

Accepted Manuscript

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

Dr Raelia Lew

PII: S1521-6934(18)30127-5

DOI: [10.1016/j.bpobgyn.2018.05.005](https://doi.org/10.1016/j.bpobgyn.2018.05.005)

Reference: YBEOG 1835

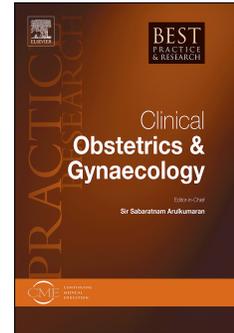
To appear in: *Best Practice & Research Clinical Obstetrics & Gynaecology*

Received Date: 7 February 2018

Accepted Date: 4 May 2018

Please cite this article as: Lew R, “Natural history of ovarian function including assessment of ovarian reserve and premature ovarian failure”, *Best Practice & Research Clinical Obstetrics & Gynaecology* (2018), doi: 10.1016/j.bpobgyn.2018.05.005.

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Title Page

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

Author affiliations

Dr Raelia Lew

CREI, FRANZCOG, PhD, MMed, MBBS(Hons)

Department of Obstetrics and Gynaecology, Faculty of Medicine, University of Melbourne, Level 7 Royal Women’s Hospital, 50 Flemmington Parade, Parkville 3052

Melbourne IVF, 340 Victoria Parade, East Melbourne, 3002

Royal Women’s Hospital, 50 Flemmington Parade, Parkville, 3052

E-mail: raelia.lew@mivf.com.au; rmskalicky@gmail.com

Abstract

This chapter describes ovarian anatomy and embryology in the human. 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 periovulatory follicles. Paracrine and autocrine factors play critical roles in oocyte maturation and follicular development and research relating 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. At the chapters 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 cancer patients.

Keywords

Ovary, oocyte, physiology, folliculogenesis, fertility preservation, AMH

Anatomy of the ovary

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

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 (the tunica albuginea) and an inner zone where ovarian follicles surrounding oocytes are embedded within stromal tissue. 90% of ovarian cancers originate from the cuboidal surface epithelium of the ovarian cortex. Stromal interstitial cells (from mesenchyme) produce androgens in response to 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 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 fertilization

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 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 gestation until the time of menopause, a continual process of recruitment, folliculogenesis and atresia occurs. This process progresses up until the preantral phase throughout all life stages, including during pregnancy

and while using hormonal modulating agents (for example contraception, aromatase inhibitors or selective estrogen receptor modulators).

The first pre-antral phase is directed by local growth factors via 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 one 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 s, 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 luteinization.(6) One mechanism, demonstrated in mouse knock-out models, is through regulation of MTOR activation in cumulus cells.(5)

Oocytes modulate granulosa cells' FSH-stimulated estradiol and progesterone production(7) and suppress the expression of LH receptors induced by FSH.(8) Oocyte-derived BMP15 decreases progesterone production by down-regulating 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 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 prior to ovulation. The total time interval between antrum formation and the mature development of a 20mm

preovulatory follicle is approximately 60 days. Overall, a future dominant follicle starts its journey (from resting pool to release of a mature oocyte) 12 menstrual cycles prior to ovulation.(1)

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A. Gonadotrophin independent follicular recruitment: Primordial, Primary and Secondary follicles

i. Primordial follicle

A primordial follicle is a structure enveloping a small primary oocyte (~ 25µm) within a single layer of squamous granulosa cells on a basal lamina. The diameter of the human primordial follicle is approximately 30µm. 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 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 fetal life and the ovarian reserve is not restorative. Between six and nine months 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, resisting recruitment and maintaining the ovarian reserve. These include PTEN (phosphatase and tensin homolog), Foxo3a (forkhead box O3A), and SDF-1 (stromal derived factor-1). Other signalling molecules including AMH and possibly SDF-1 secreted from surrounding growing follicles and have been proposed to negatively regulate primordial follicle activation.

Primordial oocytes secrete other factors such as PDGF (platelet-derived growth factor) and bFGF (basic fibroblast growth factor) that stimulate pre-granulosa cells to secrete KL (Kit-ligand), KGF (keratinocyte growth factor), BMP-4 and BMP-7, 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**).

ii. Primary follicle

The primary follicle is an oocyte surrounded by a single layer of cuboidal granulosa cells. Its major developmental events are oocyte growth and differentiation and granulosa cell FSH receptor expression.(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, resulting in oocyte growth and development of the zona pellucida, a surrounding extracellular matrix. The transcriptionally active oocyte generates mRNA transcripts to support immediate growth, and also future processes including oocyte maturation, fertilization 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, creating a large surface area for diffusion. Gap junctions, intercellular connexin protein channels, allow diffusion of regulatory and nutrient molecules between adjacent cells.(22)

iii. Secondary follicle

Secondary follicles contain a fully grown oocyte surrounded by zona pellucida, 5 to 8 layers of granulosa cells, a basal lamina, and a 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.
- Two primary layers of theca appear; interna and externa.
- Angiogenesis allows blood to circulate around the follicle, exposing it to external influences, including nutrients and gonadotropins.
- Theca externa gains autonomic innervation.

iv. 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 1-10mm in total diameter.

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, the periantral area, and the cumulus oophorus. All express FSH receptors, but the granulosa cell subtypes have a differential response to FSH stimulation, influencing steroidogenesis and LH receptor expression. A proposed controlling influence is the morphogen gradient secreted by the oocyte.(24)

B. Gonadotrophin dependent follicular recruitment

I. Selection and Atresia

Human physiology has evolved a mechanism of mono-follicular 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 FSH rise (as plasma estradiol, inhibin A and progesterone decrease) at the end of luteal phase.

FSH levels remain elevated through the first week of the follicular phase, 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 the human remains poorly understood. It involves the complex interplay of endocrine, paracrine and autocrine factors. When negative feedback by rising estrogen and inhibins 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, via activation of apoptosis pathways (signaling 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 Signaling

FSH binds to and activates a G protein transmembrane receptor. In the human, the FSH receptor contains 678 amino acids and is organized into three domains:

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

G protein receptor action involves intracellular second messenger systems, activated or modulated via ligand binding (e.g cAMP, PKA), influencing downstream gene expression. It is now 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 auto-activation. Further research lead 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 estradiol.(3)

Aromatase enzyme expression

FSH mediated induction of P450AROM gives granulosa cells of the dominant follicle the ability to produce high concentrations of estradiol 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. 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 prior to 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 up-regulating 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 Signaling

In the human, 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, protein kinase C phosphorylation sites).

Downstream effects of LH receptor activation, are mediated by second messenger systems (eg. GS/adenylate cyclase/cAMP/PKA). Phosphorylation of target proteins modulates gene transcription, 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 theca cell androgen production.

ii. Two cell, two gonadotrophin hypothesis of ovarian steroidogenesis

LH and the theca: LH receptor present, P450AROM absent

- LH receptors are present on theca cells (and initially absent on granulosa cells).
- LH stimulates steroidogenesis and 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 has diffused into granulosa cells (via the follicular fluid) is aromatized to estrone, and converted to estradiol (by 17β hydroxyl steroid dehydrogenase).
- Granulosa cells release estradiol, which diffuses back towards the thecal layer and into the peripheral blood stream.

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

iii. Ovulation and oocyte meiosis

Ovulation:

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

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 higher 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 ovum pick 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, 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 remodeling occurs and stigma (macula pellucida) formation occurs, creating a hole in the surface of the ovary through which the ovum is released. Ovulation involves processes of apoptosis, proteolysis, vascular remodeling and cell migration (through disruption of theca cell gap junctions) 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 fertilization. The LH surge invokes post receptor effects that modify intra-follicular molecular factors, that, up until 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 again arrests. Meiosis will not proceed further until the oocyte is fertilized.

iv. Luteinisation and the corpus luteum

Following ovulation, the follicle wall becomes the corpus luteum. The corpus luteum is a transient endocrine gland, producing abundant amounts of progesterone and estradiol 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 to by the membrana granulosa, theca interna, theca externa, and invading vasculature.

Immediately prior to ovulation, granulosa cells express StAR, P450SCC, 3 β -HSD, and P450AROM in high concentration. 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 estradiol synthesis operates in the human corpus luteum.

Steroidogenesis is initially LH and subsequently HCG dependent. Progesterone levels peak around 8 days after ovulation. If pregnancy does not occur, the corpus luteum degenerates by luteolysis to form a corpus albicans. Estrogen 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 fetal development, AMH produced by the 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 to 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 the ovarian reserve.

In oncology patients, AMH can be serially measured to assess the impact of chemotoxic agents on ovarian function, to predict future fertility and onset of premature ovarian insufficiency.(36)

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

Premature Ovarian Failure

Premature ovarian failure (POI) is a term used to describe women who lose ovarian function and transition to an early menopause, at an age below 40 years. Ovarian failure is associated with progressive oligomenorrhoea to an eventual hypergonadotrophic 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 centered approach to longterm management of POI affected patients.(38) One element of this management is optimized hormone replacement, which should continue until the average age of natural menopause (50-55 years of age).(39)

At least 50% of POI patients 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). Gene 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, 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 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, vitelligo, 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 cancer patients, 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 a FSH stimulated treatment cycle and oocyte collection procedure within an acceptable timeframe, so patients' oncology management planning and ultimate prognosis for survival is 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 recognised 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 fertility preservation patients 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 to 8 days into active treatment. GnRH antagonist regimens are associated with a lower 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, 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 prior to oocyte retrieval, compared with follicular phase start. In a meta-analysis of 8 studies, no differences in peak oestradiol levels were detected and FET (frozen embryo transfer) pregnancy rates were not statistically different 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 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 (eg letrozole) can be used in luteal phase stimulation, to reduce circulating estradiol levels and minimise inhibitory effects of both estradiol and progesterone on endogenous gonadotropin secretion. This is because estradiol itself acts as a modulator of progesterone production.(46) Aromatase inhibitors also serve to reduce circulating estradiol levels during ovarian stimulation in women with estrogen sensitive cancers.(47)

ACKNOWLEDGEMENTS

The author appreciates the support and mentorship of Associate Professor John McBain in the formation of this chapter.

Conflicts of interest

The author has no conflicts of interest.

PRACTICE POINTS

- Ovarian folliculogenesis involves a complex system of autocrine, paracrine and endocrine regulation, where oocytes continually progress from 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 a 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 longterm 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 signaling pathways.

References

1. Gougeon A. Human ovarian follicular development: from activation of resting follicles to preovulatory maturation. *Ann Endocrinol (Paris)*. 2010;71(3):132-43.*
2. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev*. 1996;17(2):121-55.
3. Knight PG, Glister C. TGF-beta superfamily members and ovarian follicle development. *Reproduction*. 2006;132(2):191-206.
4. Juengel JL, McNatty KP. The role of proteins of the transforming growth factor-beta superfamily in the intraovarian regulation of follicular development. *Hum Reprod Update*. 2005;11(2):143-60.*
5. Guo J, Shi L, Gong X, Jiang M, Yin Y, Zhang X, et al. Oocyte-dependent activation of MTOR in cumulus cells controls the development and survival of cumulus-oocyte complexes. *J Cell Sci*. 2016;129(16):3091-103.
6. Pangas SA, Matzuk MM. The art and artifact of GDF9 activity: cumulus expansion and the cumulus expansion-enabling factor. *Biol Reprod*. 2005;73(4):582-5.
7. Vanderhyden BC, Tonary AM. Differential regulation of progesterone and estradiol production by mouse cumulus and mural granulosa cells by A factor(s) secreted by the oocyte. *Biol Reprod*. 1995;53(6):1243-50.
8. Eppig JJ, Wigglesworth K, Pendola F, Hirao Y. Murine oocytes suppress expression of luteinizing hormone receptor messenger ribonucleic acid by granulosa cells. *Biol Reprod*. 1997;56(4):976-84.

9. Chang HM, Cheng JC, Taylor E, Leung PC. Oocyte-derived BMP15 but not GDF9 down-regulates connexin43 expression and decreases gap junction intercellular communication activity in immortalized human granulosa cells. *Mol Hum Reprod*. 2014;20(5):373-83.
10. el-Fouly MA, Cook B, Nekola M, Nalbandov AV. Role of the ovum in follicular luteinization. *Endocrinology*. 1970;87(2):286-93.
11. Huang Z, Wells D. The human oocyte and cumulus cells relationship: new insights from the cumulus cell transcriptome. *Mol Hum Reprod*. 2010;16(10):715-25.
12. Fragouli E, Bianchi V, Patrizio P, Obradors A, Huang Z, Borini A, et al. Transcriptomic profiling of human oocytes: association of meiotic aneuploidy and altered oocyte gene expression. *Mol Hum Reprod*. 2010;16(8):570-82.
13. Zhao HC, Ding T, Ren Y, Li TJ, Li R, Fan Y, et al. Role of Sirt3 in mitochondrial biogenesis and developmental competence of human in vitro matured oocytes. *Hum Reprod*. 2016;31(3):607-22.
14. Chang HM, Qiao J, Leung PC. Oocyte-somatic cell interactions in the human ovary-novel role of bone morphogenetic proteins and growth differentiation factors. *Hum Reprod Update*. 2016;23(1):1-18.
15. SRM R. The vasculature of the ovary and ovarian function. *Recent Prog Horm Res*. 1950;5(65).
16. Bouilly J, Beau I, Barraud S, Bernard V, Azibi K, Fagart J, et al. Identification of Multiple Gene Mutations Accounts for a new Genetic Architecture of Primary Ovarian Insufficiency. *J Clin Endocrinol Metab*. 2016;101(12):4541-50.*

17. Hansen KR, Knowlton NS, Thyer AC, Charleston JS, Soules MR, Klein NA. A new model of reproductive aging: the decline in ovarian non-growing follicle number from birth to menopause. *Hum Reprod.* 2008;23(3):699-708.
18. Oktay K, Briggs D, Gosden RG. Ontogeny of follicle-stimulating hormone receptor gene expression in isolated human ovarian follicles. *J Clin Endocrinol Metab.* 1997;82(11):3748-51.
19. Findlay JK, Drummond AE. Regulation of the FSH Receptor in the Ovary. *Trends Endocrinol Metab.* 1999;10(5):183-8.
20. Fortune JE, Cushman RA, Wahl CM, Kito S. The primordial to primary follicle transition. *Mol Cell Endocrinol.* 2000;163(1-2):53-60.
21. Albertini DF, Combelles CM, Benecchi E, Carabatsos MJ. Cellular basis for paracrine regulation of ovarian follicle development. *Reproduction.* 2001;121(5):647-53.*
22. Bruzzone R, White TW, Paul DL. Connections with connexins: the molecular basis of direct intercellular signaling. *Eur J Biochem.* 1996;238(1):1-27.*
23. Edwards RG. Follicular fluid. *J Reprod Fertil.* 1974;37(1):189-219.
24. Erickson GF, Shimasaki S. The role of the oocyte in folliculogenesis. *Trends Endocrinol Metab.* 2000;11(5):193-8.
25. Baker SJ, Spears N. The role of intra-ovarian interactions in the regulation of follicle dominance. *Hum Reprod Update.* 1999;5(2):153-65.
26. Desai SS, Roy BS, Mahale SD. Mutations and polymorphisms in FSH receptor: functional implications in human reproduction. *Reproduction.* 2013;146(6):R235-48.*

27. Simpson ER. Aromatase: biologic relevance of tissue-specific expression. *Semin Reprod Med.* 2004;22(1):11-23.
28. Speroff MAFaL. *Clinical Gynecologic Endocrinology and Infertility.* 8th Edition ed: Wolters Kluwer Health, Lippincott Williams and Wilkins; 2011 2011.
29. Mehlmann LM, Jones TL, Jaffe LA. Meiotic arrest in the mouse follicle maintained by a Gs protein in the oocyte. *Science.* 2002;297(5585):1343-5.
30. Norris RP, Freudzon M, Mehlmann LM, Cowan AE, Simon AM, Paul DL, et al. Luteinizing hormone causes MAP kinase-dependent phosphorylation and closure of connexin 43 gap junctions in mouse ovarian follicles: one of two paths to meiotic resumption. *Development.* 2008;135(19):3229-38.
31. Crisp TM, Dessouky DA, Denys FR. The fine structure of the human corpus luteum of early pregnancy and during the progesterational phase of the menstrual cycle. *Am J Anat.* 1970;127(1):37-69.
32. Alvaro Mercadal B, Imbert R, Demeestere I, Gervy C, De Leener A, Englert Y, et al. AMH mutations with reduced in vitro bioactivity are related to premature ovarian insufficiency. *Hum Reprod.* 2015;30(5):1196-202.
33. Pankhurst MW, Chong YH. Variation in circulating antimullerian hormone precursor during the periovulatory and acute postovulatory phases of the human ovarian cycle. *Fertil Steril.* 2016;106(5):1238-43 e2.
34. Tadros T, Tarasconi B, Nassar J, Benhaim JL, Taieb J, Fanchin R. New automated antimullerian hormone assays are more reliable than the manual assay in patients with reduced antral follicle count. *Fertil Steril.* 2016;106(7):1800-6.

35. Jung S, Allen N, Arslan AA, Baglietto L, Brinton LA, Eggleston BL, et al. Demographic, lifestyle, and other factors in relation to antimullerian hormone levels in mostly late premenopausal women. *Fertil Steril*. 2017;107(4):1012-22 e2.*
36. Freour T, Barriere P, Masson D. Anti-mullerian hormone levels and evolution in women of reproductive age with breast cancer treated with chemotherapy. *Eur J Cancer*. 2017;74:1-8.
37. Grisendi V, La Marca A. Individualization of controlled ovarian stimulation in vitro fertilization using ovarian reserve markers. *Minerva Ginecol*. 2017;69(3):250-8.
38. Kovanci E, Schutt AK. Premature ovarian failure: clinical presentation and treatment. *Obstet Gynecol Clin North Am*. 2015;42(1):153-61.
39. Sullivan SD, Sarrel PM, Nelson LM. Hormone replacement therapy in young women with primary ovarian insufficiency and early menopause. *Fertil Steril*. 2016;106(7):1588-99.
40. Jankowska K. Premature ovarian failure. *Prz Menopauzalny*. 2017;16(2):51-6.
41. Thakur M, Feldman G, Puscheck EE. Primary ovarian insufficiency in classic galactosemia: current understanding and future research opportunities. *J Assist Reprod Genet*. 2018;35(1):3-16.
42. Betterle C, Rossi A, Dalla Pria S, Artifoni A, Pedini B, Gavasso S, et al. Premature ovarian failure: autoimmunity and natural history. *Clin Endocrinol (Oxf)*. 1993;39(1):35-43.

43. Robertson DM, Gilchrist RB, Ledger WL, Baerwald A. Random start or emergency IVF/in vitro maturation: a new rapid approach to fertility preservation. *Womens Health (Lond)*. 2016;12(3):339-49.*
44. Baerwald AR, Adams GP, Pierson RA. Characterization of ovarian follicular wave dynamics in women. *Biol Reprod*. 2003;69(3):1023-31.
45. Boots CE, Meister M, Cooper AR, Hardi A, Jungheim ES. Ovarian stimulation in the luteal phase: systematic review and meta-analysis. *J Assist Reprod Genet*. 2016;33(8):971-80.*
46. Endo T, Henmi H, Goto T, Kitajima Y, Kiya T, Nishikawa A, et al. Effects of estradiol and an aromatase inhibitor on progesterone production in human cultured luteal cells. *Gynecol Endocrinol*. 1998;12(1):29-34.
47. Keskin U, Ercan CM, Yilmaz A, Babacan A, Korkmaz C, Duru NK, et al. Random-start controlled ovarian hyperstimulation with letrozole for fertility preservation in cancer patients: case series and review of literature. *J Pak Med Assoc*. 2014;64(7):830-2.*

Table 1: Oocyte, granulosa and stromal signalling molecules and pathways implicated in follicle recruitment, growth 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 (Sox11) • Sox12 	Regulate gene expression
<ul style="list-style-type: none"> • FIGLA • LHX8 • NOBOX • GDF-9 • BMP-15 • PDGF • bFGF • Kit ligand 	Regulate gene expression Promote primordial follicle survival and activation Regulate follicle development Promote granulosa cell proliferation
<ul style="list-style-type: none"> • Phosphatidylinositol 3-OH-kinase (PI3K)-AKT-Foxo3a 	Restrain follicle activation

signaling	
<ul style="list-style-type: none">• PTEN• TSC1 and TSC2• mTOR	
<ul style="list-style-type: none">• SDF-1• KGF• BMP-4• BMP-7	Promote primordial to primary follicle transition
<ul style="list-style-type: none">• AMH	Negatively regulates recruitment