by a side effects from oral oestrogen, a transdermal preparation may be the answer, particularly for those with concurrent hypertension or with additional risk factors for thrombosis. Those women with particularly prominent vaginal symptoms may benefit from the additional topical oestrogen in the form of a pessary, vaginal tablet or cream. The place of oestrogen implants is greatly diminished now that the formulation of transdermal patches has improved making them more widely acceptable. None of these HRT formulations are contraceptive. Even if pregnancy is very unlikely in POF, contraception is still advisable. An unwanted pregnancy for a young woman with POF can be a very traumatic experience - it may be the only spontaneous pregnancy in that

woman's life. If HRT is chosen then it can be combined with barrier contraception or an intrauterine device.

Progesterone is required for all women with an intact uterus in order to avoid endometrial hyperplasia induced by unopposed oestrogen. While some women might find the continuous combined form of oestrogen and progesterone replacement attractive as a way of avoiding menstruation, it must be remembered that the use of these preparations in young women has not been studied over the long-term.

Testosterone supplements are rarely required when the adrenal gland continues to supply androgens. For those women with combined ovarian failure and Addison's disease who have no source of androgens, it seems reasonable to offer testosterone replacement. Recent trials have shown that testosterone replacement improves mood, well being and libido although its use is limited by the side effects of unwanted hair growth and acne.

Options for fertility

Spontaneous return of ovarian function can occur in women with a firm diagnosis of POF and no particular feature predicts this rare event with great accuracy. It is clear however, that medical treatments do not make this rare event more likely. For instance, conventional ovulation induction treatment with, or without, suppression of gonadotrophins, does not raise the fertility rate above background levels in women with POF. Hormonal replacement therapy has a neutral effect on fertility in this group of women - in fact, the rare conception usually occurs during HRT treatment. Uncontrolled studies have suggested that treatment with glucocorticoids might promote fertility, particularly in women who have an autoimmune aetiology for ovarian failure. Given the fact that good autoimmune markers have yet to be developed for ovarian failure and that the risks of this treatment have not been evaluated, it seems premature to promote this option for anyone outside of a controlled research setting.

The advent of ovum donation has been a major breakthrough for women with ovarian failure over the past ten years. As the number of assisted reproduction centres offering a ovum donation increases, more women will be able to take up with this option. In the United Kingdom, the success rate of ovum donation is somewhat higher than that for a standard *in vitro* fertilisation giving each couple a 30% chance of a pregnancy for each cycle of treatment. Embryo donation offers an alternative strategy for achieving a normal pregnancy while some couples choose surrogacy or adoption.

There is increasing expectation that advanced assisted reproduction techniques might make it possible to utilise the very few eggs remaining in the ovary at the time of diagnosis of ovarian failure. At the time of writing, there have been no consistent successes with cryopreservation of ovarian material. Frozen sections of ovary might be used for later autotransplantation with a view to *in vivo* ovulation or alternatively, dispersed ova may be matured *in vitro* in preparation for fertilisation. Until the methodology of these techniques are proven, cryopreservation should be preserved for rare circumstances. One such circumstances is a young woman facing chemotherapy which is likely to destroy the ovary. The starting material in this situation is a normal healthy ovary which might be able to sustain the damaging effects of the freezing process. It is premature to offer cryopreservation to women presenting with non-iatrogenic ovarian failure in whom the very little ovarian tissue remains at the time of diagnosis. Instead, women with established ovarian failure will have to wait for the establishment of *in vitro* maturation of their few remaining oocytes.