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REVIEW

Molecular crosstalk between insulin-like growth factors and follicle-stimulating hormone in the regulation of granulosa cell function

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Abstract

Background: The last phase of folliculogenesis is driven by follicle-stimulating hormone (FSH) and locally produced insulin-like growth factors (IGFs), both essential for forming preovulatory follicles.

Methods: This review discusses the molecular crosstalk of the FSH and IGF signaling pathways in regulating follicular granulosa cells (GCs) during the antral-to-preovulatory phase.

Main findings: IGFs were considered co-gonadotropins since they amplify FSH actions in GCs. However, this view is not compatible with data showing that FSH requires IGFs to stimulate GCs, that FSH renders GCs sensitive to IGFs, and that FSH signaling interacts with factors downstream of AKT to stimulate GCs. New evidence suggests that FSH and IGF signaling pathways intersect at several levels to regulate gene expression and GC function.

Conclusion: FSH and locally produced IGFs form a positive feedback loop essential for preovulatory follicle formation in all species. Understanding the mechanisms by which FSH and IGFs interact to control GC function will help design new interventions to optimize follicle maturation, perfect treatment of ovulatory defects, improve in vitro fertilization, and develop new contraceptive approaches.

KEYWORDS
FSH, granulosa cells, insulin-like growth factors, ovary, steroidogenesis

1 | INTRODUCTION

Over the last 4 decades, groundbreaking research has systematically enhanced our understanding of the intricate molecular interplay between insulin-like growth factors (IGFs) and gonadotropin signaling involved in the regulation of ovarian function and female fertility. Meticulous in vitro studies involving granulosa cells (GCs) from humans, rodents, nonhuman primates, and farm animals, coupled with innovative genetic approaches, have unveiled IGFs as pivotal intraovarian regulators. IGFs wield unparalleled influence over critical facets of follicle dynamics, including growth, selection, atresia, cellular differentiation, steroidogenesis, oocyte maturation, and cumulus expansion. Furthermore, the discerned actions of IGFs have illuminated a profound interdependency with gonadotropins, underscoring their status as indispensable paracrine regulatory factors beyond their initially described role as "co-gonadotropins." The

implications of these findings extend far beyond the realm of basic research, holding the promise of transformative insights into fertility treatments and reproductive health interventions. Recent outstanding reviews have covered the roles of IGFs in ovarian development and their effects on reproductive tissues in general.[1,2] This review provides a comprehensive account of the effects of IGFs and the molecular interaction of IGF and follicle-stimulating hormone (FSH) signaling in GCs.

2 | FOLLICULOGENESIS OVERVIEW

The ovary fosters the development and maturation of the female gamete, the oocyte. The ovarian functional unit is the follicle, which consists of an oocyte surrounded by one or several layers of GCs enclosed by a basal lamina. The development of the ovarian follicle to the point at which a mature oocyte(s) is ovulated is termed folliculogenesis, a process that in humans lasts up to 1 year.[3] It starts with primordial follicle activation, which involves the transformation of squamous GCs into cuboidal cells to form primary follicles. In primary follicles, GCs start to proliferate slowly, forming several layers of cells to form secondary follicles. Secondary follicles acquire a layer of highly steroidogenic cells around the basal lamina known as the theca layer. Blood vessels develop within the theca layer, allowing systemic hormones to regulate follicle growth.

Further follicle development is driven by FSH, which is essential for the formation of antral follicles and, ultimately, their maturation to the preovulatory stage (Figure 1). During the last stages of maturation, GCs differentiate into mural cells, which produce high levels of estradiol, progesterone, and inhibins. These hormones coordinate oocyte maturation and ovulation with uterine receptivity. As we will describe in this review, the growth beyond the early antral stage and hormone production of follicles depends on the actions of FSH and IGFs. This review will focus on the molecular crosstalk of the FSH and IGF signaling pathways in the regulation of follicle maturation to the preovulatory stage.

2.1 | Overview of FSH signaling

FSH is produced by the gonadotrophs of the anterior pituitary and travels in blood to the gonads, where it acts exclusively on the GCs via binding to its receptor, the FSH receptor (FSHR). FSHR expression, and therefore FSH actions, are limited to GCs.[4] The FSHR is a G protein-coupled receptor linked to the activation of stimulatory Gα. Gα activation stimulates adenylyl cyclase that catalyzes the conversion of ATP to cAMP. Thus, FSH activation of GCs increases intracellular cAMP, which in turn activates protein kinase A (PKA). One well-known target of PKA is the cAMP response element-binding protein (CREB), a transcription factor. CREB binds to cAMP response element sequences, boosting transcription of FSH-regulated genes.[5,6] In addition to CREB, several transcription factors, including LRH1 and GATA4, participate in the regulation of GC steroidogenesis.[7,8] By inhibiting dual-specific phosphatases, PKA mediates the activation of ERK,[9] a regulatory component of GC survival. PKA also promotes p38 MAPK and JNK signaling.[10] A well-known intracellular target of the FSHR is AKT (acute transforming retrovirus thymoma protein kinase).[11] Downstream of AKT signaling are FOXO and HIF1α factors that are involved in GC proliferation.[12–14] This review will focus on the mechanisms involved in the regulation of AKT by FSH and IGFs (Figure 2). For a complete description of all signaling pathways and intracellular effectors of FSH in the GCs, see the following references.[15–17]

2.2 | Ovarian steroidogenesis

From the early antral to the preovulatory stage, the mural GCs and adjacent theca cells produce increasing levels of progestins, androgens, and estrogens. Mural GCs and theca cells cooperate to produce estradiol, which is critical for GC proliferation, follicle development, and the regulation of gonadotropin secretion.[18] Theca and GCs express the enzymes necessary to convert cholesterol into progesterone,[19] including steroid acute regulatory protein (StAR), which transports cholesterol into the mitochondria where cholesterol side-chain cleavage enzyme (CYP11A1) catalyzes the first step in the steroidogenic pathway. Subsequent reactions by 3β-Hydroxysteroid dehydrogenase/$\Delta^{5\text{-}4}$ isomerase (3βHSD) leads to the production of progesterone. GCs are unable to convert progestins into androgens. However, theca cells specifically express cytochrome P450 Family 17 Subfamily A Member 1 (CYP17A1), which converts progesterone into androgen. Androgens then diffuse to the granulosa layer where aromatase (CYP19A1), which is exclusively expressed in GCs, converts androgens into estradiol[20] (Figure 3).

3 | INSULIN-LIKE GROWTH FACTORS

IGF1 and IGF2 are polypeptides with structural similarity to insulin comprised of α and β chains linked by disulfide bonds. IGF1, initially known as somatomedin C, is produced mainly in the liver under the control of growth hormone (GH) and secreted into the systemic circulation.[21] However, IGF1 is also expressed locally in most tissues, including the ovary, exerting paracrine and autocrine actions.[22] IGF2 is considered the major growth factor during fetal development, but it is also expressed in various adult tissues, including the liver, although its actions in adult target tissues are largely unknown.[23]

3.1 | IGF receptors and signaling

The type 1 IGF receptor (IGF1R) mediates the stimulatory effects of IGF1 and IGF2. This receptor belongs to the family of receptor tyrosine kinases, and its activation triggers autophosphorylation of specific tyrosine residues in the receptor β-subunits, thus promoting their tyrosine kinase activity (Figure 4). Tyrosine phosphorylation

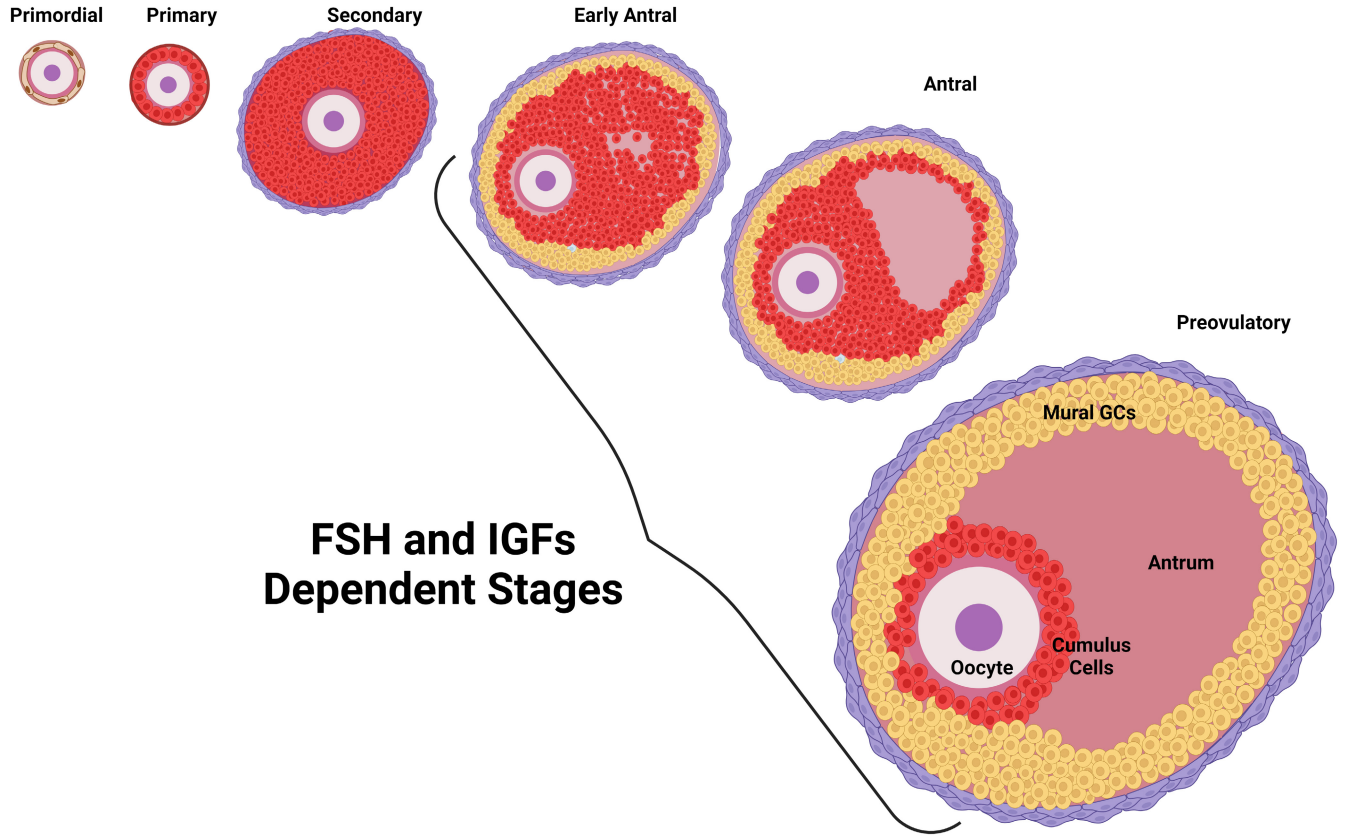

FIGURE 1 Follicle development. The oocyte is initially surrounded by a flattened layer of GCs in the primordial follicle. As the follicle progresses to the primary stage, the GCs change morphology to become a single layer of cuboidal cells. These GCs proliferate to form multiple layers in the secondary/preantral follicle. A fluid-filled cavity then begins to form within the follicle, known as the antrum. In mono-ovulatory species, a single follicle becomes dominant and progresses to the preovulatory stage. As the antrum grows, GCs differentiate into the mural GCs lining the wall of the follicle and cumulus GCs immediately surrounding the oocyte.

facilitates the binding of adaptors containing phosphotyrosine-binding domains, such as insulin receptor substrate (IRS)1 and IRS2, which in turn are tyrosine phosphorylated (pY) by the receptor.[24]

Class 1A phosphoinositide 3-kinases (PI3Ks) are recruited to the membrane via the interaction of their p85 regulator subunit with pY-IRSs[25] bringing the PI3K p110 catalytic subunit to the membrane where it converts phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3). PIP3 serves as a docking site for signaling proteins that include phosphoinositide-dependent kinase 1 (PDK1). Phosphatase and tensin homolog 10 (PTEN) is a negative regulator of PI3K that converts PIP3 back to PIP2. AKT also binds to PIP3 causing the translocation of AKT to the plasma membrane and its colocalization with PDK1. Therefore, PIP3 brings PDK1 and AKT together allowing PDK1 to phosphorylate AKT at T308.[26] AKT becomes fully active after phosphorylation on S473 by the mammalian target of rapamycin complex 2 (mTORC2).[27] Although IGF1R activation has been shown to stimulate mitogen-activated protein kinases (MAPK),[28] IGFs do not seem to be a significant stimulus for MAPK activation in rat and human GCs.[29–31]

The type 2 IGF receptor (IGF2R) is a single-chain polypeptide with large extracellular domains with high affinity for IGF2.[32] It is largely accepted that the IGF2R does not propagate intracellular signaling within cells. The primary role of the IGF2R is to mediate the internalization of IGF2 upon binding to the receptor. Additionally, the IGF2R can be released from the cell membrane,[33] which suggests it could block IGF2 actions in the intercellular space.

In addition to the expression of IGFs and their receptors, the bioavailability of IGFs dramatically changes during follicle growth. The bioavailability of IGFs is regulated by IGF-binding proteins (IGFBPs) that bind IGF1 and IGF2 with high affinity and can both inhibit (mostly) and potentiate their actions. Since the primary focus of this review is the intracellular molecular crosstalk between FSH and IGFs, the regulation by IGFBPs will not be described. Recent excellent reviews on the role of IGFBPs in the ovary can be found in the references cited here.[34,35]

3.2 | Role of IGFs in GC function across species

3.2.1 | Rats

Expression

The mRNA of IGF1 but not IGF2 was first detected in rat ovaries by Northern blot in 1987.[36] Later, in situ hybridization studies identified

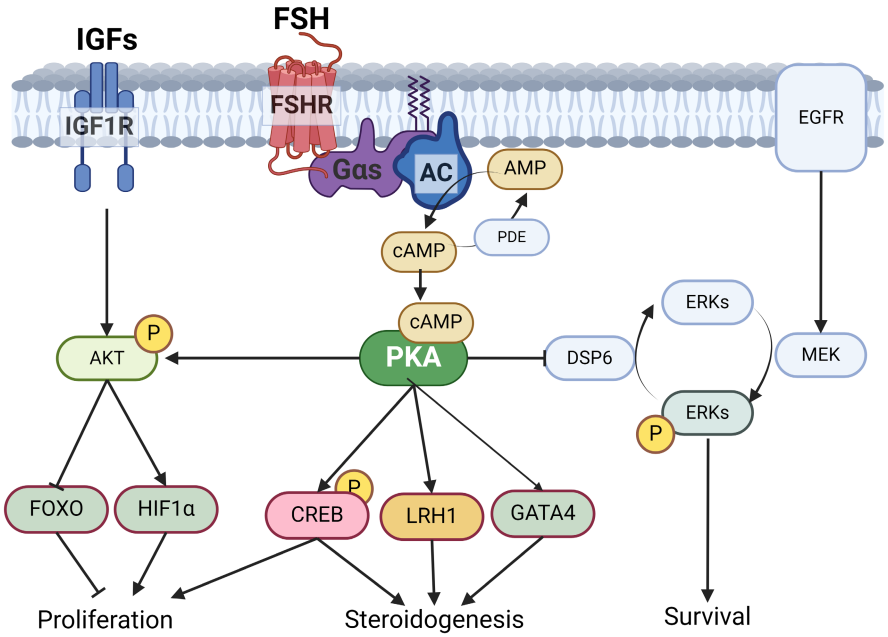

FIGURE 2 Simplified scheme of major FSH intracellular signaling. FSH binds and activates the FSHR, which stimulates adenylyl cyclase (AC) activity and increases intracellular cAMP. cAMP activates PKA, ERK, and AKT, which regulate the expression of FSH-targeted genes.

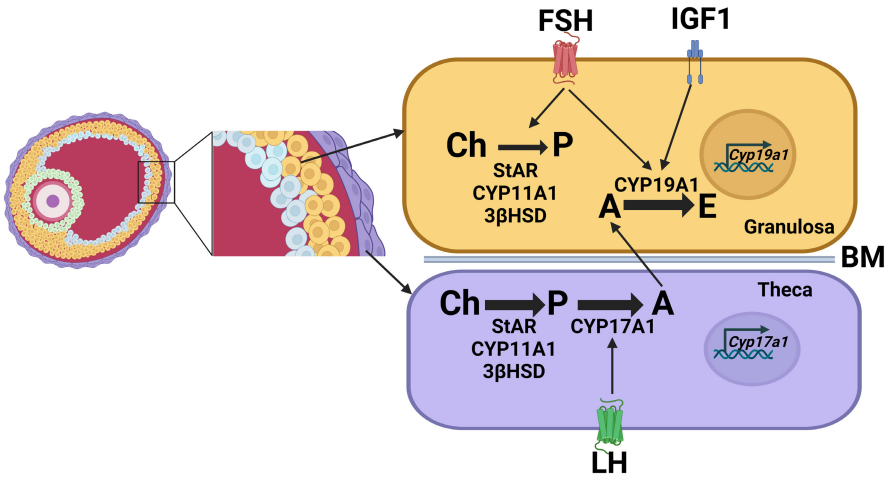

FIGURE 3 Granulosa and theca cell steroidogenesis. A, androgens; BM, basal membrane; Ch, cholesterol; E, estrogens; P, progesterone.

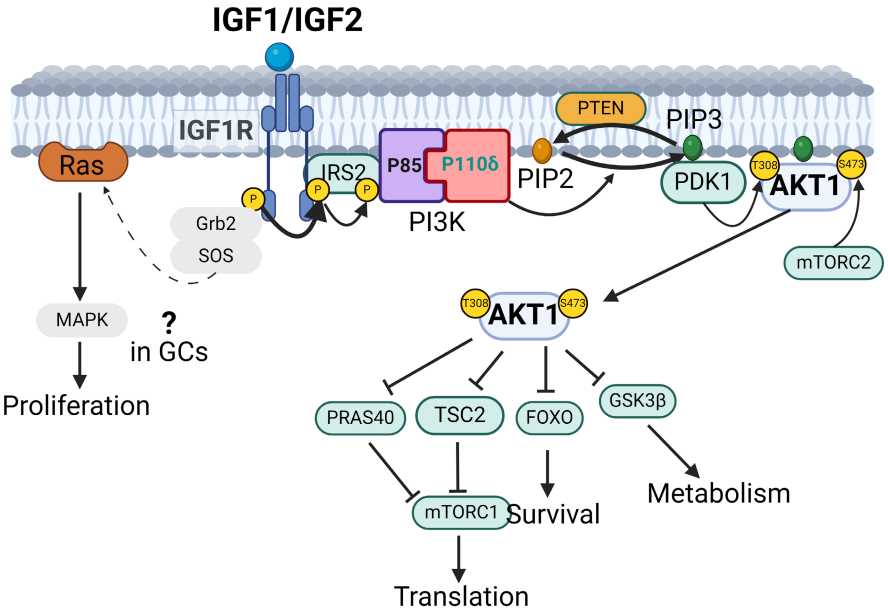

FIGURE 4 IGFs signal through the IGF1R in GCs. IGF1 and IGF2 are both ligands for the IGF1R. After activation by either ligand, tyrosine residues in the intracellular region of the IGF1R are phosphorylated. This leads to the recruitment of insulin receptor substrate (IRS) that acts as a scaffold for proteins necessary for activating MAPK and PI3K/AKT pathways.

the GCs of healthy developing follicles as the primary site of IGF1 synthesis.[37] In large preovulatory follicles, IGF1 mRNA is found primarily on GCs lining the antrum and the cumulus cells but not in the mural GCs.[38,39] Thus, there is a progressive decrease in IGF1 expression in the GCs from the early antral to the preovulatory stage that starts in the mural GCs. The factors involved in the regulation of IGF1 expression are not known but seem to be gonadotropin-independent since IGF1 mRNA levels remain unchanged after hypophysectomy with or without gonadotropin replacement.[38] Estradiol stimulates IGF1 in GCs but not in hepatocytes, indicating tissue-specific regulation.[40] In vitro, IGF1 (but not IGF1R) mRNA expression rapidly decreases once GCs are placed in culture; however, its expression is maintained in the presence of insulin and dexamethasone.[41] In the corpus luteum, IGF1R is expressed at higher concentrations in early pregnancy, while IGF1 increases close to parturition[42] due to the stimulatory effects of prolactin.[42,43] Thus, estradiol, insulin, glucocorticoids, and prolactin may regulate IGF1 expression in rat GCs.

Earlier studies showed that GCs bind IGFs[44] and that binding is enhanced by FSH.[45,46] Accordingly, hypophysectomy decreases, whereas FSH treatment increases IGF1R expression without changes in IGF1 levels.[38,47] IGF1 and IGF1R expression is insensitive to growth hormone (GH) control[38]; therefore, the ovarian IGF1 system is autonomous from circulating GH.

Paracrine roles of GC-derived IGF1

IGF1 is restricted to the GC compartment as no IGF1 expression has been detected in rat theca cells.[37,40] However, early studies also showed that IGF1 can enhance basal and LH-supported androgen biosynthesis in rat theca-interstitial cells.[48,49] Similarly, in vivo, IGF1 acts synergistically with LH to induce hyperandrogenism in rats.[50] IGF1 stimulates CYP11A1 and 3βHSD in theca cells.[51–53] In contrast, IGF1 stimulates the expression of CYP17A1 only in the presence of LH.[54] Thus, IGF1 acts on the steroidogenesis of theca cells by augmenting and mimicking LH actions. IGF1 is also a potent stimulator of theca cell proliferation, increasing the number and proportion of steroidogenically active theca cells.[55] However, although IGF1R is highly abundant in the granulosa and luteal cells, it is not detected in rat theca cells.[38,56] Therefore, how IGF1 regulates theca cell function in rats remains unknown. Despite this, the evidence raises the possibility that IGF1 from GCs acts in a paracrine manner in the adjacent theca cells, enhancing ovarian androgen production to guarantee the estrogen requirements of the developing follicle (Figure 5).

Interestingly, the IGF1R is present in oocytes.[38] Therefore, mutual actions between the oocyte and GCs can be speculated in which the oocyte itself induces IGF1 expression in GCs, whereas the IGF1 produced by GCs could support the metabolic activity of the oocyte. Similar relationships appear to be at play in humans; see below. On the other hand, the knockdown of the IGF1R in the oocyte of mice has no consequences on fertility[57]; therefore, the role of the IGF1R in oocyte physiology is unclear. Effects: Earlier in vitro reports demonstrated that low concentrations of IGF1 (~4.0 ng/mL) strongly synergize with FSH on the stimulation of progesterone,[58–60] estradiol,[60,61] and GC proliferation.[39,62] IGF1 also synergizes with FSH to stimulate

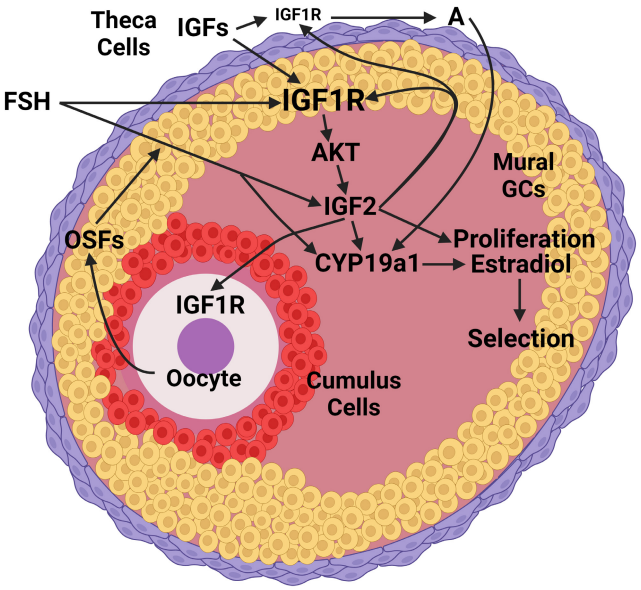

FIGURE 5 Intrafollicular autocrine and paracrine interactions between the oocyte, GCs, and theca cells mediated by the IGF system (see text for details).

luteinizing hormone (LH) receptor,[63] CYP19A1,[64] and cAMP production[60,65] as well as proteoglycan biosynthesis.[66] Eventually, it was shown that IGF1 enhances the FSH-induced up-regulation of StAR, CYP11A1, 3βHSD, and CYP19A1 expression.[31,67] However, IGF1 alone does not impact any of these genes. As an exception, IGF1 alone and synergistic with FSH stimulates inhibin[68,69] and cartilage link protein expression.[70] Interestingly, IGF1 increases LH receptor and FSHR mRNA stability but not gene transcription.[71,72] These experiments demonstrated that IGF1 has an important role in the process of GC differentiation from the preantral to the large antral follicle stage by potentiating FSH actions.

3.2.2 | Mice

Expression

In the ovary of mice, the patterns of IGF1 and IGF1R expression are similar to those described in the rat.[73] Interestingly, IGF1 mRNA is present exclusively in a subset of follicles, while all follicles express IGF1R.[73] Later, it was shown that IGF1 is detected only in FSHR-positive follicles. However, the specific pattern of IGF1 expression remains unaltered in the ovaries of FSH knockout (KO) mice.[74] Similarly, hypophysectomy in the presence or absence of FSH replacement does not affect IGF1 expression,[74] indicating that FSH also does not regulate IGF1 expression in mice.

Knockout mice

Most newborn IGF1 KO pups die after birth, though their survival rate is dependent on genetic background; about 10% of pups with the C57BL/6J background survive to adulthood, whereas 68% with the MF1 background survive.[75] Surviving IGF1 KO mice are infertile

and exhibit delayed bone development and growth retardation, reaching only 30% of the typical adult weight.[76] In contrast, mice lacking IGF2 are fertile with normal ovarian function.[77]

IGF1 KO female mice have hypoplastic gonads containing primordial, primary, and secondary antral follicles but only rare small antral follicles.[73] Moreover, IGF1 KO females fail to ovulate even after administration of gonadotropins, suggesting that ovarian dysfunction is the primary cause of infertility.[73] It was later shown that GCs of IGF1 KO mice have reduced glucose transporter 1 expression[78] and show impaired proliferation in the absence of apoptosis, although BAX expression is higher in these cells.[79] Thus, IGF1 KO mice demonstrated that IGF1 is required for antral follicle formation. Whether or not this effect of IGF1 is ovary-specific was evaluated using conditional knockdown mice.

The IGF1R gene is essential for normal embryonic development and pup survival after birth.[75] Notably, the neonatal lethality of IGF1R KO mice is invariable and independent of the genetic background. However, female mice heterozygous for IGF1R (IGF1R+/−) and mice with only a reduction in IGF1R expression are fertile.[80,81] Similarly, most IGF2R KO mice die perinatally and exhibit overgrowth due to high levels of IGF2 in the circulation. The few IGF2R KO females that survive are infertile due to an imperforate vagina.[82] Strikingly, female mice lacking IGF1R and IGF2R are fertile but have small litter sizes.[82] The excess of IGF2 in the absence of IGF2R and IGF1R may partially rescue ovarian function by activating the insulin receptor (INSR) or another unidentified receptor, as proposed by Ludwig et al.[82]

To explore the ovarian roles of IGFs without causing severe systemic defects, Baumgarten et al.[56] produced mice that lack IGF1R, specifically in the GCs ($IGF1R^{GCKO}$). $IGF1R^{GCKO}$ female mice are viable and grow normally but are infertile; their ovaries lack antral follicles and do not ovulate even after treatment with exogenous gonadotropins.[56] GCs from $IGF1R^{GCKO}$ mice are prone to enter apoptosis and have a significant reduction in AKT activation and steroidogenic gene expression but normal levels of FSHR.[56] Ablation of INSR and/or IGF1R in the oocyte has no impact on fertility.[57] However, the knockdown of the INSR and IGF1R in GCs of preovulatory follicles leads to infertility due to a lack of luteal support.[83] Thus, only GCs require the IGF1R to undergo cell differentiation upon FSH stimulation in vivo.

What is the role of GH and liver-derived IGF1 in ovarian function?

GH receptor (GHR) KO mice that lack endocrine IGF1 are subfertile,[84,85] and their ovaries contain atretic follicles and few preovulatory follicles.[84] IGF1 treatment of GHR KO mice increases the number of healthy antral follicles and decreases the percentage of atretic follicles but does not rescue fertility or the responsiveness to gonadotropins.[84,86] Similarly, IGF1 KO mice expressing IGF1 only in the liver can conceive and produce pups, although with difficulty, suggesting subfertility.[87] These findings suggest that GH regulates follicular growth independently of IGF1 and that in the absence of IGF1 production in the ovary, circulating (liver or exogenous) IGF1 can only partially sustain ovarian function.

3.2.3 | Humans/primates

Expression

In humans, IGF1R is expressed in GCs of antral follicles and colocalizes with phosphorylated AKT and the FSHR.[88] IGF2 is highly expressed in these cells, but IGF1 is not detectable.[30,89–92] Consequently, the follicular fluid of dominant follicles contains 3- to 10-fold more IGF2 than IGF1.[93–96] There is also a positive association between intrafollicular IGF2, follicular function,[94,96] and early embryo development.[97] As GH administration to pre-menopausal women does not affect ovarian IGF2 expression,[98] and serum levels of IGF1 and IGF2 do not vary across the menstrual cycle, it is unlikely that systemic IGFs play any role in the regulation of human GCs.[96] High IGF2 production by human GCs explains the normal follicular development and fertility of Laron dwarfism patients with extremely low blood levels of IGF1.[99,100] Similarly, the high levels of IGF2 in primate follicles explain why exogenous administration of IGF1 does not affect follicle maturation in monkeys.[101] The preferential expression of IGF2 by dominant follicles suggests that IGF2 plays a critical role in follicle selection and maturation.

Effects

Although IGF1 is not expressed in human GCs, IGF2 and IGF1 stimulate steroid production by upregulating StAR, CYP11A1, and CYP19A1 expression in luteinized human GCs.[30,102–111] In preantral undifferentiated GCs, IGF2 also stimulates sex steroid synthesis,[112–114] increases survival,[115] and suppresses IGFBP1.[116] IGFs also act synergistically with FSH to stimulate steroid synthesis and proliferation in GCs from normal[30,107] and PCOS[117] follicles. Therefore, unlike rodents, IGF1 or IGF2 can stimulate aromatase expression in the absence of FSH in human GCs.[30]

In human GCs, FSH and cAMP stimulate IGF2 expression[30,112,118,119] by activating promoter 3 of the IGF2 gene,[30] although the factors involved remain to be determined. Oocyte-secreted factors (OSFs), such as growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15), potentiate the stimulatory effect of FSH on IGF2 but have no effect by themselves.[120,121] These findings suggest an intricate interplay between OSFs, IGF2, and FSH is established in human follicles during GC differentiation. This interplay involves FSH stimulation of IGF2 that is strongly enhanced by OSFs. In turn, IGF2 autocrine actions activate the IGF1R in the GCs, leading to the activation of AKT, which is essential to promote the expression of IGF2 and CYP19A1 and sustaining proliferation. Thus, it is possible to postulate the formation of a positive loop interaction within the follicle, which is critical for follicle growth and possible selection of the dominant follicle (Figure 5).

Paracrine action of GC-produced IGF2 in humans: In human primordial follicles, the IGF1R is restricted to the oocyte; however, in preantral follicles, the IGF1R is present in GCs and theca cells.[122] As follicles grow, IGF1R in granulosa and theca cells increases.[122] In small antral follicles, IGF1 and IGF2 mRNAs can also be found in theca cells. Although IGF1R is detectable only in GCs, IGF2R is expressed in granulosa and thecal cells.[90] In dominant follicles, IGF2,

IGF1R, and IGF2R are only found in GCs.[90] However, some reports also found that human GCs and theca cells express the IGF1R.[88,122] Moreover, recent single-cell RNA sequencing data showed that granulosa and theca cells express the IGF1R.[123] Thus, the relative distribution of IGFs and their receptor suggests that autocrine and paracrine IGF actions between GCs, the oocyte, and thecal cells are tightly coordinated during follicle maturation (Figure 5).

3.2.4 | Bovine/swine/ovine

IGF2 and IGF1 have been detected in bovine GCs.[124,125] IGF1 protects GCs from apoptosis[126] and increases GC proliferation[127,128] via the IGF1R. In bovine GCs from small follicles, IGF1 acts synergistically with FSH to increase GC proliferation and CYP19A1 expression via the PI3K and AKT pathway.[129]

IGF1 is produced by swine GCs[130] probably under the control of gonadotropins.[131] Accordingly, in the swine ovary, IGF1 and FSHR show selective follicular coexpression.[132] GCs also express IGF1R[133] and respond to IGFs with an increase in proliferation and steroid secretion.[133–135] IGFs synergize with FSH to increase steroid production, steroidogenic gene expression, and lipoprotein uptake[134–142] and promote FSH-stimulated synthesis of hyaluronic acid and hyaluronan synthase 2.[143]

In the ovine ovary, IGF1 is present in the GCs from secondary and antral follicles,[144] and its expression seems to be stimulated by GH or FSH.[145] IGF1 may also participate in cumulus expansion in response to FSH in vitro.[146] In addition, IGF1 and FSH synergistically increase oocyte growth and LH receptor expression in sheep follicle cultures.[144]

4 | IGF1R SIGNALING AND GC FUNCTION

This section outlines the effects that deletion or mutation of components of the IGF1R signaling pathway have on the regulation of GC function and female fertility.

4.1 | IRS2

In rodents, targeted deletion of IRS1 does not affect fertility.[147] In marked contrast, deletion of IRS2 leads to female infertility and disturbances in glucose metabolism.[148,149] IRS2 KO female mice show reduced pituitary gonadotrophs and lower gonadotropin levels at proestrus; however, ovulation cannot be rescued by exogenous gonadotropins,[148,150] suggesting ovarian dysfunction. Thus, the reproductive defects observed in IRS2 KO mice confirm that IRS1 is insufficient to preserve ovarian function.[150] In *Drosophila melanogaster*, the deletion of chico, a homolog of IRS2, causes infertility.[151,152] Finally, ovarian IRS2 levels in humans are higher than those found in the liver or pancreas.[153–155] Thus, IRS2 seems crucial in regulating ovarian function, ranging from Drosophila to humans.

In preovulatory luteinizing GCs from FSH-treated animals, FSH, cAMP, and IGF1 stimulate IRS2 expression[156,157] and phosphorylation.[158] Conversely, FSH stimulation of AKT, StAR/CYP11A1, and glucose uptake decreases in IRS2-deficient GCs.[156,157] Strikingly, FSH weakly stimulates IRS2 expression in GCs from women with polycystic ovary syndrome (PCOS).[156] In mouse GCs, IRS2 expression closely relates to AKT activation. Thus, in vivo, treatment with an FSH analog increases IRS2 and AKT phosphorylation effects paralleled by a decrease in miR-431.[159] MiR-431 was previously identified as a regulator of IRS2 and IGF1R.[160] Conversely, miR-431 overexpression decreases IRS2 expression and attenuates AKT activation.[159] In light of this evidence, FSH may control IGF1R signaling by regulating IRS2 activity via phosphorylation or miR-431 expression.

4.2 | SHP2

The role of phosphatases in the regulation of GCs remains understudied. Of particular interest are SHP1 and SHP2 (protein tyrosine phosphatase non-receptor type 6 and type 11, respectively) since they can dephosphorylate IRSs.[161,162] SHP1 KO mice are markedly insulin-sensitive because of enhanced IRS-PI3K-AKT signaling.[163] SHP1 and SHP2 are expressed in the ovary,[164] although SHP1 is present only in atretic follicles.[164] Mice lacking SHP2 in the GCs appear to have increased follicle growth and ovulation.[165] Thus, inhibition of SHP activity by FSH may enhance IGF1R signaling.

4.3 | PI3K

The class IA PI3K enzymes are heterodimeric lipid kinases made up of a regulatory subunit, p85, and a catalytic subunit, of which three isoforms are known: p110α, p110β, and p110δ.[166] Of the catalytic subunits, p110δ (encoded by the Pik3cd gene) is highly expressed in the mouse ovary and plays a crucial role in the regulation of ovarian function.[167] Pik3cd KO females are subfertile, exhibit fewer growing follicles, have more atretic follicles, and respond poorly to exogenous gonadotropins.[167] Interestingly, primordial follicle activation and oocyte meiotic maturation are unaffected in Pik3cd KO mice. The findings indicate that the p110δ catalytic subunit is a critical component of the PI3K pathway and is needed for FSH stimulation of follicle growth.

4.4 | PTEN

PTEN dephosphorylates PIP3 and inhibits PI3K signaling. Human, rat, and bovine GCs of large preovulatory follicles express higher levels of PTEN than GCs of small follicles, suggesting that PTEN expression increases during terminal follicular growth.[168–170] In human GCs, PTEN knockdown increases AKT phosphorylation and cell proliferation in response to IGF1.[171] Moreover, in

human ovaries, there is an inverse relationship between PTEN and phospho-AKT, confirming that PTEN antagonizes the activity of PI3K on AKT.[168] This was confirmed in mice lacking PTEN in GCs, which show increased ovulation, GC proliferation, and antral follicle formation.[172] Thus, the evidence indicates that PTEN controls AKT activation in GCs and promotes conditions favoring atresia/apoptosis of growing follicles. It is possible to foresee that an inhibitory effect of FSH on PTEN activity could enhance IGF1R signaling.

4.5 | AKT

AKT activity is essential for the induction of GC differentiation by FSH. Thus, FSH actions are abolished by overexpression of dominant-negative AKT[173] or treatment with AKT inhibitors.[31] It is also well-known that FSH induces AKT phosphorylation in GCs via cAMP.[174–176] However, the mechanisms mediating the effects of cAMP on AKT remain controversial[177] since some reports showed that inhibition of PKA prevented AKT phosphorylation,[175] but others showed that PKA inhibition augments AKT phosphorylation by FSH.[11,70,176,178] As discussed below, FSH indirectly stimulates AKT activity by regulating the IGF1R signaling pathway.

Of the three isoforms, AKT1 and AKT2 are the major isoforms found in the ovary and GCs of rats and humans.[168,179] Genetic studies showed that AKT1KO mice are subfertile and display abnormal cyclicity, with ovaries containing fewer growing and preovulatory follicles refractory to exogenous gonadotropins.[180] At the molecular level, AKT1KO ovaries have decreased expression of the cell cycle regulators Ccnd3 and Ccnd1.[180] In contrast, fertility is not significantly affected in female mice lacking AKT2.[181] Thus, AKT1 is crucial for normal ovarian function and for the response of GCs to gonadotropins.

Recently, proteomic and genomic analyses of AKT1-deficient GCs revealed defects in metabolism, apoptosis, cell cycle, and cytoskeleton dynamics. The report shows a decrease in the phosphorylation of proteins involved in intracellular transport and mitochondrial physiology, such as β-catenin, RTN4, EEF1D, and CALM1, and an increase in phosphorylation of GOT2, EIF2S2, SRRM2, and PTMA, suggesting possible direct and indirect effects of AKT1 silencing on protein phosphorylation.[182] How these proteins regulate GC and ovarian function requires further investigation.

5 | INTERDEPENDENT EFFECTS OF FSH AND IGFs IN GCs

As described thus far, IGFs have been considered to act as co-gonadotropins functioning in an autocrine or paracrine manner to amplify FSH actions in the GCs. However, this view is not compatible with data showing that (i) FSH requires the presence of IGFs and IGF1R to stimulate GCs, (ii) FSH renders GCs sensitive to IGFs by supporting/controlling the IGF1R signaling pathway, and (iii) FSH signaling interacts with factors downstream of AKT to maximally stimulate GC differentiation.

(i) If IGFs only potentiate FSH actions, it is expected that blocking the availability of IGFs or IGF1R activity should partially but not completely block FSH stimulation of GC differentiation markers. In contrast, growing evidence shows that in the absence of IGFs, FSH is unable to stimulate GC differentiation. For instance, it was shown early on that blocking IGF availability with IGFBPs or anti-IGF1 antibodies dose-dependently and completely inhibits steroid and inhibin α production in rat[31,69,183,184] and human[112,113] GCs. IGFBPs also inhibit cAMP-stimulated steroidogenesis, suggesting that the effects of blocking IGF1R activation are independent of FSHR activation. Moreover, the addition of IGF1 reverses the inhibitory effect of IGFBPs.[69,183] The crucial role of IGF1 on GC differentiation is further revealed by comparing the ovarian phenotypes observed in FSHKO, FSHRKO, and IGF1KO mice. FSHKO and FSHRKO mice are infertile due to a block in folliculogenesis at the preantral stage,[185,186] which is the same phenotype observed in IGF1KO and IGF1R^{GCKO} mice,[56,73,74] demonstrating that in vivo IGF1 is also essential for the stimulation of antral follicle growth by FSH.

Similarly, a crucial role of IGF1R activation was demonstrated in experiments showing that FSH actions in GCs are inhibited by general tyrosine kinase inhibitors,[69,187] IGF1R-specific inhibitors, or IGF1R knockdown.[30,31] Inhibition of IGF1R activity also completely blocks FSH-induced phosphorylation of AKT in rodent and human GCs.[30,31] In vivo, FSH also fails to stimulate AKT in the absence of IGF actions, as shown in IGF1R^{GCKO} mice.[56] Genomic-wide analysis of human GCs treated with an IGF1R inhibitor revealed the full spectrum of genes regulated by IGFs and those regulated by FSH that required IGF1R activity. This report demonstrated that about 50% of FSH-regulated genes require IGF1R activity and suggested that several aspects of GC function, including cholesterol synthesis, extracellular matrix production, reactive oxygen species metabolism, and cytoskeleton are coordinately regulated by FSH and IGFs in humans.[188]

(ii) In rat GCs, IGF1 stimulates proliferation only in FSH-primed cells, suggesting that pretreatment with FSH renders GCs responsive to IGF1.[189] One of the first pieces of evidence indicating that FSH enhances IGF1R signaling was their synergistic activation of AKT.[11,30,31,70] Thus, although IGF1 treatment of GCs maximally stimulated IGF1R tyrosine phosphorylation, it failed to stimulate AKT above FSH stimulatory levels.[30,31] Moreover, in GCs cultured in serum-free media without FSH, AKT has minimal background stimulation even though GCs produce IGF1.[31] Since FSH treatment of rat or human GCs does not affect the phosphorylation of the IGF1R,[30,31] these findings suggest that FSH amplifies IGF1R signaling or removes inhibitory checkpoints located between the IGF1R and AKT.

(iii) Finally, it has been extensively demonstrated that although treatment of rodent GCs with IGF1 strongly activates AKT,[11,31] it does not stimulate the expression of genes involved in GC differentiation in the absence of FSH.[31,187] Moreover, the overexpression of constitutively active AKT (caAKT) alone is not enough to mimic the effects of FSH.[173] Hence, despite the presence of active AKT, GC differentiation fails in the absence of FSH, indicating a collaborative relationship between FSH signaling and AKT downstream targets in orchestrating GC differentiation. In agreement with this idea, the expression of caAKT is enough to amplify gene expression responses to FSH[30,31,173] and is further supported by findings demonstrating that in cells lacking IGF1R activity, FSH-induced differentiation can be fully rescued by caAKT.[30]

6 | PLAUSIBLE MOLECULAR CROSSTALK BETWEEN FSH AND IGF INTRACELLULAR SIGNALING PATHWAYS

Evidence suggests that FSH and IGF1 signaling pathways intersect upstream and downstream of AKT to regulate gene expression and GC differentiation. In this section, we will integrate the above-described findings and propose molecular mechanisms that may contribute to better understand the interaction between FSH and IGF signaling in GCs (Figure 6).

6.1 | IGF1R to AKT

6.1.1 | FSH inhibition of tyrosine phosphatases

Tyrosine phosphorylation of the IGF1R and IRS2 is essential for the formation of signaling complexes. Whether IGF1R and IRS2 interaction is affected by FSH remains to be determined. However, it has been shown that FSH increases IRS2 stability in GCs,[156] an effect that posttranslational modifications, including phosphorylation, could mediate. In fact, in the presence of FSH, higher levels of tyrosine-phosphorylated IRS2 have been detected in GCs.[156] The mechanisms involved and whether IGF1R is required for IRS2 phosphorylation by FSH are currently not known. Since the FSHR does not directly regulate tyrosine kinases, it is most likely that FSH stimulates tyrosine phosphorylation of IRS2 by inhibiting tyrosine phosphatases such as SHP2, which have been shown to bind to and dephosphorylate IGF1R[190–192] and IRSs.[161,162]

As described above, SHP2 negatively impacts GC function as SHP2 knockdown increases ovulation.[165] It is currently accepted that SHP1 and SHP2 are active under basal conditions, presumably by constitutive association with phosphoproteins, and that specific cell stimuli lead to their inactivation. SHP2 can be phosphorylated by Rous sarcoma oncogene kinase (SRC), protein kinase C (PKC), and PKA.[193–195] FSH targets each of these kinases[176]; therefore, it may also regulate the activity of SHP2. Thus, in the absence of FSH, SHP2 dephosphorylates IRS2 blocking IGF1 signaling (Figure 6). This hypothesis infers that SHP2 participates in the mechanisms controlling IRS2 interaction with the IGF1R or PI3K and that FSH controls these interactions, options that remain unexplored.

PTEN silencing in GCs increases proliferation, antral follicle formation, and fertility.[172] Therefore, following the idea that FSH removes factors inhibiting IGF1R signaling, it is also possible that inhibition of PTEN activity by FSH amplifies IRS2 signals by removing its adverse effects on the PI3K pathway. However, the role of FSH in PTEN activity remains to be explored.

6.1.2 | FSH inhibition of serine kinases

Although Tyr phosphorylation of IGF1R and IRS proteins is a key step in the activation of downstream signaling, IGF1R and IRS activity is

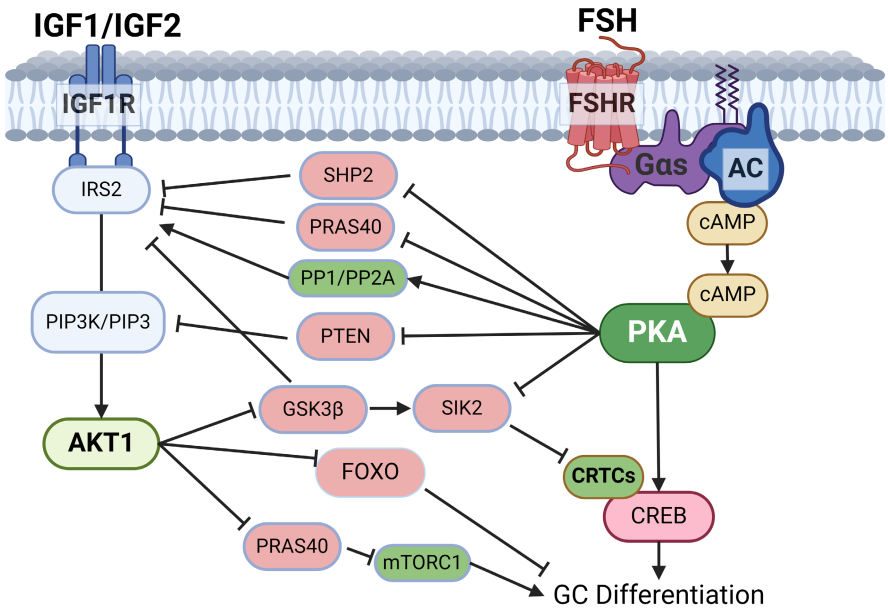

FIGURE 6 Possible interactions between FSH and IGF1R signaling in GCs.

also predetermined by serine/threonine phosphorylation levels. In contrast to the positive effect of tyrosine phosphorylation, serine/threonine phosphorylation of IGF1R and IRS has adverse effects on PI3K and AKT activation. Thus, an additional point of interaction between FSH and the IGF1R signaling pathway may be the phosphorylation of serine/threonine residues in IGF1R or IRS2 that prevent the activation of downstream signaling. For example, IRS phosphorylation on Ser residues by PKC, $p70^{S6K}$, JNK, or GSK3β impairs IGF1R signaling and PI3K-AKT activation.[196–204]

The inhibitory effect of serine/threonine phosphorylation on IRS2 may explain the synergistic effect that H89 has on FSH-induced AKT activation.[11] H89 is generally used as a PKA inhibitor; however, H89 also inhibits $p70^{S6K}$.[205] Therefore, H89 may prevent $p70^{S6K}$ serine/threonine phosphorylation of IRS2 causing an increase in IGF1R signaling and potentiating the effect of FSH on AKT. Serine/threonine phosphorylation of IRS2 may also explain the inhibitory effect of PKC on FSH-induced phosphorylation of AKT.[11]

Evidence supports the role of serine phosphorylation in the regulation of IRS2 in GCs. For instance, FSH treatment of luteinized rat GCs decreases serine-phosphorylated IRS2.[158] This effect could be mediated by serine/threonine phosphatases protein phosphatase 1 (PP1) and PP2A since okadaic acid and calyculin A, two inhibitors of PP1/PP2A, inhibit FSH-stimulated steroidogenesis.[206–209] Accordingly, it has been shown that PP2A activity is stimulated by FSH and PKA.[210,211] Thus, FSH may contribute to PP1 and PP2A activation, which in turn dephosphorylate inhibitory serine residues on IRS2 or IGF1R stimulating their activity (Figure 6).

Finally, GSK3β has been shown to phosphorylate IRS at serine residues, an effect that attenuates downstream signaling.[203,204] GSK3β is a serine/threonine kinase inhibited via phosphorylation at serine 9.[212] In rat and porcine GCs, GSK3β phosphorylation at serine 9 increases after FSH treatment via a PI3K-dependent mechanism.[213–215] It has been recently shown that a more robust phosphorylation at Ser9 occurs in GCs treated with both FSH and IGF1 and that inhibition of IGF1R activity prevents FSH phosphorylation of GSK3β.[215] These findings show that FSH and the IGF system interact to inactivate GSK3β. This idea is further supported by findings showing that the overexpression of constitutively active GSK3β blocks the synergistic stimulation of aromatase by FSH and IGF1, whereas inhibition of GSK3β activity potentiates the effects of FSH.[215] Therefore, it is possible to postulate that the strong inhibitory effects that FSH and IGF1 have on the activity of GSK3β may play an essential role in upregulating IRS2 activity (Figure 6).

6.2 | AKT to target genes

Although AKT is essential, its activation alone is insufficient to stimulate GC differentiation, demonstrating that AKT interacts with FSH signaling independently of the IGF1R. AKT and FSH signaling interaction points include regulating CREB cofactors, inhibiting GSK3β/FOXO1, and eliminating PRAS40 activity (Figure 6). However, the full array of AKT downstream targets that could interact with the FSH-activated signaling cascades warrants further investigation.

6.2.1 | Regulation of CREB cofactors by AKT

Upon FSH activation, PKA phosphorylates CREB, enhancing its association with cofactors such as CBP and p300[216,217] and CREB-regulated transcriptional coactivators (CRTCs),[218] which in turn modulate CREB activity. Of these cofactors, CRTCs appear to play a significant role in GCs. Indeed, CRTCs are highly expressed in GCs, and FSH stimulates CRTC2 nuclear localization,[219,220] contributing to activating gene expression.[221] On the other hand, CRTC activity is inhibited after phosphorylation by kinases such as salt-inducible kinases (SIKs), which induce nuclear exclusion of CRTCs.[222,223] Therefore, factors that inhibit SIK may stimulate CRTCs and CREB. SIK activity increases upon phosphorylation by GSK3β[224] or decreases after phosphorylation by PKA.[225] As mentioned above, FSH inhibits GSK3β and stimulates PKA. Thus, CRTC/CREB activity may be controlled by a decrease in SIK activity following GSK3β inhibition or PKA stimulation (Figure 6).

In agreement with the negative effect of SIK activity on GC function, it was recently reported that SIKs participate in the regulation of folliculogenesis, ovulation, and fertility in rodents and the control of GC differentiation in humans and rodents.[226] Strikingly, it was shown that each SIK isoform has different effects on the regulation of female fertility and GC function. For instance, the knockdown of SIK2 enhances FSH-induced GC differentiation and ovulation. In sharp contrast, the knockdown of SIK3 causes anovulation and female infertility.[226] Therefore, it is expected that if FSH regulates the activity of SIK kinases, this effect must be specific to SIK2. Further research is needed to determine this possibility.

6.2.2 | FOXO1 inhibition

FOXO1 is highly expressed in GCs of growing follicles,[179] and its inhibition is a well-known effect of AKT.[227] In rat GCs, FSH and IGF1 rapidly phosphorylate FOXO1 via activation of the PI3K/AKT signaling pathway, leading to its exclusion from the nucleus.[13,228,229] However, whether FSH and IGFs synergize to phosphorylate FOXO1 in GCs is not known. Mice lacking FOXO1 in the GCs are infertile, lack corpora lutea, and show follicles with abnormal appearances indicating that FOXO1 is essential for GC maturation and luteinization. At the molecular level, FOXO1 inhibition enhances the expression of the LH receptor, inhibin α, cyclin D2, epiregulin, LRH1, and several *steroidogenesis* genes.[228–230] This evidence suggests that inhibition of FOXO1 activity by AKT could be a crucial step needed to fully activate the differentiation program stimulated by FSH (Figure 6). Further research is needed to determine whether the detrimental effects of IGF1R or AKT inhibition could be rescued, at least partially, by the simultaneous knockdown of FOXO1.

6.2.3 | PRAS40

The proline-rich Akt substrate of 40 kDa (PRAS40, also known as AKT1S1) inhibits the mechanistic target of rapamycin (mTORC1). mTORC1 is a crucial regulator of protein synthesis known to be regulated by the IGF system via AKT.[231] In this pathway, AKT phosphorylates and inhibits PRAS40,[232,233] relieving mTORC1 inhibition. Inactivation of PRAS40 in *Drosophila melanogaster* increases egg laying and fertility.[234] Therefore, inhibition of PRAS40 by AKT could be a crucial step needed to stimulate protein synthesis by FSH and significantly enhance GC proliferation (Figure 6).

PRAS40 could also target mechanisms upstream of AKT. In *Drosophila*, inactivation of PRAS40 rescues the infertility of IRS2KO female flies (see above).[234] Moreover, in mice, the knockdown of PRAS40 causes a 4-fold increase in IRS levels in hepatocytes,[235] indicating that PRAS40 inhibits IRS expression and could be a negative regulator of the IGF1R signaling in GCs. The capacity of PKA to phosphorylate PRAS40[236] supports the idea that FSH may also participate directly in the control of PRAS40 activity. Thus, PRAS40 may reduce IRS2 levels, thus inhibiting both the FSH and IGF1R pathways. This is an attractive hypothesis, but further experiments are needed to establish whether this occurs in GCs (Figure 6).

7 | IGF1R AND FSHR CROSSTALK AND FEMALE INFERTILITY

Over 40% of female infertility cases stem from ovarian dysfunction due to a lack of oocyte maturation or due to anovulation.[237] Addressing this challenge involves weeks of hormone treatments, particularly the administration of exogenous FSH, aimed at fostering the maturation of multiple ovarian follicles to maximize the collection of mature oocytes for in vitro fertilization. Regrettably, up to 24% of women exhibit poor responses to FSH stimulation, yielding very few mature oocytes for collection.[238] This insufficiency in egg production accounts for over 80% of canceled IVF cycles.[239] The mechanisms underlying FSH-induced follicle maturation in the human ovary remain poorly understood, partly due to the absence of a robust model for studying human GC differentiation, further complicating these investigations.

Intraovarian IGF system inefficiencies correlate with decreased ovarian function in women. Thus, components of the IGF gene family are downregulated in GCs of women with diminished ovarian reserve.[240] Clinical data support close interactions between FSH and IGFs in follicle development by showing that IGF levels are low in the follicular fluid of poor-responding patients.[241] On the other hand, IGF concentrations in follicular fluid are higher in women who respond better to follicular stimulation.[242] In addition, serum IGF1 levels decline with age, which correlates with the well-known decrease in fertility due to aging.[243] These findings underscore the close interaction between FSH and IGFs in regulating human follicle development.

Defects in IGF1R signaling have also been implicated in the development of PCOS, which is characterized by follicle growth disorders, chronic anovulation, hyperandrogenemia, and polycystic ovaries. Thus, PCOS is associated with AKT and GSK3β polymorphisms downstream of IGF1R.[244] Defective expression of the IGF1R in GCs and increased IGF1R expression in theca cells are common features in PCOS ovaries.[245] This imbalance may be responsible, at least in part, for impaired insulin and IGF1 actions in GCs and enhanced actions in thecal stromal cells in PCOS ovaries leading to hyperandrogenism.[122] IGFR1 expression in preantral follicles, particularly in early preantral PCOS follicles, suggests a role for IGFs in regulating the initiation of human follicle growth. Thus, accelerated preantral follicle growth in PCOS may result from increased endogenous IGF activity.[245] Therefore, understanding IGF1R signaling pathways may help elucidate GC and theca cell defects driving follicle growth changes in PCOS.

8 | CONCLUSIONS

We propose that ovarian follicles exposed to increasing levels of FSH benefit from locally produced IGFs, and only follicles with high endogenous IGF production can fully respond to FSH. Thus, a positive feedback loop between the endocrine effects of FSH and the autocrine and paracrine actions of IGFs plays an essential role in the establishment of follicle dominance and follicle survival in all species. Understanding the complexity of the molecular and signaling pathways coordinating the actions of FSH and IGFs in the GCs could reveal novel therapeutic targets to optimize follicle growth in subfertile patients. These targets can also be used to develop novel contraceptive approaches.

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The authors declare no conflict of interest could be perceived as prejudicing the impartiality of the research reported.

ETHICS STATEMENT

Human/Animal rights: This review article contains no studies of human and animal subjects directly performed by authors. There is also no clinical trial involved.

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