



Review Article

The Ovarian Folliculogenesis – A Mini Review

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ABSTRACT

Follicle formation is a critical determinant of the size of the primordial follicle pool and is essential for fertility. This process occurs during definitive histogenesis. Follicles secrete essential hormones required for proper endocrine functioning and are also the chief suppliers of steroid sex hormones. Additionally, they also produce other local regulators. Throughout childhood and puberty, the human ovary presents differences in the follicle population. Ovarian granulosa cells play a significant role in every stage of follicular growth through proliferation, steroidogenesis and production of autocrine and paracrine factors. This review outlines the events of folliculogenesis at different stages and the factors influencing this complex pathway.

Keywords: Antral follicles, Follicular cells, Granulosa cells, Primary follicles, Primordial follicles, Primordial germ cells

INTRODUCTION

The development of gonads involves three sequential steps: gonadal morphogenesis, primary sex differentiation and definitive histogenesis.^[1] During definitive histogenesis, the primitive ovary transforms into a definitive ovary with the formation of follicles.^[2] This stage comprises the compartmentalisation of the organ. Moreover, in this stage, the parenchyma is divided into a primitive outer cortex and inner medulla.^[3] The follicles, which consist of oocytes and supporting somatic cells, are the basic structural and functional unit of the ovary.^[4] To understand folliculogenesis, a comprehension of the events related is necessary. The entire event commences at 5–6 weeks when the primordial germ cells from the yolk sac migrate to the gonadal ridge and settle there, which results in the formation of a bipotential gonad. The bipotential gonad either transforms into a testis or an ovary.^[5,6] In the absence of the sex-determining region Y and other factors, the bipotential gonad will develop into an ovary.^[7]

The study states that the primordial germ cell morphology is distinguished by its large size and round nucleus with a conspicuous nucleolus. It also consists of abundant glycogen particles and a considerable number of lipid droplets in the cytoplasm.^[8] To aid pluripotency and also to prevent differentiation of primordial germ cells, the important step is the activation of the Wnt3/ β -catenin signalling pathway, which stimulates the necessary transcription factors. A stem cell growth factor known as Kit (KIT) ligand (also called Steel factor) is secreted by the hindgut. This factor binds to the KIT receptor on the primordial germ cells. While the primordial germ cells are migrating, the prevention of apoptosis and halting of mitosis are very important. This is facilitated by the activation of KIT signalling, which will activate the tyrosine kinase that aids survival factors such as NANOS 3 and DND 1.9 Another noteworthy apoptosis factor that

has a substantial role in controlling the oocyte population, especially in earlier periods of ovarian development, is BAX. Later on, the oogonia develop into oocytes before entering meiosis. Some of the oocytes undergo apoptosis after the period of mitosis and sex determination. It is suggested that the apoptotic process is to eliminate abnormal or inferior quality cells, as they may not produce healthy, fertilisable eggs.^[9,10] Oocytes give rise to clusters known as germ cell nests, which are formed during the apoptosis period.

PRIMORDIAL FOLLICLES

Studies state that in rodent models, the final stage of development of the ovary takes place when the germ cell nest breaks down and primordial follicles are formed. While the germ cell nest undergoes the breakdown, the granulosa cells will encircle the oocyte to form a primordial follicle.^[11]

The characteristic feature of primordial follicles is a single oocyte, which is larger than the one in a nest and is surrounded by a single layer of flattened, squamous-shaped granulosa cells. The primordial follicles have a larger cytoplasm-to-nucleus ratio compared with oocytes seen in germ cell nests.^[12] It is estimated that there are 60,000–700,000 follicles present at birth, which undergo depletion in number until only a few thousand follicles remain by menopause.^[3] The primordial follicle formation is also followed by a significant cell loss, as the majority of the primordial follicles remain quiescent and die in this dormant state. As the event progresses, the ones that survive will form and become part of the ovarian follicle reserve.^[13,14] Studies suggest that if apoptosis is suppressed by deleting genes involved in regulating apoptosis, such as caspase 2 or BAX, the number of primordial follicles formed increases.^[15] Another key factor identified in association with these events is PUMA (p53 upregulated modulator of apoptosis).^[16] The human primordial follicle is approximately 40 µm in diameter and comprises a 30 µm oocyte in the centre with roughly 10 squamous pregranulosa cells.^[17] These follicles are typically located in the peripheral cortex of the ovary.^[3] The Balbiani body which is an assembly of cytoplasmic organelles. It consists of mitochondria and Golgi bodies associated with the nucleus of germ cells, which are found in these follicles. Interestingly, this striking feature is absent in mouse models. There were early studies suggesting that the foetal and neonatal mouse oocytes contain a perinuclear Golgi body consisting of small vesicles similar to a Balbiani body. However, successive studies state that mouse oocytes lack these mitochondrial aggregates.^[18] It is reported that in zebrafish and frogs, the Balbiani body serves as an important part of a vegetal transport pathway, which is believed to entrap mRNAs and other gene products required for germ cell formation as well as embryo patterning.^[18,19]

PRIMARY FOLLICLES

Interestingly, one more time oocyte degeneration occurs while the establishment of the ovarian pool is going on, and only a minority are transformed into primary follicles. The primary follicles are characterised by transformation from flattened cells to cuboidal cells surrounding the oocyte.^[14] In the cortex of the ovary, the dormant primordial follicles are gradually activated into a second wave of activated follicles.^[20] The oocytic enlargement and granulosa cell proliferation occur as the primordial follicles become primary follicles.^[21,22]

ANTRAL FOLLICLES

The primary follicles transform into secondary follicles when the oocyte is surrounded by two or more proliferating granulosa cells. During the transition into the antral phase, the stroma of the primary follicles develops its blood supply and differentiates into theca interna and theca externa. The theca interna develops LH receptors. Both FSH and LH receptors facilitate the follicle to respond to their corresponding gonadotropins.^[21] The successive maturation of these secondary follicles is gonadotropin dependent [Figure 1]. In the granulosa cell, the FSH binds to the respective receptor. In response to this event, the follicles grow and start developing a fluid-filled space called an antrum. LH binds to the receptors in the theca interna and forms androgens. These androgens are crucial for follicular growth as well as follicular loss via apoptosis.^[22–24] According to Williams and Erikson, the preantral or Class 1 phase is divided into the primordial, primary, and secondary follicle stages. The antral phase is divided as follows: small (Class 2, 3, 4, 5), medium (Class 6), large (Class 7), and preovulatory (Class 8) Graafian follicle stages.^[25] The development of the preantral stage from the primordial stage is interestingly not dependent on gonadotropin stimulation; rather, it is controlled by local signals from the oocyte and somatic cells. The oocyte helps in regulating the proliferation and differentiation of granulosa cells and theca cells, while the somatic cells support the growth of the oocyte.^[26] The fluid-filled preantral follicle spaces coalesce to form an antrum. The main hormone affecting the antral follicle is FSH. It is important for the survival and proliferation of granulosa cells, as well as the LH receptor expression and estradiol production.^[27]

REGULATORS OF FOLLICULOGENESIS

Although numerous factors are held responsible for the regulation of folliculogenesis, a few important and novel factors that play a key role are discussed here.

HIPPO SIGNALLING PATHWAY

This pathway is primarily found in mammals and has a significant role in follicular growth, activation and steroidogenesis. Studies

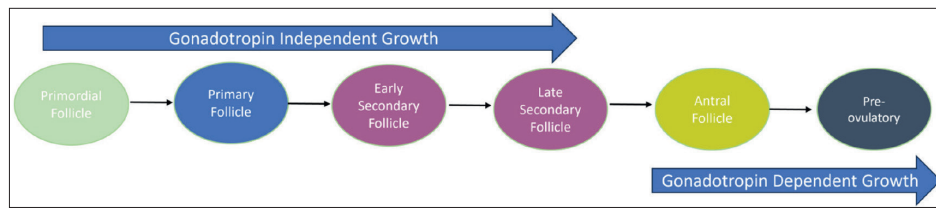


Figure 1: Schematic diagram of the stages of folliculogenesis.

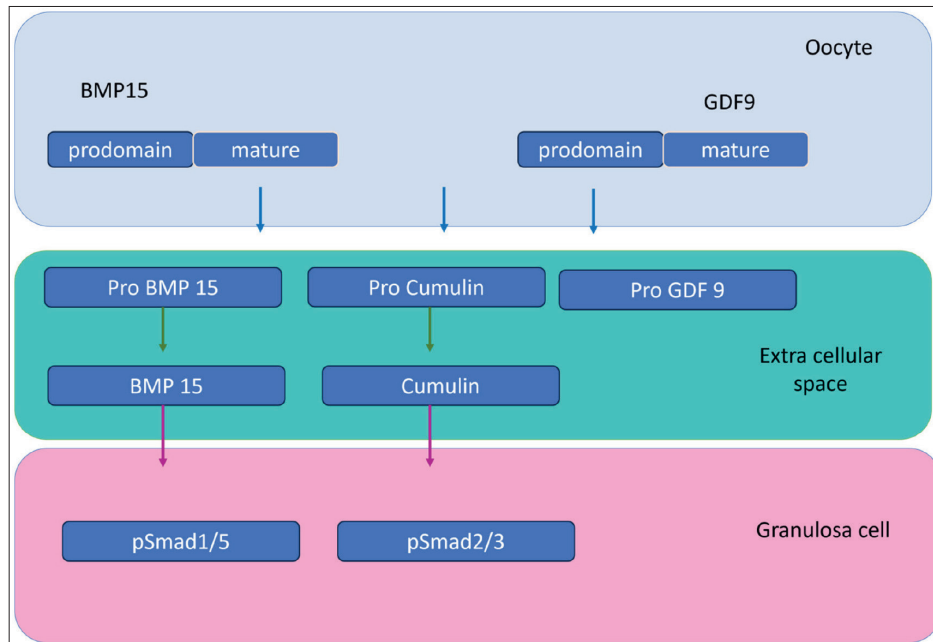


Figure 2: Schematic model of GDF9 and BMP15 formation and signalling in mammalian oocytes. Both GDF9 and BMP15 are co-expressed during the process of folliculogenesis. BMP: Bone morphogenetic protein, GDF: Growth and differentiation factor.

suggest that any dysregulation of the Hippo pathway may lead to reproductive disorders such as polycystic ovary syndrome, premature ovarian insufficiency, and ovarian cancers because of the loss of follicular homeostasis.^[28]

This pathway comprises a core protein kinase cascade involving many negative regulators of growth, such as MST1/2, SAV1 and MOB kinase activator 1 (MOB1).^[15,28] Under the optimal conditions, the MST/SAV1 complex phosphorylates and activates LATS1/2-MOB1. This will phosphorylate and inactivate YAP1 and TAZ.^[29] Many studies recognise the Hippo signalling as a crucial regulator of ovarian physiology and pathology; however, the exact and elaborate role of the mechanisms involved remains obscure.^[28]

INSULIN-LIKE GROWTH PEPTIDE FACTOR 3

Insulin-like peptide 3 (INSL3) is a member of the insulin-like group of peptide hormones, which in humans, besides insulin, IGF1 and IGF2, also includes relaxin and the

structurally related peptides H1-relaxin, INSL3, INSL4, INSL5, INSL6 and INSL7.^[30] The theca interna cells of growing follicles produce INSL3, as well as its associated receptor, RXFP2.^[31] The INSL3–RXFP2 system is believed to be an important pathway for the proper functioning of antral follicles. INSL3 is not only secreted into the bloodstream by these follicles but also accumulates in the fluid of the follicular antrum.^[32] Studies suggest that the expression of INSL3 is downregulated during the preovulatory LH surge.

Though numerous studies are emerging with regard to INSL3 in its role in folliculogenesis, it cannot be overlooked that many aspects regarding its mechanism remain unexplored.

NEUROPEPTIDE PHOENIXIN (PNX)

PNX, a novel neuropeptide, is derived from the Smim20 protein.^[33] The PNXs are most abundantly expressed in the hypothalamus and less in the ovary. PNX binds to its receptor, G-protein coupled receptor 173. It plays a significant role in



Figure 3: Role of GDF9 and BMP15 on the ovarian physiology and pathology. BMP: Bone morphogenetic protein, GDF: Growth and differentiation factor.

both the central nervous system and the female reproductive system, where it induces LH secretion. Moreover, it stimulates oocyte maturation.^[34] A study by Guvenc *et al.*^[35] states that PNx and nesfatin-1 have similar distribution patterns in the brain. Both of them significantly increased the secretion of FSH, LH, and testosterone in rat plasma. These changes were without promoting any changes in plasma GnRH. This shows that both these neuropeptides play a synergistic role in regulating male sex hormones.^[34,35] A study by Ullah K *et al.* implies that the concentrations of LH, FSH, and nesfatin-1 in patients with PCOS were positively correlated with the level of this neuropeptide.^[36] Though these studies show a wide range of significance of PNx in its role in the reproductive pathway, more studies need to be conducted to categorise its exact function.

GDF9 AND BMP15

The TGF- β superfamily is a family of protein secretors in mammals. They consist of TGF- β s, anti-Mullerian hormone, activins, inhibins, bone morphogenetic proteins (BMPs), and growth differentiation factors (GDFs).^[37] Studies state that the BMPs and GDFs of this family play crucial roles in the regulation of folliculogenesis.^[38,39] These growth factors secreted by the oocyte are very important for granulosa cell proliferation. Any mutation to either of these may hamper the normal follicular development. They may lead to arrest in the early antral stage.^[40,41] A study by Reader *et al.* mentions that the SMAD 2/3 pathway is essential for the synergistic action of both GDF9 and BMP15 [Figure 2].^[42] The results from

many other studies also emphasise that BMP15 and GDF9 are substantial regulators of follicular development in ruminant models.^[43]

A change in any of the autocrine, paracrine or endocrine factors can lead to reduced oocyte quality or cystic follicles, which may result in infertility. The GDF9 and BMP15 are crucial for ovarian and follicular development. It is noteworthy that certain ovarian pathologies are associated with mutations in GDF9 and BMP15, such as primary ovarian insufficiency and polycystic ovary syndrome, as well as dizygotic twinning [Figure 3].^[44] The study by Shah *et al.*^[45] states that the activation of the Hippo pathway is linked to increased proliferation of small follicles. Thus, in patients with PCOS, the abnormal follicular proliferation may be related to the Hippo pathway.^[45] Studies have also shown the association between the Hippo pathway activation and the iron-dependent cell death, which in turn is related to follicular genesis.^[46,47] The novel regulator INSL3 also throws some promising insights into understanding its role in the female reproductive pathologies. The knockout studies in mice show that the loss of INSL3 or its receptors in females may result in partial fertility with reduced folliculogenesis.^[30,48] A study by Pelusi *et al.*^[48] mentions that INSL3 corresponds closely with circulating Antimüllerian Hormone (AMH) concentration. Both these factors are correlated with each other in women with PCOS, as they are increased in the presence of amenorrhoea. INSL3 and AMH may reflect a dysfunction of the thecal and granulosa cells in PCOS cases in which there is increased androgen production and anovulation.^[48]

CONCLUSION

The human oocyte development is a highly complex process which involves paracrine, endocrine and autocrine regulators playing highly significant roles. As new factors and pathways are emerging with regard to folliculogenesis, understanding their mechanism is necessary, as many factors can help us to comprehend the associated female infertility pathologies and their possible role in the therapeutics involved.

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