From Primary Follicle to Ovulation

Primer Follikülden Ovulasyona

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ABSTRACT

Folliculogenesis is a very complex process not clearly understood yet. Contributions of many hormones, growth factors, regulatory proteins and different types of cells make this process more confusing. However, important milestones of understanding female reproductive life are hidden in this process. Investigations on this issue may yield important data which may enable us to improve assisted reproduction techniques or prevent/treat ovarian failure.
We aimed to summarize new aspects of this issue and proposed new hypotheses in the light of recent literature.

Key Words: Folliculogenesis, recruitment, granulose, theca, oocyte, gene.

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ÖZET

Follikülogenez, hala net olarak anlaşılamamış karmaşık bir süreçtir. Birçok hormonun, büyüme faktörlerinin, düzenleyici proteinlerin ve farklı türde hücrelerin katılımı bu süreci daha da karmaşık hale getirir. Ancak kadının reprodüktif çağını anlamak için önem taşıyan kilometre taşları bu süreçte saklıdır. Bu konudaki çalışmalar üremeye yardımcı teknikleri geliştirmemize veya ovaryen yetmezliği engellememize/tedavi etmemize olanak verecek önemli bilgiler sağlayabilir. Bu konunun güncel görünümünü, ileri sürülen yeni hipotezler ve yayınlar ışığında özetlemeyi amaçladık.

Anahtar Sözcükler: Follikülogenez, recruitment, granüloza, teka, oosit, gen

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INTRODUCTION

Primary goal of the folliculogenesis is to release one healthy follicle capable of being fertilized. This requires a healthy communication of somatic (theca and granulosa) and germ cells (oocyte) since activation of primordial follicle till ovulation.

Primordial germ cells originate from yolc sac, allantois, and hindgut endoderm and migrate to genital ridge in 5-6th gestational weeks. Here they reach maximum of their number (6-7 millions) in 16-20th weeks via mitosis. Primordial follicle stable in diplotene phase of meiotic prophase surrounded by single layer of flat granulose cells constitute a primary follicle.

Activation of a primary follicle

Primordial follicles silently wait until recruitment (Fig 1). Traditionally a multidirectional complex communication is thought to exist between germinal and somatic cells mediated by extracellular matrix components and autocrine/paracrine growth factors (1). New research demonstrated some inhibitory signals keeping primordial follicles silent, loss of which can cause early activation, and therefore early depletion, of primordial follicle pool (2). Only Fox12 (Forkhead Box Protein 12), mutation among investigated genes such as Tsc-1 (Tuberous sclerosis-1), PTEN (Phosphatase and tensin homolog), Fox03a (Forkhead Box Protein O3), p27 and Foxl2 is found to be related to premature ovarian failure (3).

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Figure 1: Primordial follicles (arrows).

Selection of silent primordial follicles to growing pool starts in intrauterine 5-6th weeks and continues in all ages including menopause until ovarian reserve is completely depleted (when approximately 1000 primordial follicles are left, in menopause) (4). This is a continuous process different from recruitment of a single antral follicle cohort under FSH (Follicle Stimulating Hormone) effect in every cycle. Pregnancy, ovulation and unovulation periods do not interfere with growth and atresia. Decrement is proportional to total follicle count, therefore is most prominent in intrauterine life. Approximately 2 millions of oocytes are present at birth and 300.000 in puberty.

How it is determined which follicles will grow in a cycle is unclear, however their count is related to inactive primordial follicle count. Effects narrowing residual follicle pool such as unilateral oophorectomy can cause early menopause.

It takes 85 days for a silent primordial follicle to become preovulatory. Follicle to ovulate is determined in last luteal to follicular phase shift (in first few days of the cycle). Development since then is independent from gonadotropins (5). Some among the growing follicle pool are rescued from atresia with increasing FSH (6) and others are eliminated via apoptosis. As becoming primary follicle, flat granulose cells of primordial follicle gain cuboidal shape and proliferate. Oocyte enlarges above 60µm diameter and zona pellucida occurs (Fig 2) (7).

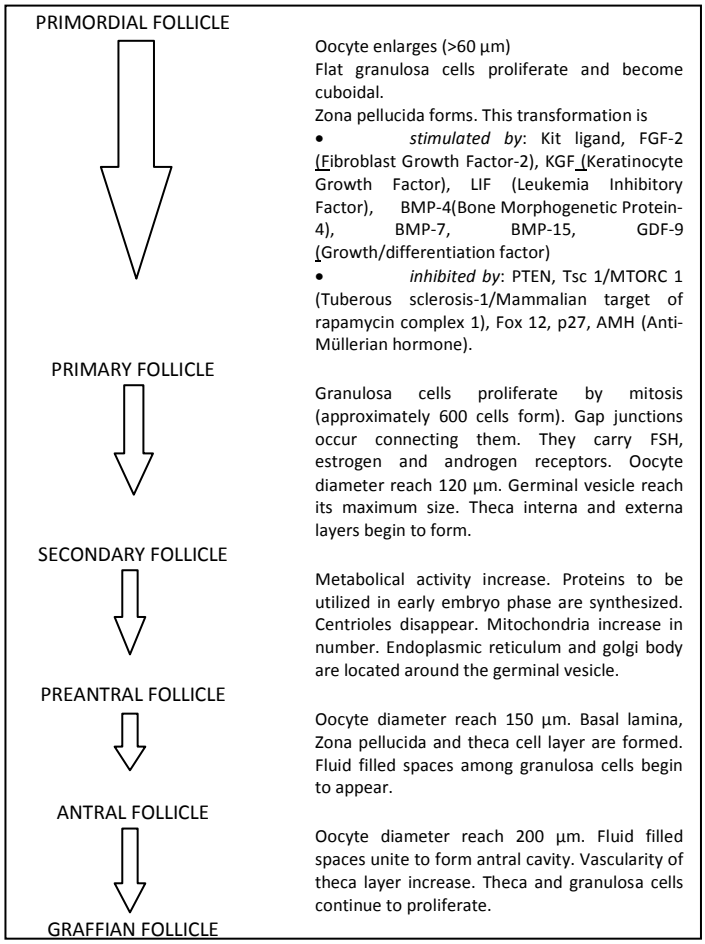

Figure 2: Developmental stages of the follicle

During follicular activation, transcription factor Fox 12 plays an important role in conversion of pregranulosa cells to granulosa cells. Channels made of Connexin proteins enable metabolite and intermediary molecule transfer between oocyte and granulosa cells (8). Expression of Connexin increases with FSH stimulation and decreases with LH stimulation (9). After ovulation these channels function in corpus luteum and are regulated by local oxytocin (10).

Except inhibitory signals blocking premature activation of primordial follicles, activatory signals encouraging primordial follicle to grow into primary follicle. These signal originating from oocytes, somatic cells, and stroma exert a coordinated and sinergistic effect to trigger growth. This explains survival of primordial follicles in in situ ovarian tissue culture despite isolated primordial follicles cannot survive in conventional cultures (11). As primordial follicles donot carry FSH receptors, FSH is not required for transition of primordial to primary follicles (12).

Studies on transgenic animal models and human ovarian tissues demonstrated important roles of BMP-4 and BMP-7, TGF-β superfamily members produced by ovarian stromal and/or theca cells (13, 14), and GDF-9 produced by oocytes (15, 16). Growth factors such as Kit ligand and LIF produced in granulosa cells and acting paracrinally, and FGF-7 and FGF-2 produced in theca and stromal cells and cytokines play roles in primary follicle growth (17, 18). Promoting effect of insulin in association with Kit ligand and LIF on primordial to primary follicle transition is also demonstrated in rat ovarian culture (17, 20). Other genes newly demonstrated to have roles in initiation of follicle growth are nobox and Foxo3 (21, 22).

Progression to preantral and antral phases

Primary follicle surrounded by single layer of granulosa cells grows into secondary follicle surrounded by multiple layers of granulosa cells which increase in numbers by mitosis. Oocyte diameter increases to 80 µm and follicle diameter to 120 µm with approximately 600 granulosa cells. Germinal vesicle also reaches its maximum size. FSH, estrogen, androgen receptors and gap junctions appear on granulosa cells. At the end of primary follicle stage, early theca interna occurs as a result of follicular blood flow. Theca externa occurs as the follicle grows and compresses surrounding stroma. In lack of GDF-9 originating from oocyte, theca layer does not exist. (23).

Oocyte is metabolically active in secondary follicle stage, it matures and differentiates. It synthesizes mRNA and proteins to be used in early implantation embryo stage. Necessary nutrients, growth factors, and other molecules are shared through gap junctions on transzonal processes. Morphologically, centrioles disappear and mitochondria increase in number. Endoplasmic reticulum and golgi body are placed surrounding germinal vesicle in this stage. During growing phase, oocyte matures for meiosis. Regular disjunction of chromosomes during meiosis depends on healthy folliculogenesis and oocyte development (24).

Oocyte diameter reaches 120–150 µm in pre-antral stage and 200 µm in antral stage. Basal lamina, zona pellucida and theca cell layer occur in this stage (25). Fluid filled spaces among granulosa cells unite to form antral cavity (Fig 3).

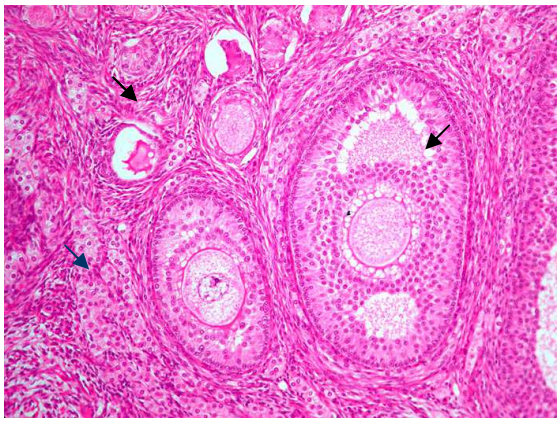

Figure 3: One primary follicle at the top, one pre-antral follicle on the bottom-left, antral follicle on the right (arrows).

Urgent fluid influx through channels made of aquaporins 7, 8, and 9 produced by granulosa cells is the major factor enlarging antrum. Osmotic gradient necessary for this influx is provided mainly by active ion transport. Hydrolysis of glycosaminoglycans in antrum also causes fluid influx by increasing osmolarity of follicular fluid.

Antrum is important not only for transfer of nutrients and waste products, but also for development of cumulus-oocyte complex and ovulation. Vascularity of theca cell layer increases, oocyte growth and proliferation of theca and granulosa cells continue. This stage is independent from gonadotropins and can last for months. Contribution of FSH to preantral follicle development is debatable. Although not absolutely essential, it may enable preantral follicle growth probably by interacting with other ovarian factors. This hypothesis is based on the facts that this stage follicles are rarely found in ovaries of hypogonadotropic hypogonadism patients (26) despite ovarian grafts transplanted to hypoganadal rats show follicular development up to antral stage (5) however this effect is not observed in isolated pre-antral follicles cultured with FSH (27).

BMP-4 and 7, TGF-β, GDF-9 and BMP-15 produced by granulosa and/or theca cells are known to play important roles in this stage (28). Roles of theca cells on follicular growth are diverse and important. They are the primary place of androgen synthesis in the ovaries and provide precursors for estrogen synthesis in granulosa cells. They secrete BMP-4 and 7 to promote growth of primary follicle and modify FSH signalisation via increasing estrogen and decreasing progesteron synthesis, thus inhibit luteinisation. However BMP-4 and 7 cannot exert such an effect in lack of FSH (29). They secrete HGF and KGF to promote Kit ligand synthesis by granulosa cells which further increase HGF and KGF production by theca cells (positive feedback) (30).

Granulosa cells of preantral follicle secrete primarily estrogen, and also androgens and progestins in lesser amounts. FSH induces aromatization of androgens to estrogens. Although granulosa cells donot carry FSH receptor up to preantral stage (12), FSH is necessary afterwards to form an estrogenic micro-environment (31).

FSH and estrogen increase both number and FSH receptor synthesis of granulosa cells (32). As follicle grows, granulosa cells differentiate into many subtypes according to their closeness to the oocyte.

Granulosa cells also carry androgen receptors and are sensitive to the amount of androgens in their surrounding (33). Little amount of androgens increase aromatization and therefore estrogens, on the other hand, high amount of androgens are converted to more potent androgens resistant to aromatization via 5α reduction (34) leading the follicle to atresia. Success of a follicle depends on its ability to convert its microenvironment from androgenic to estrogenic.

Activins secreted from granulosa cells and TGF-β secreted from granulosa and theca cells are some positive regulators of preantral and antral follicle growth. Beyond primordial to primary follicle conversion, AMH is thought to have negative effect on preantral follicle development. AMH synthesis starts in primary follicle stage and continues up to mid-antral stages in humans. AMH is a dimeric glycoprotein from TGF-β family and is expressed in highest amounts in granulosa cells of secondary, pre-antral and small antral (<4mm) follicles (35). It can be seen in granulosa cells of developing pre-antral follicles in 36th gestational week (36), reaches its maximum level at puberty, and disappear after menopause (37). Inclusion of primordial follicles into rapidly growing follicle pool in AMH-null rats may designate a negative effect of AMH on conversion of primordial follicles to primary follicles (38, 39). AMH is believed to be an important indicator of ovarian reserve and IVF outcome. Its minimum variability within and between the cycles and excellent correlation with antral follicle count and number of oocytes picked-up make AMH a very precious marker (40).

Granulosa cells do not have a direct blood source. Basal lamina seperates granulosa cells from vascularized theca layer and behaves as a blood-follicle barrier. Therefore close contact between oocyte and neighbouring granulosa cells is mandatory. Gap junctions penetrating zona pellucida and connecting granulosa and oocyte cell membranes mediate their communication. Via expansive gap junctions, granulosa cells form a entegrated functional synsitium.

Follicle rapidly grows 5-6 days before the ovulation due to granulosa cell proliferation and antral fluid accumulation and moves towards the surface leading to Mittelschmerz.

Cell cycle gen Cyclin D2 expression is necessary for this enlargement. Completing this stage, follicle is ready for ovulation and called Graffian follicle (Fig 4).

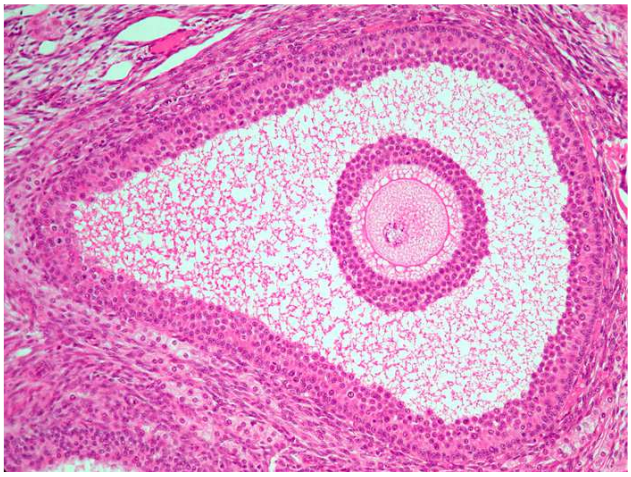

Figure 4: Graffian follicle

Recruitment and Selection of Dominant Follicle

Some follicles from antral follicle pool start their development under effect of FSH and this is called recruitment. That means rescue of an antral follicle cohort responsive to FSH from apoptosis at the beginning of the cycle (32). Follicles in this pool can proceed either to ovulation or to atresia. Traditionally it is thought that only one follicular development wave occurs in one cycle however new ultrasonographic studies proposed presence of multiple follicular development waves (41). Selected follicle and other members of the cohort are morphologically same. However mitotic index of granulosa cells of the selected follicle is high and its follicular fluid has a respectable amount of estrogen and measurable amount of FSH. Estrogen, FSH and local growth factors effect dominant follicle via autocrine, paracrine and endocrine ways. Estrogen increases effect of hypophyseal FSH on the follicle, on the other hand, it decreases hypophyseal FSH secretion via negative feedback limiting FSH effect on less developed follicles. Microenvironment of these follicles become androgenic and atresia starts. Ovarian steroids and other local factors also add to this period. First event in this period is diminishing FSH receptors on the granulosa cells (32). High intracellular estrogen and high number of FSH receptors are 2 features of dominant follicle which rescue itself from diminishing effect of high estrogen secreted by itself on hypophyseal FSH. Better vascularized theca layer due to VEGF also enable high amount of gonadotropins to reach dominant follicle.

Ovulation of follicle gains dominance 5-7 days after disappearance of corpus luteum of the preceeding cycle. Length of menstrual cycle is determined by the dominant follicle (follicular phase) and corpus luteum (luteal phase), not the hypothalamus or hypophysis. If the selected dominant follicle is damaged, no other member of the cohort can replace it.

Intrafollicular estrogen/androgen ratio is low when follicle is smaller than 8 mm^2. In midfollicular phase this ratio is reversed. Recruited follicle is capable of producing enough amounts of estrogens and estrogen level in the vein of dominant ovary is higher than the contralateral ovary in 5-7th days of the cycle. As a result of granulosa and theca cell activity, antral fluid can include higher levels of steroids than plasma. In late follicular phase, intrafollicular estradiol level is correlated with follicle size, and estradiol level in systemic circulation reaches its maximum level which is approximately 1mg/ml (42). High FSH and high intrafollicular estrogen level increase LH receptor concentration in granulosa cells. LH is critical for late maturation of dominant follicle (43). Following LH peak, intrafollicular estradiol and androstenedione levels decrease. Reflecting early granulosa cell luteinization, progesterone and 17-OH-progesterone levels increase. In preovulatory follicle fluid, estradiol and progesterone levels are high and androgens are low. In contrast, in small follicles in late follicular phase, androgen levels are high and estrogen and progesterone levels are low.

These findings suggest that follicular hormone levels are controlled by the microenvironment of the follicle individually. In presence of FSH, estrogens are dominant in follicular fluid and in lack of FSH, androgens become dominant (44). Early rise in LH slows down mitosis in granulosa cells, degeneration starts and follicle remains in androgenic micro environment.

Granulosa cells produce peptides called activin, inhibin, and follistatin and secrete into systemic circulation under FSH stimulation. Hypophyseal secretion of FSH is stimulated by activin and inhibited by inhibin. Follistatin binds activin and block its stimulatory effect. With follicular maturation, inhibin A level increases, and inhibin B, activin A and free follistatin stay stable. Prolaktin can also be determined in follicular fluid everytime but its physiologic role is not known (32). Inhibin secreted to systemic circulation FSH level acting on other follicles and guaranty dominance. Inhibin B is highest in midfollicular phase and just after ovulation. As corpus luteum forms, Inhibin-A increases under LH stimulation.

Inhibin A increases FSH receptor synthesis in granulosa cells, suppresses early phases but induces latter phases of follicular development, and plays roles in control of aromatase activity. It decreases LH effect, therefore androgen production in theca cells (45). When activin effect is blocked, follicular development stops (46, 47). In small follicles, secretion of activin A is prominent than inhibin A. In bigger antral follicles ınhibin A secretion is prominent (48). Inhibin A gives substrate to aromatization by increasing androgen production in theca cells under LH stimulation.

AMH weakens response of preantral and small antral follicles to FSH. TGF-β show effects similiar to activin effects. BMP-6 from granulosa cells and BMP-4 and 7 from theca cells lower androgen synthesis in response to LH limiting substrates of aromatization (28).

CONCLUSION

Better understanding actors of folliculogenesis can enable us to prevent premature ovarian failure, to develop effective in vitro maturation techniques and to improve ovarian stimulation protocols.

Conflict of Interest

No conflict of interest was declared by the authors.

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