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# Characteristic of factors influencing the proper course of folliculogenesis in mammals

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## Abstract

Folliculogenesis is the process of ovarian follicle formation,, taking presence during foetal period. During the follicular development, oogoniums undergo meiosis and oocytes are formed. In the ovaries of new born sows, primary and secondary follicles are present and, 90 days after birth, tertiary follicles appear. During development in the ovarian follicles growth of granulosa cells and differentiation of the thecal cells can be observed. A cavity filled with follicular fluid appears. Granulosa cells are divided into: mural cells and corona radiata, which together with the oocyte form the cumulus oophorus. Corona radiata cells, mural layers and oolemma contact each other by a network of gap junctions. Secreted from the pituitary gland, FSH and LH gonadotropin hormones act on receptors located in granular and follicular cells. In the postnatal life tertiary follicles and Graafian follicles are formed. When the follicle reaches a diameter of 1 mm, further growth depends on the secretion of gonadotropins. Mature ovarian follicles produce: progestins, androgens and oestrogens. The growth, differentiation and steroidogenic activity of ovarian follicles, in addition to FSH and LH, is also affected by prolactin, oxytocin, steroid and protein hormones, numerous proteins from the cytokine and interleukin family, metabolic hormones like insulin, glucocorticoids, leptin, thyroid hormones and growth hormones. Despite numerous studies, many processes related to folliculogenesis have not been discovered Learning the mechanisms regulating reproductive processes would allow to easily distinguish pathological processes and discover more and more genes and mechanisms of their expression in cells that build ovarian follicles.

**Running title:** regulation of folliculogenesis

**Keywords:** mammals, folliculogenesis, physiological factors

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## Physiological basis of folliculogenesis in mammals

Folliculogenesis is the process of formation of follicles, which are the basic structures of ovaries in mammals. It is one of the most important stages in the development of female reproductive organs, beginning in fetal life [1]. Ovaries in pigs arise in the early stages of embryogenesis, around the 26th day of pregnancy. The first ovarian follicles appear in the fetus about 40 days after fertilization [2]. The process of developing ovarian follicles coincides with the beginning of meiosis in the oogoniums and the formation of the oocyte. The somatic elements of the ovarian follicle move towards the ovum. The oocyte is initially surrounded by a single layer of flat granulosa cells, which are supported by a basement membrane. The structures formed in this way are primary follicles, which undergo further growth and differentiation processes provides the oocyte with an optimal environment and protects it against harmful substances secreted into the blood stream [3], [4].

A significant pool of oocytes accumulated in the porcine ovary (about 60%) is not dislocated into the follicles and degenerates in the early stages of fetal life [5], [6]. In the ovaries of newborn females, follicles are present, the primary (about 0.12 mm) and secondary (up to 0.4 mm in diameter), whose total pool is estimated at approximately 500,000 [7]. On the 90th day after birth, the first antral follicles (tertiary follicles) appear on the surface of the gilt's ovaries. In ovarian follicles there is a growth of granulosa cells from 10 to 30 layers and differentiation of covering cells (thecal) [4]. In the continuing stages of follicular development, the volume of the oocyte and the adjacent granulosa cells, whose shape changes from a flat to cubic, increases. In addition, the female gamete produces glycoproteins such as ZP1, ZP2, ZP3, which are the building blocks of the zona pellucida and receptors for sperm necessary for fertilization process [8]–[10]. At the same time, at the basement membrane of the follicle, the follicular envelope is formed, which under the influence of blood vessels penetrating its structure, is gradually differentiated into the theca externa and theca interna [11]. Then a cavity filled with follicular fluid appears, surrounded by a layer of granulosa cells. After the creation of the cavity, the granular layer of the cells is divided into: mural cells, lining the inside of the vesicle wall and corona radiata, which surround the egg together with the cumulus oophorus [12]. The cumulus cells, due to the close presence of the oocyte, distinguish from the mural layer with their very low expression of LH receptors, high proliferation, weak steroidogenesis and the synthesis of a significant amount of hyaluronic acid and components of the intercellular substance. Cells of corona radiata, mural layer and oolemma contact each other with the help of numerous junctions, creating the proper environment for the development of the oocyte [12]–[18].

Gonadotropin hormones, secreted from the pituitary, such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) interact with receptors localized in the granulosa cells surrounding the oocyte, and thecal cells [19]. Research by Erickson et al. showed that the growth of primary follicles is controlled mainly by local growth factors and proceeds independently of the level of hormones secreted from the pituitary gland [20]. On the other hand, the subsequent increase in the size of the antral follicles is primarily influenced by the vesicular fluid accumulated in them [21], [22]. The components of this fluid are granulosa cells in the ovarian follicles, the plasma filtrate and the inner lining of the vesicle. Follicular fluid contains many substances that stimulate mitotic divisions in cells, such as glycosaminoglycans, proteins, amino acids, sugars, hormones, or non-steroid factors [23]. Further development of ovarian follicles is already taking place in postnatal life. Tertiary and ovulatory vesicles (Graafian vesicles) are formed. The formation of antrum is also significantly influenced by androgens, for which receptors are found in all cells of the granular layer of growing vesicles, whereas in Graafian vesicles they occur only in cumulus cells [24]–[26].

In gilts, at the 4th week after birth, the first follicles with a diameter of approximately 1 mm are observed on the surface of the ovaries. Some, in the next 2-3 weeks reach a diameter of 7 mm, and later, in the preovulatory stage, 8-10 mm. This phase lasts for a short time – from 4 to 6 days. In sows, unlike cows, no wave-like development of the ovarian follicles is observed. The number of follicles remains essentially unchanged up to day 15 of the sexual cycle. Between the 15-20 day of the cycle, the small follicular pool (<3mm) is gradually reduced, while the number of medium (3-5 mm) and large (> 5mm) follicular increases. From a hundred follicles reaching a diameter of up to 4 mm, "recruited" is a pool of about 50, of which only 10-25 are ovulated, while the others undergoes atresia [2], [27]. However, the population of ovulatory follicles in pigs is inhomogeneous in terms of size (individual vesicles differ in size) and biochemical features (vesicles show different sensitivity to LH) [28].

When the follicle reaches a diameter of 1 mm its further growth depends on the secretion of gonadotropins (FSH, LH), while the earlier stages of growth occur independent of the effects of these hormones [29]. The resulting cavity induces its further development. The membranes of granulosa cells increase the number of receptors for FSH and LH, and the synthesis of estrogen receptors in nucleus and cytoplasm is increased [24], [30]. The development of an appropriate number of receptors for FSH leads to the activation of enzymes from the aromatase family converting testosterone into estradiol. Folliculotropic hormone is defined as the primary inducer of granulosa cell maturation, regulating their

proliferation and differentiation. Further activity of these processes is shaped by the increase in estradiol levels, inhibin or the formation of an appropriate number of receptors for lutropin (LH) in granulosa cells [31]. According to Hunter et al. ,the minimum diameter of the vesicles in which the LH receptors were found is 5 mm [32].

At different stages of development, most of the ovarian follicle pool degenerates. At the biochemical basis atresia follicular cells are aromatase activity loss and the production of estradiol and the reduced secretion of steroid hormones [4], [33].

## Follicular steroidogenesis

The ability to synthesize sex hormones in ovarian follicles is influenced by the development of receptors for pituitary gonadotropin hormones. The steroidogenic activity of ovarian follicles is already noticeable during fetal life. It is influenced by the growth and differentiation of the follicular cells, the stage of follicular development and the phase of the estrous cycle [1], [34], [35]. Mature ovarian follicles produce three sex steroids: progestins, androgens and estrogens, which in turn act as regulators of ovarian development. The precursor of all steroid hormones in gonadal cells is cholesterol. In the ovary its main source are blood lipoproteins (LDL) and high density lipoprotein (HDL) [31]. Cholesterol can also be synthesized de novo from acetate, or derived from lipid esters accumulated in cholesterol cells. Through the endocytosis processes, lipoprotein molecules enter the cytoplasm of follicular cells. After esterification and hydrolysis, cholesterol travels to the mitochondria. Within this cellular structure, cholesterol is converted under the influence of P450SCC aromatase into pregnenolone, and at a later stage in the endoplasmic reticulum it is converted to progesterone. Progesterone is subject to hydroxylation, resulting in the formation of androgens. In the next step, under the influence of cytochrome oxidase, aromatization of androgens, resulting in creation of estrogens, occurs.. This leads to the conversion of testosterone to estradiol, and androstenedione to estrone [3], [35]. The fluid harvested from mature follicles exhibited high activity of aromatase and concentration of estradiol [28]. In the synthesis of estrogen hormones, both the internal and the granular tissues of the ovarian follicle are involved. The cells of the inner casing produce androgens (under the control of LH), which during aromatization under the influence of cytochrome P450AROM oxidase (induced by FSH) are converted into estrogens [36].

Studies by Conley et al., however, points to the additional independent production of estradiol in the cells of the inner lining of the follicle, under the influence of P450c17α oxidase [34]. It is suggested that estradiol produced in the granular cell layer is collected in the follicular fluid, and it is synthesized in the inner membrane, it is delivered to the bloodstream. Just before ovulation, the oxidase activity decreases in the ovarian follicles, which results in a decrease in the level of androgens and estradiol. Granulosa cells begin the synthesis of progesterone and, in a further stage of the cycle, luteinization of the follicle occurs. The ability to synthesize androgens from progesterone is proprietary only to the cells of the internal envelope of the vesicle, from which androstenedione (androgen) is transferred to granular layer cells, where it is transformed into testosterone and further aromatized to estradiol [37].

## Factors influencing follicular steroidogenesis

The growth, differentiation and steroidogenic activity of ovarian follicles, in addition to FSH and LH, are also affected by prolactin, oxytocin and steroid and protein hormones produced directly in the vesicle, which in the auto- and paracrine pathway affect the follicular cells [26], [38]. Important follicular regulators include numerous proteins from the cytokine and interleukin family [4]. Metabolic hormones (insulin, glucocorticoids, leptin, thyroid hormones and growth), are also noteworthy, directly affecting the oocyte or hypothalamic-pituitary axis and thus regulating ovarian function [38].

Thyroid hormones interact through receptors located in granulosa cells and regulate the activity of ovarian follicles. These hormones, together with FSH, affect the function of granulosa cells by stimulating the formation of receptors for LH / CG (luteinizing hormone / chorionic gondotropin) and morphological differentiation. At the same time, these hormones significantly inhibit apoptosis during in vitro cultivation of porcine granular layer cells. They also participate in the regulation of steroidogenesis by inducing or inhibiting the effect on steroidogenic enzymes [39] [40].

The biosynthesis of prostaglandins (PG) in the ovary is associated with the stimulation of follicular activity and the process of ovulation. They are also responsible for regulating the flow of blood through the ovary. The dominant role in the development of ovarian follicles is played by prostaglandin E2. In addition, PGE2 stimulates the oocyte maturation process. Internal PGF2a vesicles induce morphological changes (resumption of meiosis) and biochemical changes (release of hydrolases in ovarian epithelial cells). Prostaglandins also show luteotropic and luteolytic activity in the corpus luteum. The luteolysis process is associated with the increasing concentration of PGF2α, with a concurrent decrease in PGE2 concentration, which is accompanied by increased uterine contractile activity [41].

Among the non-steroidal factors regulating the development of follicles, GnRH (gonadotropin-releasing hormone) is distinguished, mainly secreted by the hypothalamus. It stimulates the synthesis and secretion of FSH and LH from the pituitary

gland [42]. In preantral follicles, it inhibits steroidogenesis, formation of LH receptors and growth processes. At a later stage of ovarian development, it influences the synthesis of progesterone, ovulation processes and the resumption of meiosis [26]. GnRH is produced by the hypothalamus, but there are two other forms of this hormone, GnRH-I and GnRH-II, in various ovarian compartments, also in granulosa cells [42].

Nitric oxide is synthesized in the blood vessels of the follicle and ovarian lining through endothelial NO synthase (eNOS). This free radical affects cytotoxic cells on the granular layer, which in combination with vasodilatation, facilitates ovulation. Free oxygen radicals act contrary to NO, inhibiting maturation and delaying follicle breakage [41].

Cytokines are a group of immune modulators with low molecular weight, which regulate ovarian activity through auto- and paracrine pathway. Cytokines affecting folliculogenesis include interleukin-1, 6, 8 (IL-1, -6, -8), tumor necrosis factor (TNF), interferon-γ (INF-γ). This group of modulators also includes growth factors such as insulin-like growth factor (IGF), transforming growth factors β (TGFβ), epidermal growth factor (EGF) and fibroblast growth factor (FGF). The function and location of cytokines varies. Present in the follicular fluid, IL-1 inhibits the release of GnRH and gonadotropin and stimulates steroidogenesis, whereas IL-6 blocks the proliferation of granulosa cells. TNF-α inhibits the synthesis of estradiol by lutein cells, influences the regression of the corpus luteum and stimulates the synthesis of progesterone in granular cells. INF-γ induces class II antigen complex (MHC) and is a signal to initiate luteolysis, also blocking the production of progesterone and estrogens by granulosa lutein cells. IGF-2 is responsible for strengthening the reaction of the follicular structures to the action of gonadotropins. In turn, EGF in the ovary regulates the proliferation of granulosa cells, including inhibiting gonadotropin-independent differentiation of these cells. In contrast, FGF is involved in the processes of the formation and luteolysis of the corpus luteum, with the us apoptotic processes [41], [43]–[47].

Protooncogenes are naturally occurring cellular factors that control growth and differentiation processes. The proto-oncogenes include: G protein from the GP6 (guanosine triphosphate) binding protein subfamily, tyrosine-like receptors and nuclear proteins. These factors prevent apoptosis, prolonging the life of cells and affect the maintenance of the corpus luteum during pregnancy [41].

The function of the ovarian follicle also depends on the oocyte in it. The ovum synthesizes factors that have a significant impact on the development of the oocyte itself, as well as the formation and activity of ovarian follicles [48]. Communication between oocyte and the somatic cells is reciprocal and important for both structures [49]. The matured ovum secretes mainly proteins belonging to the broad family of transforming growth factors (TGFs), such as bone morphogenetic proteins (BMPs), differential growth factor (GDF-9) and transforming growth factor alpha (TGFα). They affect the normal growth of granulosa cells and ovarian follicles, regulate their proliferation and development, and any abnormalities in their expression can be dangerous for developing follicles and oocytes. It has recently been discovered that nerve growth factor (NGF) can also stimulate the expression of FSH and LH, and influence the secretion of estrogen and progesterone in GCs [50], [51]. The control of granulosa cell function by the oocyte takes place on the basis of feedback between both cell types. The gamete- somatic cell relation affects both the development of oocyte and ovarian follicles [19], [32].

During oogenesis oocyte undergoes a wide dynamic change of gene expression, and these are controlled by mostly by the hormonal interactions and factors synthesized by the somatic cells surrounding it. Transcription regulators that play an important role in the formation of antral follicles in the process of folliculogenesis include: Foxo3, Foxl2, Figla, Lhx8, Nobox, Sohlh1 and Sohlh2. In the case of mutation in any of these genes occurs, they can cause ovarian failure, and ultimately infertility in mammals [19].

## Perspectives in research of mammalian folliculogenesis

This article discusses the issue of folliculogenesis in mammals, mainly in pigs and related processes. This is a very interesting subject in terms of scientific research. Today, obtaining research material in the form of the ovaries of female mammals, including model animals such as pigs or cows does not require major efforts, which opens the possibility of large scale studies of reproductive systems closely resembling those of human origin. However, despite numerous studies on the reproduction of mammals, many processes related to folliculogenesis have still not been discovered. Learning about all the mechanisms regulating reproductive processes would ease distinguishing pathological processes that impede fertility in mammals, especially humans. Continuous development of the science of folliculogenesis allows us to discover more and more of genes and their expression mechanisms in the cells that build follicles. Such studies may enable more effective diagnosis and treatment of ovarian associated diseases, development of new assays using freshly discovered markers, and provide reference for further clinical research [52].

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**Conflicts of Interest**
The authors declare they have no conflict of interest

This paper does not contain any studies with human participants or animals performed by any of the authors

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