**—Mini Review—**

# *Oocyte-thecal Cell Regulatory Loop in the Control of Preantral Follicle Development*

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***Abstract:*** *Oocyte-somatic cell interaction plays a crucial role in preantral folliculogenesis. Although topical research has focused on oocyte-granulosa cell interaction over the past decade, an oocyte-thecal cell regulatory loop may also play an important role during the preantral stage. Formation of the thecal cell layer is a key event that occurs during preantral folliculogenesis. Granulosal factors (e.g. IGF-I and KL) appear to stimulate the recruitment of thecal cells from stromal cells. Oocyte-derived GDF9 appears to indirectly modulate thecal cell differentiation, perhaps through regulating the granulosal IGF-I and KL expression. Theca-produced androgens stimulate granulosa cell proliferation and preantral follicle growth. GDF9 enhances preantral follicle growth by up-regulating thecal androgen production, suggesting that a threshold level of androgens derived from thecal cells is necessary for preantral follicle growth, and that the androgen production may be controlled by the oocyte-derived GDF9. The challenge ahead is not to only understand the precise nature of these interactions, and to elucidate how dysregulation in these interactions may lead to ovarian pathologies such as polycystic ovary syndrome and gonadotropin poor-responsiveness.*

***Key words:*** *GDF9, Androgen, IGF-I, Kit ligand*

## Introduction

Oocyte-somatic cell interaction plays a crucial role in preantral folliculogenesis [1–3]. Classically, it was thought that the oocyte was passively carried along the developmental process, and its maturation was controlled entirely by the production of endocrine hormones and surrounding somatic cell factors. However, the latest concept in reproductive biology is that the oocyte itself is actively involved in regulating the surrounding somatic cells in order to provide an environment suitable for its own growth, maturation, and ovulation. Over the past decade, research has focused on granulosa cells and their interaction with the oocyte. While the thecal cell is also an essential component of follicular growth and ovulation, we do not yet fully understand the control of the recruitment and function of thecal cells, an important consideration, since their function appears to be altered in certain causes of infertility [4]. This review focuses on recent progress that has been made in understanding the possible importance of the intraovarian oocyte-thecal cell regulatory loop in the control of preantral follicle development.

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## Thecal Layer Formation is a Key Event that Occurs during Preantral Folliculogenesis

Preantral folliculogenesis is characterized by oocyte growth, granulosa cell proliferation, and the acquisition of an additional somatic cell layer, the theca. The appearance of a thecal cell layer at the preantral stage is an important physiological event in early follicular development (Fig. 1) [5], as evidenced by: 1) the concurrence of the organization of the thecal layer and the increased follicular growth and the steroidogenic response to gonadotropins [6, 7]; 2) the increased structural support provided by the thecal layer and blood supply carrying ovarian regulators to the developing follicle [8, 9]; and 3) the increase of thecal aromatizable androgen production for granulosa cell estrogen biosynthesis and the enhancement of early follicular growth by the androgenic products of the thecal cell [10–14].

Thecal layer formation is gonadotropin-independent because thecal precursor cells lack LH receptors (LHR)

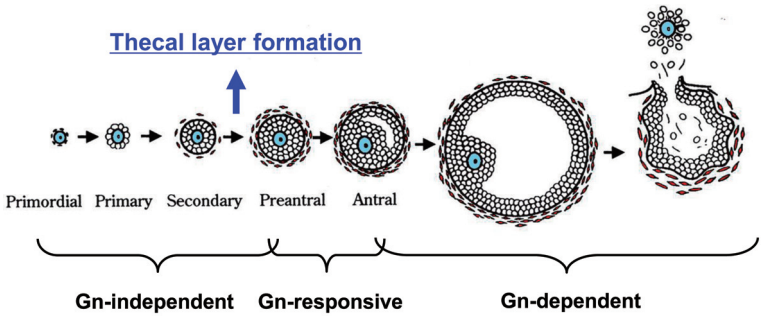


**Fig. 1.** Thecal layer formation is a key event that occurs during preantral folliculogenesis.

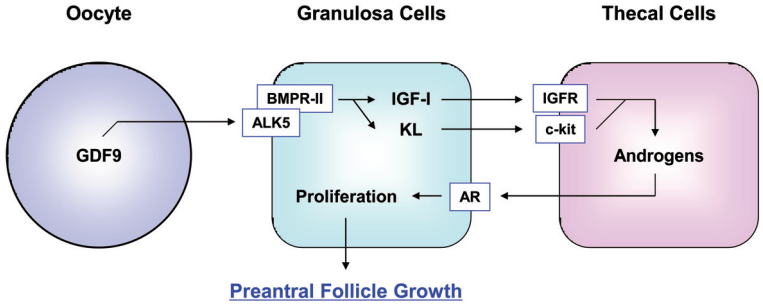


**Fig. 2.** Oocyte-thecal cell regulatory loop in the control of preantral follicle development.

[15] and the thecal layer still forms in the ovaries of FSH-deficient mice [16]. As thecal cells are only associated with growing follicles, one would assume that the follicle itself would produce factors that signal to the stroma to recruit the cells that form the thecal cells. A recent *in vitro* study using bovine ovarian tissue showed that ovarian stromal cells, cultured in the presence of granulosa cells collected from small antral follicles, transformed into putative thecal cells with increased lipid droplets, androgen production, and LH receptor (LHR) expression [17]. Using neonatal mouse ovaries, putative thecal stem cells were purified and induced to differentiate *in vitro* [18]. When these stem cells were treated with conditioned media from granulosa cells, the cells differentiated into thecal cells [18]. These results suggest that granulosa cells are involved in the functional differentiation and the acquisition of LH responsiveness in thecal precursor cells. Theca cells maintain an epithelial-like appearance and androgenic capacity when co-cultured with granulosa cells, but become fibroblastic and produce less androgen when cultured alone [19], suggesting that the presence of granulosal factor(s) is also indispensable for theca cells to sustain their morphology and function.

Granulosal factors that may contribute to thecal cell recruitment and/or differentiation include insulin-like growth factor-I (IGF-I) and kit ligand (KL; also known as stem cell factor) (Fig. 2) [18, 20]. Both IGF-I and KL are secreted by granulosa cells, and their receptors, IGF-1 receptor and c-kit, are present in thecal cells, respectively. IGF-I increased thecal cell proliferation and LHR expression *in vitro* [21–23]. In bovine, KL also stimulated ovarian stromal cell proliferation [24]. When putative thecal stem cells were treated with IGF-I and KL, the cells differentiated into thecal cells and produced androgens [18]. In rat theca-interstitial cells, a culture combination of IGF-I and KL increased the expression of androgenic factors (*i.e.* StAR, CYP11A, CYP17, HSD3$\beta$, and LHR), and hence androgen synthesis *in vitro* [20], providing strong evidence that the granulosa cell-derived IGF-I and KL may act synergistically to regulate thecal cell recruitment and differentiation into steroid-producing cells.

## Oocyte-derived GDF9 Stimulates Thecal Cell Differentiation through Granulosal Factors

Growth differentiation factor 9 (GDF9) is an oocyte-derived factor and a member of the TGF-$\beta$ superfamily, which includes TGF-$\beta$, activin, and bone morphogenetic proteins (BMPs) [25, 26]. Ovaries from GDF9 null mice exhibit a developmental block at the primary follicle stage, which is characterized by failed thecal layer formation in early follicles, which also show a lack of the thecal cell markers, CYP17, LHR, and c-kit [27]. Based on these observations, it is possible to infer that oocyte-derived GDF9 also stimulates thecal cell recruitment, proliferation and differentiation, and induces the formation of thecal layer at the preantral stage. Nevertheless, GDF9 may be more important in thecal cell differentiation than in thecal recruitment, because the double-mutant (GDF9 and inhibin α) mice form a morphological thecal layer, but the specific thecal cell markers (CYP17 and LHR) are not expressed in this layer [28].

We recently indicated that GDF9 augments androgen production and CYP17 expression in rat preantral follicles, whereas down-regulation of GDF9 by intra-oocyte injection of GDF9 Morpholino antisense oligos suppressed these responses, indicating that GDF9 is important in thecal cell differentiation during the preantral stage [29]. Nevertheless, the observed effect of GDF9 may be mediated, not through a direct action on thecal cells, but indirectly through granulosa cells. GDF9 signals through a complex of type I (activin-like receptor kinase-5; ALK5) and type II (BMP receptor type II; BMPR-II) membrane serine/threonine kinase receptors (Fig. 2) [30], resulting to phosphorylate and activate of the Sma- and Mad-related protein (Smad)-2 and Smad3 [30–32]. In rodents, ALK5 mRNA/protein and Smad2/3 proteins are expressed in the oocyte, granulosa, and thecal cells [33], whereas BMPR-II mRNA expression is observed only in granulosa cells, but not in thecal cells [34]. These results suggest that thecal cells are not capable of responding to GDF9 and that GDF9 indirectly modulates thecal cell function through a granulosal factor(s).

Recombinant GDF9 has been shown to up-regulate IGF-I in cultured granulosa cells [35, 36]. Reportedly, GDF9 stimulates KL expression in rat neonatal ovaries [37], whereas GDF9 suppressed KL expression in mouse granulosa cells [38], suggesting that GDF9 may have species-specific effects on KL expression. Nevertheless, these results raise the possibility that oocyte-derived GDF9 regulates thecal cell differentiation through these granulosal factors; *i.e.* IGF-I and KL (Fig. 2).

## GDF9 Promotes Preantral Follicle Growth by Up-regulating Thecal Androgen Production

Oocyte-somatic cell interaction plays a crucial role in preantral folliculogenesis [1–3]. Deletion of GDF9 in the oocyte results in decreased granulosa cell proliferation and failure of follicles to develop past the primary stage [39], demonstrating the importance of this growth factor in early follicular development. GDF9 stimulates rat granulosa cell proliferation and preantral follicle growth *in vitro* [40]. GDF9 promotes preantral follicle survival by suppressing granulosa cell apoptosis and follicular atresia [41]. GDF9 is also required to maintain FSH receptor expression in the granulosa cells [41].

We have recently shown that the oocyte-derived GDF9 enhances rat preantral follicle growth, and augments androgen production and CYP17 expression in the preantral follicles, whereas down-regulation of GDF9 suppressed these responses [29]. The specific androgen receptor (AR) antagonist flutamide suppressed GDF9-induced preantral follicle growth *in vitro* [29]. The non-aromatizable androgen DHT, but not estradiol, rescued the follicular growth arrest by GDF9 down-regulation [29]. These results suggest that GDF9 promotes rat preantral follicle growth by up-regulating thecal androgen production (Fig. 2).

Theca-produced androgens act via AR localized to granulosa cells, stromal cells, and oocytes [42]. Although androgens have long been implicated as inhibitors of antral follicle development [43, 44], recent evidence suggests that the effect of androgens on follicular growth is dependent on the stage of follicular development and that androgens also have a growth promoting role in early folliculogenesis [5]. Global AR knockout (ARKO) female mice are subfertile, have defective folliculogenesis, and ultimately develop premature ovarian failure [45, 46], indicating that normal folliculogenesis requires AR-mediated androgen action. When AR signaling is blocked or eliminated in granulosa cells, preantral follicles do not progress to antral follicles, but are subjected to increased rate of atresia instead [47]. *In vitro* culture of granulosa cells or follicles isolated from various species also demonstrates the AR-mediated stimulatory effects of androgens on granulosa cell proliferation and follicular development [10, 11, 14, 48–52]. Furthermore, androgens enhance FSH action in the follicles by increasing the expression of FSH receptor [11, 42, 48,

51], IGF-I and IGF-1 receptor [49, 53].

Although we have focused our discussion on factors involved in normal thecal formation and function, excess thecal androgen production may contribute to ovarian dysfunction, including ovulatory defects and/or polycystic ovarian syndrome [54]. Reportedly, treatment with high doses of androgens induces granulosa cell apoptosis and follicular atresia in rat antral follicles [43, 44].

These results suggest that a threshold level of androgens derived from thecal cells is necessary for preantral follicle growth, and that the androgen production may be controlled by the oocyte-derived GDF9 (Fig. 2).

## Conclusion

Oocyte-somatic cell interaction plays a crucial role in preantral folliculogenesis. Although topical research has focused on oocyte-granulosa cell interaction over the past decade, an oocyte-thecal cell regulatory loop may also play an important role during the preantral stage. Formation of the thecal cell layer is a key event that occurs during preantral folliculogenesis. Granulosal factors (*e.g.* IGF-I and KL) appear to stimulate the recruitment of thecal cells from stromal cells. Oocyte-derived GDF9 appears to indirectly modulate thecal cell differentiation, perhaps through regulating the granulosal IGF-I and KL expression. Theca-produced androgens stimulate granulosa cell proliferation and preantral follicle growth. GDF9 enhances preantral follicle growth by up-regulating thecal androgen production, suggesting that a threshold level of androgens derived from thecal cells is necessary for preantral follicle growth, and that the androgen production may be controlled by the oocyte-derived GDF9. The challenge ahead is not to only understand the precise nature of these interactions, and to elucidate how dysregulation in these interactions may lead to ovarian pathologies such as polycystic ovary syndrome and poor-responsiveness to gonadotropins. In addition, identification of the factor(s) that promote preantral follicle growth might provide important information for the identification of intra-follicular biomarkers for the selection of healthy oocytes and embryos in assisted reproduction.

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