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Natural history of ovarian function including assessment of ovarian reserve and premature ovarian failure



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This chapter describes ovarian anatomy and embryology in humans. The formation of the ovarian reserve is discussed, and events of folliculogenesis are described, including description of developmental events in primordial, primary, secondary, antral and peri-ovulatory follicles. Paracrine and autocrine factors play critical roles in oocyte maturation and follicular development, and research related to the hypothesised roles of individual factors is discussed. Gonadotrophin-dependent events relating to dominant follicle selection are discussed. The two-cell, two-gonadotrophin hypothesis of ovarian steroidogenesis is explained. The clinical role of AMH is outlined. Premature ovarian failure and known associated aetiological factors are described. In the conclusion, with an understanding of the principle events of ovarian folliculogenesis, the follicular wave theory is described, and it is explained how adaptation of ovarian stimulation regimens may achieve time-efficient fertility preservation treatment options for patients with cancer.

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Anatomy of the ovary

The human ovary has three major regions: the hilum (rete ovarii), the outer cortex and the central medulla.

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The hilum contains blood vessels, nerves and hilus cells and is the point at which the ovary attaches to the mesovarium.

The ovarian cortex has an outer zone (tunica albuginea) and an inner zone where ovarian follicles surrounding oocytes are embedded within the stromal tissue. Ninety per cent of ovarian cancers originate from the cuboidal surface epithelium of the ovarian cortex. Stromal interstitial cells (from the mesenchyme) produce androgens in response to Luteinizing Hormone (LH) receptor activation.

The central medulla is derived from mesonephric cells.

The ovarian reserve

Human germ cells originate in the primitive ectoderm and migrate to the gonadal ridges by 5 weeks of gestation. At this stage, paired ‘indifferent’ gonads exist as gonadal ridges, alongside the mesonephric ducts (the urogenital ridges). During their migration, germ cells proliferate, multiplying by mitosis.

Differentiation of the indifferent gonad into an ovary (or testis) involves the expression of many genes, directed by the sex chromosomes.

In the early ovary, rapid mitotic germ cell multiplication results in peak numbers of oogonia (6–7 million) by 20 weeks of gestation.

Oocyte formation

Oogonia are transformed into oocytes as they enter Meiosis I (arresting in the diplotene stage of prophase). Each oogonium is destined to become a single oocyte. Meiosis will resume before ovulation to be completed at the time of fertilisation.

A massive loss of oocytes physiologically occurs in the second half of pregnancy during the phase of follicle formation.

Follicle formation occurs from around 20 weeks of gestation. Secondary sex cords (vascular projections from the medulla) penetrate and divide the ovarian cortex. Perivascular cells (with origins from the mesonephros/coelomic epithelium) give rise to spindle-shaped pre-granulosa cells that surround oocytes in a single layer, surrounded by a basement membrane (primordial follicles).

Folliculogenesis

From 20 weeks of gestation until the time of menopause, a continual process of recruitment, folliculogenesis and atresia occurs. This process progresses until the preantral phase throughout all stages of life, including during pregnancy and while using hormone-modulating agents (for example contraception, aromatase inhibitors or selective oestrogen receptor modulators).

The first preantral phase is directed by local growth factors through autocrine/paracrine mechanisms. The second developmental phase is gonadotropin dependent and proceeds to the endpoint of either ovulation or atresia [1]. The entire transition from a primordial to an ovulating follicle takes approximately 1 year [2].

Studies in several species have demonstrated that members of the transforming growth factor- β (TGF- β) superfamily (including bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), TGF- β , activins and inhibins and anti-Müllerian hormone (AMH)), are expressed in the ovary and involved in the regulation of folliculogenesis through autocrine and paracrine mechanisms [2–4].

Oocyte–somatic cell interaction

Oocytes actively participate in directing follicular development and ovulation. Within the micro-environment of the ovarian follicle, oocytes and their supportive somatic cells coordinate both folliculogenesis and oocyte meiotic competence [5].

Oocyte-derived paracrine factors have been shown to promote the development and survival of cumulus–oocyte complexes. Particularly implicated are GDF9 (growth differentiation factor-9) and

GDF9-BMP15 heterodimer, which also modulate the expression of target genes involved in ovulation and luteinisation [6]. One mechanism demonstrated in mouse knockout models is through the regulation of mTOR activation in cumulus cells [5].

Oocytes modulate FSH-stimulated oestradiol and progesterone production in granulosa cells [7] and suppress the expression of LH receptors induced by FSH [8]. Oocyte-derived BMP15 decreases progesterone production by downregulating the expression of *StAR* in human granulosa cells [9].

These findings are compatible with the longstanding proposed hypothesis that the oocyte is capable of inhibiting follicular luteinisation [10].

Transcriptome assessment has shown that bidirectional communication between the oocyte and cumulus cells is essential for the production of a competent oocyte [11,12]. It is hoped that further research into the complex molecular events in cumulus–oocyte interactions will enable the design of clinical in vitro maturation (IVM) protocols [13,14].

From resting pool to ovulation

It takes approximately 290 days or 10 regular menstrual cycles for a primordial follicle to develop to the secondary stage. The process of atresia begins to impact at this stage. At the antral follicle stage, a dominant follicle is selected approximately 20 days before ovulation. The total time interval between antrum formation and the mature development of a 20 mm preovulatory follicle is approximately 60 days. Overall, a future dominant follicle starts its journey (from resting pool to the release of a mature oocyte) 12 menstrual cycles before ovulation [1].

Gonadotrophin independent follicular recruitment: primordial, primary and secondary follicles

Primordial follicle

A primordial follicle is a structure enveloping a small primary oocyte (~25 µm) within a single layer of squamous granulosa cells on the basal lamina. The diameter of the human primordial follicle is approximately 30 µm. There is no direct contact between the cells of the primordial follicles and other cells. Primordial follicles lack any independent blood supply, and their access to the endocrine system is very much limited [15]. The oocyte within a primary follicle is arrested in the dictyate stage of meiosis I.

Several genes have been identified as important in the formation of primordial follicles, including *FIGLA* and *Forkhead box L2 (Foxl2)* [16].

A woman forms her reserve of primordial follicles during foetal life, and the ovarian reserve is not restorative. Between 6 and 9 months of gestation, as primordial follicles are forming, a profound loss of oocytes occurs through apoptosis. Throughout the first 30 years of a woman's life, primordial follicles are serially activated at a steady rate and recruited to progress through the stages of folliculogenesis towards their destiny of either atresia or ovulation. Beyond this time, the rate of loss of follicles to atresia accelerates. This physiological process contributes to a loss of ovarian reserve that has been linked to declining fecundity over the age of 35 years [17].

Intrinsic paracrine and autocrine factors have been implicated as important in maintaining the majority of primordial follicles in a resting state, thus resisting recruitment and maintenance of the ovarian reserve. These include *PTEN* (phosphatase and tensin homolog), *Foxo3a* (forkhead box O3A) and *SDF-1* (stromal cell-derived factor-1). Other signalling molecules including *AMH* and possibly *SDF-1* are secreted from surrounding growing follicles and have been proposed to negatively regulate primordial follicle activation.

Primordial oocytes secrete other factors such as platelet-derived growth factor (*PDGF*) and basic fibroblast growth factor (*bFGF*), and these factors stimulate pre-granulosa cells to secrete *KL* (Kit-ligand), *KGF* (keratinocyte growth factor), *BMP-4* and *BMP-7*, thereby promoting oocyte growth and follicle activation. It has been proposed that circulating insulin, *GDF-9* and *BMP-15* promote granulosa cell proliferation.

Insulin from the circulation may promote follicle activation. Several oocyte, granulosa and stromal cell proteins have been identified to be critical for primordial follicle survival, inhibition and recruitment (Table 1).

Primary follicle

The primary follicle is an oocyte surrounded by a single layer of cuboidal granulosa cells. Its major developmental events are growth and differentiation of oocyte and FSH receptor expression in granulosa cells [18].

FSH receptor expression is stimulated by FSH, activin, TGF β and cyclic AMP [19].

Follicle recruitment and initial growth stages are gonadotropin independent, but FSH is requisite for primary to preantral stage follicular transition. Higher FSH plasma levels accelerate primary follicle development [20].

The oocyte genome reactivates, thus resulting in oocyte growth and development of the zona pellucida, a surrounding layer of extracellular matrix. The transcriptionally active oocyte generates mRNA transcripts to support immediate growth and also future processes including oocyte maturation, fertilisation and early embryogenesis.

Follicular growth and development is regulated by a complex autocrine and paracrine relationship between oocyte, granulosa and stromal cell protein expression [21].

In primary follicles, intercellular connections (cytoplasmic projections and interdigitating microvilli) develop between the oocyte and granulosa cells, thus creating a large surface area for diffusion. Gap junctions, that is intercellular connexin protein channels, allow diffusion of regulatory and nutrient molecules between adjacent cells [22].

Secondary follicle

Secondary follicles contain a fully grown oocyte surrounded by zona pellucida, five to eight layers of granulosa cells, a basal lamina and theca interna and externa with associated blood vessels.

Events of the primary-to-secondary transition:

- Oocyte completes its growth but meiosis remains suspended.
- Granulosa cells progress from simple cuboidal epithelium to stratified columnar epithelium.
- The two primary layers of theca interna and externa appear.
- Angiogenesis allows the blood to circulate around the follicle, thus exposing it to external influences including nutrients and gonadotropins.
- Theca externa gains autonomic innervation.

Table 1

Oocyte, granulosa and stromal signalling molecules and pathways implicated in the recruitment and growth of follicle and inhibition of adjacent follicular development.

Oocyte, granulosa and stromal signalling molecules and pathways	Molecular role
<ul style="list-style-type: none"> • Spermatogenesis- and oogenesis-specific basic helix-loop-helix 1 (Soxhlh1) 	Regulate gene expression
<ul style="list-style-type: none"> • Soxhlh2 • FIGLA • LHX8 • NOBOX • GDF-9 • BMP-15 • PDGF • bFGF • Kit ligand • Phosphatidylinositol 3-OH-kinase (PI3K)-AKT-Foxo3a signalling • PTEN • TSC1 and TSC2 • mTOR • SDF-1 • KGF • BMP-4 • BMP-7 • AMH 	Regulate gene expression Promote primordial follicle survival and activation Regulate follicle development Promote granulosa cell proliferation Restrain follicle activation Promote primordial to primary follicle transition Negatively regulates recruitment

Antral follicle

An antral follicle develops an 'antrum', a cavity containing follicular fluid, a plasma exudate that functions as a regulatory microenvironment of secretory products from both the oocyte and granulosa cells [23].

Antral follicles grow to a total diameter of 1–10 mm.

The relative number and size of antral follicles vary between individuals and as a function of age and menstrual cycle stage. The 'antral follicle count' (AFC, determined by ultrasound) can assess the number of visible follicles present in a woman's ovaries early in her menstrual cycle. AFC can be used clinically as a measure of ovarian reserve.

The theca interstitial cells express receptors for LH and insulin and have a capillary network that surrounds antral follicles. High levels of androgens are produced in response to LH and insulin stimulation.

The granulosa cells of antral follicles differentiate into subtypes influenced by their position: the membrana, periantral area and cumulus oophorus. All the subtypes express FSH receptors, but the granulosa cell subtypes have a differential response to FSH stimulation, thus influencing steroidogenesis and LH receptor expression. A proposed controlling influence is the morphogen gradient secreted by the oocyte [24].

Gonadotrophin-dependent follicular recruitment

Selection and Atresia

Human physiology has evolved a mechanism of monofollicular ovulation in the majority of ovulatory menstrual cycles. Behind this process is the selection and preferential maturation of a single antral follicle from a cohort of follicles that had up to a critical point, developed in parallel.

The mechanism of selection is gonadotrophin dependent and involves the secondary increase in FSH (as plasma oestradiol, inhibin A and progesterone levels decrease) at the end of the luteal phase.

FSH levels remain elevated through the first week of the follicular phase, thus resulting in progressively increased follicular fluid levels of FSH in the dominant follicle, inducing selection.

The mechanisms by which a single follicle attains dominance over subordinate follicles under physiological circumstances in humans remain poorly understood. It involves the complex interplay of endocrine, paracrine and autocrine factors. When negative feedback by increasing oestrogen and inhibin levels results in falling concentrations of circulating FSH, dominant follicle(s) survive and proceed to develop to the Graafian stage [25].

The fate of subordinate follicles is to die by atresia through the activation of apoptosis pathways (signalling pathways coupled to programmed cell death) in the oocyte and granulosa cells. Atresia may be prevented by exposure to prolonged high concentrations of FSH, the basis for IVF stimulation regimens.

FSH signalling. FSH binds to a G-protein transmembrane receptor and activates it. In humans, the FSH receptor contains 678 amino acids and is organised into three domains:

- 1) An extracellular NH₂-terminal ligand-binding domain that has six potential N-linked glycosylation sites.
- 2) A transmembrane domain: seven hydrophobic alpha helices (heptahelical domain) secure the receptor in the plasma membrane.
- 3) An intracellular COOH-terminal domain. Regulated phosphorylation of amino acids is involved in FSH receptor desensitisation and downregulation.

G-protein receptor action involves intracellular second messenger systems, activated or modulated through ligand binding (e.g. cAMP and PKA), thereby influencing downstream gene expression. It is currently acknowledged that multiple second messenger pathways are involved in post-receptor effects. Case studies have shown that heptahelical domain mutations can result in FSH receptor autoactivation. Further research leads to the acknowledgement of FSH receptor polymorphisms that

may be associated with differential response to iatrogenic FSH stimulation and tendency to develop ovarian hyperstimulation syndrome [26].

Granulosa mitosis. Granulosa cells of the dominant follicle rapidly proliferate during the follicular phase of the menstrual cycle in response to FSH stimulation and the influence of local factors including GDF-9, BMP-15, BMP-6, activin, TGF- β isoforms and oestradiol [3].

Aromatase enzyme expression. FSH-mediated induction of P450AROM provides granulosa cells of the dominant follicle the ability to produce high concentrations of oestradiol from theca-derived androstenedione [27].

Luteinisation potential. During the follicular phase, granulosa cells acquire the potential to produce progesterone (luteinisation potential), but the process remains suppressed. When preparing for luteinisation, under the influence of FSH, granulosa cells of the dominant follicle express steroidogenic acute regulatory protein (StAR), P450 side-chain cleavage enzyme (P450SCC) and 3 β -hydroxysteroid dehydrogenase (3 β -HSD). Inhibition of progesterone production until just before ovulation is achieved by oocyte-derived inhibitors. Candidate molecules include GDF-9, BMP-6 and BMP-15.

LH receptor expression. The LH receptor is a G-protein-coupled heptahelical transmembrane receptor, structurally similar to the FSH receptor. Its activating ligands are LH and HCG.

The dominant follicle acquires competence to respond to the LH/FSH surge by upregulating granulosa cell expression of LH and FSH receptors. FSH itself induces this process, and it is thought that oocyte-derived factors inhibit mature LH receptor expression (glycosylation, transit to the cell surface) until the late follicular phase.

LH signalling. In humans, the LH receptor contains 675 amino acids and is organised into three domains:

1. An extracellular ligand-binding domain
2. A heptahelical transmembrane domain
3. An intracellular domain (G-protein interactions and protein kinase C phosphorylation sites).

Downstream effects of LH receptor activation are mediated by second messenger systems (e.g. GS/adenylate cyclase/cAMP/PKA). Phosphorylation of target proteins modulates gene transcription, thereby promoting androstenedione biosynthesis.

Insulin and androgen production. All antral follicles have the potential to produce androstenedione. Insulin receptors are expressed by human theca cells. Insulin and LH can synergistically upregulate androstenedione biosynthesis, and insulin resistance is associated with clinical and biochemical hyperandrogenism in women. HDL is also a potent stimulator of androgen production in theca cells.

Two-cell, two-gonadotrophin hypothesis of ovarian steroidogenesis

LH and the theca cells: LH receptor present, P450AROM absent

- LH receptors are present on theca cells (and initially absent on granulosa cells).
- LH stimulates steroidogenesis and the secretion of androstenedione and testosterone by theca cells. The amount of androgen secretion can be influenced by other endocrine and paracrine factors (e.g. insulin, IGF-1, activin and inhibin).
- Androstenedione/testosterone diffuses and accumulates in follicular fluid.

FSH and the granulosa: LH receptor initially absent, P450AROM present

- FSH induces aromatase enzyme (P450AROM) activity in granulosa cells.
- Androstenedione/testosterone that diffused into granulosa cells (through the follicular fluid) is aromatised to oestrone and converted to oestradiol (by 17 β -hydroxysteroid dehydrogenase).
- Granulosa cells release oestradiol, which diffuses back towards the thecal layer and into the peripheral blood stream.

(Granulosa cells acquire LH receptor expression as the dominant follicle grows.)

Ovulation and oocyte meiosis

Ovulation. Ovulation is the release of a mature metaphase II oocyte (enclosed within a cumulus complex) from the dominant follicle for possible fertilisation.

The achievement of ovulation requires co-ordinated and co-operative actions of the anatomical compartments of the ovarian follicle, the endocrine system and intraovarian paracrine factors.

Events leading to ovulation

1. Antral fluid accumulation and cavity distension
2. Mural granulosa cell differentiation: oocyte morphogen gradients result in high LH receptor concentration in mural granulosa cells, which mediate the response to the mid-cycle LH surge signal.
3. Cumulus cells undergo expansion and mucification (secreting a viscoelastic extracellular matrix composed of hyaluronan and associated proteins). The matrix created is important for the ovum to be picked up by the fallopian tube and sperm/oocyte interaction *in vivo*.
4. A cascade of signalling pathways are activated (e.g. Gs/cAMP/PKA, ERK and MAPK) with complex downstream effects. Numerous proteins generated in the mural granulosa cells are likely to be required for successful ovulation and prevention of premature luteinisation. The physical expulsion of the oocyte is dependent on a pre-ovulatory surge in intra-follicular prostaglandin synthesis [28].
5. Theca remodelling occurs and stigma (macula pellucida) formation occurs, thus creating a hole in the surface of the ovary through which the ovum is released. Ovulation involves apoptosis, proteolysis, vascular remodelling and cell migration (through the disruption of gap junctions in theca cells) initiated in response to the LH surge.

Resumption of meiosis. Meiotic resumption is a critical event in ovulation because it is a prerequisite for normal fertilisation. The LH surge invokes post-receptor effects that modify intra-follicular molecular factors, which, up to this point, prevented meiosis I from progressing. Mouse models have implicated intra-oocyte cAMP concentration and gap junction changes in this process [29, 30]. The oocyte nucleus (germinal vesicle) breaks down, meiosis I resumes and the first polar body is emitted. Meiosis II progresses to metaphase and is again arrested. Meiosis will not proceed further until the oocyte is fertilised.

Luteinisation and the corpus luteum

Following ovulation, the follicle wall becomes the corpus luteum. The corpus luteum is a transient endocrine gland that produces abundant amounts of progesterone and oestradiol during the first week of the luteal phase and, if pregnancy occurs, until placental steroidogenesis is well established.

Cells of the corpus luteum are contributed by the membrana granulosa, theca interna, theca externa and invading vasculature.

Immediately before ovulation, granulosa cells express StAR, P450SCC, 3 β -HSD and P450AROM in high concentrations. After ovulation, the granulosa cells transform to granulosa-lutein cells. Cells increase in size and develop an ultrastructure typical of differentiated steroidogenic cells with abundant smooth endoplasmic reticulum, mitochondria featuring tubular cristae and large lipid droplet cytoplasmic inclusions [31].

Theca-lutein cells also exhibit the ultrastructure of steroidogenic cells and produce androstenedione. It is thought that the two-cell, two-gonadotropin mechanism for oestradiol synthesis operates in the human corpus luteum.

Steroidogenesis is dependent initially on LH and subsequently on HCG. Progesterone levels peak around 8 days after ovulation. If pregnancy does not occur, the corpus luteum degenerates by luteolysis to form corpus albicans. Oestrogen and progesterone levels fall, and a new menstrual cycle begins.

The clinical role of measuring AMH. Anti-Mullerian Hormone (AMH) is a dimeric glycoprotein produced by granulosa and Sertoli cells. In male foetal development, AMH produced by Sertoli cells of the testes induces regression of the Mullerian structures. In the absence of AMH, the uterus, fallopian tubes and upper vagina arise from the Mullerian ducts.

In women, AMH is produced by the primary ovarian follicles and acts to negatively regulate the progression of earlier resting follicles into active and progressive folliculogenesis. AMH mutations with reduced in vitro bioactivity have been linked to premature ovarian insufficiency [32].

AMH can be measured from the serum at any stage of the menstrual cycle [33]. Early assays were less reliable than the current standard [34].

AMH levels are temporarily dampened by oral contraceptive use [35].

AMH has several clinical applications. AMH is helpful for the early diagnosis of disorders of sexual differentiation, cryptorchidism and anorchia, precocious and delayed pubertal onset, the diagnosis of granulosa cell ovarian tumours, the diagnosis of polycystic ovarian syndrome (PCOS) and the assessment of ovarian function and ovarian reserve.

In patients with cancer, AMH can be serially measured to assess the impact of chemotoxic agents on ovarian function to predict future fertility and the onset of premature ovarian insufficiency [36].

Before ovarian stimulation for IVF and oocyte cryopreservation, AMH may be utilised to guide regimen choice and minimise the risk of the iatrogenic complication of ovarian hyperstimulation syndrome (OHSS) [37].

Premature ovarian failure. Premature ovarian failure (POI) refers to women who lose ovarian function and undergo transition to an early menopause at an age below 40 years. Ovarian failure is associated with progressive oligomenorrhoea to an eventual hypergonadotropic hypogonadal state with associated infertility.

Psychological and adverse secondary health effects of POI (such as low mood, vaginal dryness and sexual pain, vasomotor symptoms, sleep disturbance and progressive osteopaenia/osteoporosis) mandate a holistic and health promotion-centred approach for long-term management of patients with POI [38]. One element of this management is optimised hormone replacement, which should continue until the average age of natural menopause (50–55 years of age) [39].

At least 50% of patients with POI are not diagnosed with a causative underlying condition, and the aetiology of their POI remains unexplained.

POI can have genetic causes. Well-known genetic associations with POI exist in Fragile X syndrome (FRAX1), Turner's syndrome (45X0 Karyotype) and pseudohypoparathyroidism type 1a (GNAS1 gene). Genome-wide association studies and animal models have identified a large number of rare genetic mutations occurring in cases of POI, many involving proteins involved in the regulation of gametogenesis and ovarian folliculogenesis. Examples of genes involved in POF cases include FMR1, DIAPH2, POF1B, FOXL2, BMP15, NOBOX, FIGLA and NR5A1, but this list is by no means exhaustive [40]. The congenital metabolic enzymatic defect in galactosaemia is associated with POI [41].

Autoimmune causes of POI involve the formation of anti-ovarian antibodies, thus leading to the destruction of ovarian follicles. Women with autoimmune POI often suffer clustering of other autoimmune conditions such as Hashimoto's thyroiditis, Addison's disease, diabetes, coeliac disease, rheumatoid arthritis, systemic lupus erythematosus, vitiligo, albinism and myasthenia gravis [42].

Premature ovarian insufficiency or failure can occur following toxic effects of chemotherapy and radiotherapy on ovarian function (iatrogenic POI).

In patients at risk of treatment-related POI, fertility preservation treatment options may be available and fertility specialist opinion should be sought.

Wave theory, random start ovarian stimulation protocols for emergency oocyte cryopreservation

Progress in diagnosis and treatment of cancer has resulted in the need for fertility preservation to be a routine consideration in women with cancer who have not yet completed their families.

Several potential options are available to preserve fertility in patients with cancer, including ovarian tissue cryopreservation, immature or mature oocyte and embryo cryopreservation. Of these options, mature oocyte collection for embryo cryopreservation or mature oocyte cryopreservation offers the best expected live birth outcome.

A challenge for clinicians is to co-ordinate an FSH-stimulated treatment cycle and oocyte collection procedure within an acceptable timeframe, so that patients' oncology management planning and ultimate prognosis for survival are not compromised by treatment delay.

Random start IVF protocols rely on the existence of multiple waves of follicular development throughout and transcending the boundaries of the menstrual cycle [43]. Follicular wave theory has been documented across species and is observed to also occur in humans [44].

Much of the evidence surrounding IVF treatment is drawn from studies of traditional follicular phase start protocols and the development of random start rapid IVF protocols for patients requiring fertility preservation is a relatively new concept area.

In a standard follicular phase start IVF protocol, principles of management include the support of simultaneous multifollicular maturation under the influence of FSH and the prevention of spontaneous ovulation. Ovulation may be prevented using a GnRH agonist from the time of cycle commencement or a GnRH antagonist typically introduced 6–8 days into active treatment. GnRH antagonist regimens are associated with a low risk of ovarian hyperstimulation, an iatrogenic complication of ovarian stimulation that is particularly undesirable in patients planning to commence chemotherapy. GnRH antagonist protocols have the advantage that a GnRH agonist trigger may be utilised, which both reduces OHSS risk and achieves earlier post-treatment luteolysis, thus reducing progesterone exposure in women with progesterone-sensitive cancers.

Outcome studies from IVF cycles initiated in the luteal phase have been encouraging. Luteal phase cycle start was associated with longer length of FSH stimulation and higher total FSH dose use before oocyte retrieval compared with follicular phase start. In a meta-analysis of eight studies, no differences in peak oestradiol levels were detected and frozen embryo transfer (FET) pregnancy rates did not show statistical difference compared to embryos created from follicular phase start regimens [45].

When considering luteal phase ovarian stimulation, the effect of progesterone in the luteal phase must also be considered. Progesterone is potentially inhibitory to follicular recruitment and induction of luteolysis, and this effect may at least partially explain the increased length of FSH treatment in luteal phase start protocols. In a luteal phase start cycle, the immediate commencement of a GnRH antagonist is thought to induce luteolysis and may be of benefit in minimising the necessary length of treatment [43].

Aromatase enzyme inhibitors (e.g. letrozole) can be used in luteal phase stimulation to reduce circulating oestradiol levels and minimise inhibitory effects of both oestradiol and progesterone on endogenous gonadotropin secretion. This is because oestradiol itself acts as a modulator of progesterone production [46]. Aromatase inhibitors also serve to reduce circulating oestradiol levels during ovarian stimulation in women with oestrogen-sensitive cancers [47].

Summary

This chapter describes the natural history of ovarian function including assessment of ovarian reserve and premature ovarian failure. An understanding of ovarian anatomy, embryology and physiology is essential to the clinical understanding and manipulation of ovarian function for the purposes of reproductive focused fertility preservation treatment. In this chapter, the formation of the ovarian reserve is discussed and events of folliculogenesis are described. Clinical tools for ovarian reserve assessment are described and explained in the context of the physiology of follicular development and roles of markers in the regulation of follicular development. Premature ovarian failure is discussed; this failure may occur spontaneously but is often encountered in the iatrogenic context of oophorotoxic cancer treatments. Referral for fertility specialist collaboration as part of a patient's cancer

management specialist team can allow access to fertility preservation education and management. Treatment options such as ovarian tissue freezing and mature oocyte cryopreservation by vitrification can create future opportunities for patients with cancer to achieve fertility and have biologically related offspring. Appreciating the principle events of ovarian folliculogenesis, and the follicular wave theory, the chapter describes how adaptation of ovarian stimulation regimens may achieve time-efficient fertility preservation treatment options for patients with cancer. In a collaborative approach between oncology and fertility specialist teams, fertility preservation treatment can be accessed without compromising a patient's treatment for cancer and prognosis.

Practice points

- Ovarian folliculogenesis involves a complex system of autocrine, paracrine and endocrine regulation, where oocytes continually progress from the resting state towards ovulation across a woman's reproductive life.
- Follicular development can be manipulated at any stage of the menstrual cycle. Random start ovarian stimulation protocols can facilitate time-efficient protocols for oocyte cryopreservation in the setting of cancer diagnosis.
- A collaborative approach between oncology and reproductive endocrinology and fertility specialist teams allows optimal patient education and access to fertility preservation strategies and facilitates a holistic symptom management approach in the setting of premature ovarian insufficiency.

Research agenda

- Random start protocols for ovarian stimulation in fertility preservation are incompletely studied. Outcome data on long-term health outcomes from offspring conceived from cryopreserved oocytes are lacking.
- Future medical treatments to protect the ovarian reserve can be studied, which target the autocrine and paracrine factors involved in folliculogenesis and their downstream signalling pathways.

Conflicts of interest

The author has no conflicts of interest.

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