Editorial Articles

Understanding the physiology of folliculogenesis serves as the foundation for perfecting diagnosis and treatment of ovulatory defects

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Summary

Purpose: To discuss updated physiologic information concerning the mechanism of folliculogenesis. *Methods:* Physiology studies involving the growth of primordial follicular growth and pre-antral growth to the development of the corpus luteum are discussed. *Results:* Benefits in aiding fertility potential and pitfalls of these drugs in preventing embryo implantation are discussed with reference to the physiologic processes required for folliculogenesis. *Conclusions:* Knowledge of the physiology of folliculogenesis can provide further understanding of luteal function when taking follicle maturing drugs and complications, as premature luteinization and the luteinized unruptured follicle syndrome. Also, this knowledge helps to create novel therapies to prevent ovarian hyperstimulation syndrome, endometrial receptivity defects, and treating women with diminished oocyte reserve.

Key words: Antral follicle; Pre-antral follicles; Luteinizing hormone; Follicle stimulating hormone; Ovarian oocyte reserve.

Introduction

Ovulation defects have been conveniently divided into anovulation with estrogen deficiency [1], anovulation with normal estrogen [2], and ovulation defects despite regular menses [3]. Some of the subtle ovulation disorders that can be found in women who have regular menses and an adequate serum progesterone (P) one week before menses include: a short follicular phase [4, 5], premature luteinization [6], luteinized unruptured follicle syndrome [7-10], and luteal phase defects [11].

Diagnosing these subtle defects in ovulation can be challenging, and one has must to wonder in women who show a subtle ovulatory problem, and when there seems to be an adequate number of corrected cycles, why do some women still fail to conceive? Is it bad luck? Another subtle uncorrected cryptic problem outside of ovulation, such as sperm factor, or tubal factor that is not being detected. Or could there still be a subtle ovulatory defect that is not being detected?

What is frightening is that a subtle defect in the formation of the dominant follicle and a metaphase II oocyte can even lead to a normal-appearing embryo that will not successfully implant following embryo transfer (ET). A vivid example of this is the failure of many top in vitro fertilization (IVF) centers to achieve pregnancies despite the ET that appear morphologically normal, following controlled ovarian hyperstimulation (COH) in women with diminished oocyte reserve, and yet high pregnancy rates are achieved in these same women when milder drug stimulation is used [12-14]. The evidence suggests that the defect is at the oocyte level leading to an embryo that will not implant [15]. Since frozen embryos similarly fail to implant (in contrast to women with normal oocyte reserve where the adverse effect of the follicle-maturing drugs in a much lower percentage of patients seems to be at the endometrial level), the data suggest a defective implantation factor that is directly associated with the embryo [16-18]. Alternatively, high dosage follicle stimulating hormone (FSH) drugs or raising further already-elevated endogenous serum FSH levels may lead to an increased risk of meiosis errors leading to aneuploidy [16-18].

The physiology of follicular maturation and the selection of one dominant follicle out of a cohort of antral follicles is a very complex process involving a complex interaction between hormones secreted by the ovary, pituitary, hypothalamus, and other areas of the brain, hormone receptors, growth factors and cytokines. It is with the understanding of the normal physiology that new methods of diagnosing and treating infertility-related to ovulatory factors will be discovered. Furthermore by studying these factors one may learn how to mitigate some of the adverse aspects of follicle-maturing drugs or lead to the discovery of other novel methods to develop mature follicles. Thus this editorial will attempt to summarize folliculogenesis and discuss some of the newest concepts in the physiology of folliculogenesis.

Revised manuscript accepted for publication May 2, 2012

Folliculogenesis

Germ cell migration to the genital ridge begins at six to eight weeks. There are about 6 - 7 x 10^6 oocytes (maximum number) at 16 - 20 weeks. They exist as primordial follicles which are oocytes arrested in diplotene stage of meiotic prophase surrounded by single layer of spindle shaped granulosa cells. The number of primordial follicles is fixed shortly after birth.

Fate of primordial follicles

The primordial follicles either grow or undergo atresia. There are no interruptions in this process from birth to menopause. The most rapid decline by atresia of oocytes is from 20 weeks to birth (only 10% have survived, i.e., about 1 - 2 x 10^6). From birth to puberty is the next most rapid decline – only about 300,000 oocytes are left at puberty. Only 400 - 500 follicles will ovulate out of this pool during a lifetime.

Apoptosis and early pre-antral growth

Most primoidial follicles are destined for atresia (or apoptosis). Some follicles over an 85-day period develop into follicles that have the potential to become dominant follicles.

It is unknown what process inhibits the apoptosis factor of these pre-ovulation follicles, but paracrine and autocrine factors in the microenvironment seem responsible. This developmental process over these 85 days is independent of FSH. Most of these preantral follicles of two to five mm in size can develop to dominant follicles if they can be rescued from apoptosis by FSH.

The cohort of antral follicles

Those pre-antral follicles now becoming antral (five to ten mm) follicles first show an increase in oocyte size and a change in shape of the granulosa cells from squamous to cuboidal shape. Gap junctions open between the granulosa cells allowing exchange of nutrients and regulatory molecules. The granulosa cells produce factors that inhibit the final maturation of the oocyte until the luteinizing hormone (LH) surge. Follicular growth is regulated by regulatory molecules from the oocyte itself.

Functions of the gap junction

The channels that compose the gap junction are composed of proteins known as connexins. Connexin expression in ovarian follicles is up-regulated by FSH and down-regulated by LH. FSH keeps the channels of gap junctions open and LH closes them.

Development of primary follicles from primordial follicles

When mitosis of the cuboidal layer of granulosa cells in the primordial follicle reaches about 15 granulosa cells (or may begin with as few as three cells), the follicle is now considered a primary follicle. The granulosa cells are separated from the stromal cells by a basement membrane known as the basal lamina. The surrounding stromal cells differentiate into concentric layers referred to as the theca. This occurs at the time when continued mitosis produces three to six layers of granulosa cells. The stroma cells closest to the basal lamina is called the theca interna and the theca externa is the outer portion.

Role of FSH in early folliculogenesis

Although the majority of the primary follicles begin to grow, they quickly undergo atresia. By the beginning of the menstrual cycle, after about 70 days of development, there is a cohort of follicles that if exposed to sufficient FSH, have the capacity to develop into a dominant follicle.

The decline from mid-luteal phase of corpus luteum steroidogenesis (when there is maximal suppression of gonadotropins and inhibin A), allows a rise in FSH beginning a few days before menses. Bioactivity of FSH also increases.

Development of the preantral follicle

Oocyte enlargement (to 0.8 mm) and continued granulosa cell proliferation produces a multilayer effect. The oocyte and granulosa cells are now surrounded by another membrane called the zona pellucida. The theca layer continues to organize by the surrounding stroma and the follicle attains a size of two mm (about four times larger than the primordial follicle).

Steroidogeneis by the preantral follicle

FSH receptors on granulosa cells develop. Granulosa cells can produce estrogen, progesterone, or testosterone. The production of estrogen is FSH-dependent since the aromatase enzyme is FSH-dependent. Estrogen production is limited by FSH receptor content. The predominant steroid produced by the preantral follicle is estrogen.

FSH and the preantral follicle

FSH combines synergistically with estrogen to exert a mitogenic action on granulosa cells to stimulate their proliferation and also increase the number of FSH receptors. FSH receptors quickly reach a concentration of about 1,500 receptors per granulosa cell. The early appearance of estrogen within the follicle allows the follicle to respond to relatively low concentrations of FSH. This is considered an autocrine function of estrogen within the follicle. Not all granulosa cells have FSH receptors: cells with receptors can transfer a signal by gap junction causing protein kinase activation in cells lacking FSH receptors.

Androgen and the preantral follicle

Specific androgen receptors are present in the granulosa cells. They serve as precursors for estrogen through the FSH dependent aromatase enzyme. In low concentration, androgens can further enhance aromatase activity.

Androgens and the preantral follicle

With higher concentration of androgens the preantral follicle favors the conversion to five alpha reduced androgens, for example, dihydrotesterone (DHT), which cannot be converted to estrogens. 5α reduced androgens inhibit the aromatase enzyme and inhibit FSH induction of LH receptors and the follicles will eventually undergo atresia. The success of a follicle depends on its ability to convert an androgen-dominated microenvironment to an estrogen-dominated microenvironment.

Development of the antral follicle – importance of follicular fluid

Estrogen and FSH cause an increase in follicular fluid which accumulates in the intercellular spaces of the granulosa cell layer and eventually becomes a cavity. Follicular fluid provides a mechanism for nurturing the oocyte and its surrounding granulosa cells (known as the cumulus oophorus). Estrogen becomes the dominant substance in the follicular fluid in the presence of FSH vs androgens in the absence of FSH-FSH receptor interaction. Thus follicles with less FSH receptors become androgen dominant and undergo atresia. If premature luteinization occurs and LH appears in follicular fluid before mid-cycle, mitotoxic activity is activated in the granulosa layer and degeneration occurs, associated with a rise in intrafollicular androgen levels.

LH and FSH receptors – preantral follicle

LH receptors are present on theca cells only. FSH receptors are on granulosa cells only. LH stimulates theca cells to make androgen. FSH induced aromatase enzymes convert thecal androgens to estrogen in the granulosa cells. FSH and estrogen help to induce more FSH receptors in the granulosa cells.

The dominant follicle and FSH

The follicle that has the most estrogen is the dominant follicle and is destined to ovulate. Estrogen increases the sensitivity of the follicle to FSH. The negative feedback effect of estrogen on hypothalamic pituitary FSH secretion causes the other follicles with less FSH receptors to undergo atresia as serum FSH decreases. The dominant follicle is usually established between days five to seven and serum estradiol levels begin to rise by cycle day seven.

The gonadotropins and estrogen are not the only hormones involved in the creation of the dominant follicle. The following hormones also play a role: inhibin A, inhibin B, activin, follistatin, insulin-like growth (IGF) factor I, IGF II.

Inhibin B is secreted by granulosa cells in response to FSH. It directly suppresses pituitary FSH secretion. Activin originates in both pituitary and granulosa cells and augments FSH secretion and action in the early follicular phase especially aromatase activity. IGF I enhances all actions of FSH and LH.

Development of LH receptors on granulosa cells

FSH with the help of estrogen induces LH receptors on the granulosa cells of the large antral follicles. LH provides support for the final maturation and function of the dominant follicle. Not only do granulosa cells increase by day nine, the thecal vascularity is also twice as high in the dominant follicle than any other follicles.

Transition from suppression to stimulation of LH release from pituitary

The estrogen initially causes a suppression of LH release from the pituitary, but the transition from suppressor to stimulator of LH occurs as estradiol (E2) rises during mid-follicular phase. The final LH surge requires both a critical E2 level (~200 pg / ml) and certain length of exposure to this higher concentration of E2 (~50 hours).

A less sustained elevation of E2 leads to diminished or no LH surge. Estrogen increases sialic acid content of FSH and LH, thus increasing their bioactivity at mid-cycle.

Role of LH in late stages of follicular development

The increased concentration of estrogen in the dominant follicle enables FSH to shift its focus from making more FSH receptors in granulosa cells, but now increases induction of LH receptors which are critical for eventual corpus luteum function, especially progesterone secretion. Thus LH may have an important role in the final maturation and function of the dominant follicle, thus leading to a "healthier" oocyte. With the development of recombinant FSH products, some initial studies suggested that COH regimens using all recombinant FSH, produced higher pregnancy rates (this concept was partially commercially generated). However, most IVF centers have returned to adding some LH, especially in the later stages of follicular development.

Preovulatory follicle

E2 rises rapidly and usually peaks at 36 hours before ovulation, sometimes even 24 hours before ovulation. This peak in E2 induces the initiation of the LH surge.

LH binds to its receptor in the granulosa cells and initiates P secretion from the granulosa cells. LH also stimulates P receptors in granulosa cells and interaction of P with its receptor inhibits granulosa cell mitosis.

Role of progesterone in the preovulatory follicle

After the pituitary gonadotropin cells have had adequate exposure to estrogen, the small rise in P facilitates the positive feedback effect on LH rise, helping to reach a peak LH surge. If the P level exceeds 2 mg / ml, it may have a negative effect and thwart a proper LH surge. The low levels of P secreted by the dominant follicle helps facilitate the mid-cycle FSH surge. The mid-cycle FSH surge in turn helps to facilitate adequate development of LH receptors on granulosa cells.

Role of androgens on the preovulatory follicle

Some of the follicles that undergo atresia become part of the stroma again and make androgens. The rise in androgens are needed to be made into estrogens by the corpus luteum. The increase in thecal androgens is mostly related to the rise in LH, but with the drop in E2 following the LH surge inhibin increases, which enhances LH stimulation of androgens.

The LH surge (important in timing intrauterine insemination (IUI)

The onset of the LH surge is usually 34 - 36 hours before follicle rupture. The LH surge lasts 48 - 50 hours. A certain level of LH concentration must be maintained for at least 14 - 27 hours for full maturation of the oocyte to occur. The LH surge tends to occur at 3:00 a.m. and and also between midnight and 8:00 a.m. in 67% of women.

Importance of the LH surge

The LH surge initiates the continuation of meiosis (though not completed until after the sperm has entered the oocyte and the second polar body released). It also helps to expand the cumulus cells. Furthermore, it aids in luteinization of the granulosa cells. Finally, it aids in the synthesis of prostaglandins which are needed to allow the oocyte to release from the follicle.

Preovulatory follicle – factors inhibiting premature oocyte maturation and premature luteinization

LH induced cyclic adenosine monophosphate (AMP) activity overcomes the local inhibitory action of oocyte maturation inhibitor (OMI) which comes from granulosa cells. LH-induced cyclic AMP overcomes local luteinization inhibitor (possibly endothelin a product of vascular endothelial cells). Premature luteinization is also inhibited by activin which suppresses production of P by luteal cells.

The oocyte controls functions of the granulosa cells

The cumulus oophorus differs from other granulosa cells: first there are no LH receptors, and also the cumulus oophorus does not make P. The cumulus oophorus acts as a suppressor of FSH-induced LH receptors in the contiguous granulosa by the oocyte itself.

LH and progesterone interaction during the LH surge

The LH surge initiates a rise of P from granulosa cells. P may enable degeneration of collagen in the follicular wall, enabling the follicular wall to become thin and stretch, especially related to the action of plasminogen which induces collagenase.

Plasmin production

There are two plasminogen activators secreted by granulosa and theca cells in response to LH. The plasminogen activity in granulosa cells activate plasminogen in follicular fluid to make plasmin. The plasminogen activator is mainly

under LH influence, but also by the FSH surge and growth factors. The LH surge leads to suppression of plasminogen inhibitor.

Prostaglandin in the pre-ovulatory follicle

Prostaglandin E and F series and other eicosanoids increase tremendously in the preovulatory follicular fluid. Peak prostaglandin concentration is reached at the time of ovulation. A luteinized unruptured follicle may occur from suppression of prostaglandin production. The role of prostaglandin is to free proteolytic enzymes in the follicular wall. They may also aid in the extrusion of the oocyte-cumulus cell mass by causing smooth muscle contraction.

Steroids and the LH surge

Estradiol levels drop with LH surge possibly by LH down-regulation of its own receptors in the follicle. A decrease in LH may be related to negative feedback of rising serum P level. The decrease in LH may also be secondary to decreased pituitary LH and FSH synthesis secondary to change in gonadotropin releasing hormone (GnRH) pulsatility secondary to E2 and P feedback.

Another mechanism to prevent premature luteinization

FSH stimulates granulosa cells to produce a gonadotropin surge inhibiting factor (GnSIF). Its highest concentration is at mid-follicular stage. Its main function is to prevent premature luteinization.

The luteinized unruptured follicle syndrome

A follicle must be at the proper stage of maturity to enable oocyte release to a given LH surge. The rise in E2 allows for the positive effect on LH release from the pituitary. Follicle maturing drugs causing a higher serum E2 level from multiple follicles can cause a release of LH before any follicles are fully mature.

A brief summary of the corpus luteum and luteal phase

Appropriate luteal steroid secretion requires optimal preovulatory follicular development and continued tonic LH support. The corpus luteum in the early luteal phase requires vascular endothelial growth factor (VEGF) to cause increased angiogenesis. Corpus luteum regression is associated with a decrease in VEGF.

P, E2, and inhibin A suppress gonadotropins and new follicular growth. Early pregnancy produces human chorionic gonadotropin (hCG) which rescues the corpus luteum.

Future research potential gained from knowledge of physiological mechanism involved with the process of ovulation

The knowledge of growth factors required for normal ovulation and comparison of changes that occurs with these factors with follicle-stimulating drugs could lead to novel treatment regimens and a better understanding why in some instances apparent multiple ovulation does not lead to perfect embryos, or does not lead to a perfect endometrium, so that proper embryo implantation and pregnancy does not ensue.

IGF II

IGF-II is more abundant in follicles than IGF-I. IGF II is produced in thecal cells, granulosa cells, and luteinized granulosa cells. IGF II enhances FSH and LH. Also IGF II stimulates granulosa cell proliferation. Furthermore IGF II stimulates aromatase activity.

IGF I

The gonadotropins stimulate IGF I and IGF II. IGF I receptors are present in thecal and granulosa cells. IGF I is not present in luteinized granulosa cells. IGF II activates both IGF I and IGF II receptors. FSH inhibits IGF I and IGF II binding protein synthesis and thus maximizes the presence of IGF.

Epidermal Growth Factor (EGF)

EGF in conjuction with gonadotropins causes proliferation of granulosa cells. Et suppresses the up-regulation of FSH on its own receptor.

Transforming Growth Factor (TGF)

TGF-beta is secreted by theca cells. TGF-B enhances FSH induction of LH receptors on granulosa cells. TGF-B in the theca inhibits androgen production.

Fibroblast Growth Factor (FGF)

FGF has the opposite effect of TGF-B. It stimulates mitosis in granulosa cells, angiogenesis, and plasminogen activator. However FGF inhibits FSH up-regulation of its own receptor. It also ihibits FSH-induced LH receptor expression and it inhibits E2 production.

Vascular endothelial growth factor (VEGF)

VEGF is a cytokine produced by granulosa cells. It is important for vascularization of the follicle. If one blocks VEGF, this will suppress angiogenesis of the theca cells and inhibit follicular growth and development.

Luteinized granulosa cells respond to hCG with increased VEGF output, probably contributing to the increased vascular permeability associated with ovarian hyperstimulation that can occur with controlled ovarian hyperstimulation.

Other important cytokines growth factors and immunosuppressant

Angiopoietin 1 and 2 inhibit angiogensis. Platelet-derived growth factor may modify prostaglandin production within the follicle. Tumor necrosis factor alpha is produced by leukocytes and is important in apoptosis, thus aiding in follicular atresia and disintegration of the corpus luteum.

Effect of follicle stimulating drugs

The use of anti-estrogen drugs in the early follicular phase and the use of FSH or FSH/LH drugs from early follicular phase disrupts the precision and interrelationship of all these complex interactions that have been described. What is amazing is that despite the alterations of these biochemical events, the oocytes produced are reasonably normal. This is evidenced by normal or supranormal pregnancy rates per cycle in women who are anovulatory using follicle maturing drugs. Furthermore, we are all aware of the very high pregnancy rates that have been achieved by recipients using donor oocytes that have been achieved by COH.

It would appear that the main effect of the precise feedback effect, both positive and negative, and the interaction of all of these factors on each other, and on the developing dominant follicle, and the pituitary, is to select one and maybe the best follicle each month to provide the single best oocyte in the cohort.

Some problems that are increased in frequency with follicle stimulating drugs are: 1) greater need for luteal phase P supplementation, 2) a greater risk of premature luteinization, 3) a greater risk of the luteinized unruptured follicle, 4) a greater risk of ovarian hyperstimulation syndrome (OHSS) and 5) a greater risk of creating a hostile uterine environment leading to poor implantation [11-13].

Diminished oocyte reserve

There is one exception to the statement that in general COH does not adversely effect the quality of the oocyte and the ability of the resulting embryo to implant in a normal endometrial environment. This exception may be the woman with increased day three FSH and diminished inhibin B and anti-Mullerian hormone where raising the serum FSH too high leads to oocytes that fertilize normally but produce embryos that do not implant even in a good endometrial environment [14-18].

Discussion

It is hoped that the knowledge of the complex interactions of steroids, gonadotropin receptors, growth factors, and cytokines made by both the ovary and the pituitary and suprasellar structures, that we may improve the ability to identify a subtle ovulatory defect more precisely that we can do today. Similarly, we can try to determine what factors differ in stimulated vs natural cycles to gain some insight as to whether follicle maturing drugs fail to perfectly correct the ovulatory defect, and to thus fail to establish a pregnancy. Thus it is important to establish a good fund of knowledge of the hypothalamus and biogenic amines from higher brain centers, the pituitary, the ovaries, and the endometrium.

As mentioned, it seems that the main adverse effect of follicle-maturing drugs on subsequent conception is on the endometrium and not on the oocyte itself [19, 20]. However, this adverse effect is probably from the secretion of hormones, cytokines, growth factors and receptor stimulation, and suppression that leads to this adverse effect on the endometrium. It is already known that adding extra P in the luteal phase can mitigate some of the adverse effects on the endometrium [21].

The pulsatility of the gonadotropins in the precise amplitude and frequency patterns is mostly for the purpose of down-regulating and restoring FSH receptors to allow the “best” follicle in the cohort to develop. In women with gonadotropin deficiency, the use of LH/FSH gonadotropins can restore ovulation without the need for providing these drugs in a pulsatile manner. However, it is difficult to mature only one follicle. Nonetheless, the knowledge of the susceptibility of FSH receptors to down-regulation led to the idea of trying to restore ovulation by suppressing serum FSH, even in women in apparent menopause with successful pregnancies achieved [14].

There is no question that oocyte quality is more related to age than oocyte reserve [22]. Of course part of this is related to a greater tendency for oocytes from women of advanced reproductive age to be more prone to meiosis I and II errors. Comparative studies of various substances in the serum or follicular fluid of women of more advanced reproductive age versus younger women may perhaps show differences. Finding some key differences in certain cytokines, growth factors, and others in the serum follicular fluid or serum between these two groups, could lead to novel therapies that could improve success with other oocytes from women of advanced reproductive age.

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