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The effects of hormone-mediated PI3K/AKT signaling on spermatogenesis in Sertoli cells

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Abstract: The phosphoinositide-3-kinase/Akt (PI3K/AKT) signaling pathway is crucial for Sertoli cell development and completing spermatogenesis. Its main role is to promote proliferation and inhibit apoptosis. Many factors activate the PI3K/AKT pathway, like hormones, such as follicle stimulating hormone (FSH), androgen, estrogen, insulin to name a few. Many of these factors have receptors inside or on the surface of Sertoli cells (SCs). This review summarizes how these hormones directly regulate the PI3K/AKT signaling pathway in SCs, which in turn affects SC proliferation and differentiation. Further, hormone-mediated PI3K/AKT signaling also stimulates SC secretion, which is essential for germ cell development, suggesting an indirect role of PI3K/AKT signaling during spermatogenesis. These functions include promoting spermatogonia proliferation and differentiation, meiosis of spermatocytes, sperm maturation, and their release. This review also provides potential hints for clinically treating male infertility issues like cryptorchidism and Sertoli cell-only syndrome.

Introduction

The migration of primordial germ cells (PGCs) into the developing testis is the first step of seminiferous cord formation. At this point they begin to interact with SC progenitor cells called mesenchymal cells, after which seminiferous cords can be formed. Then PGCs transform into gonocytes, which are surrounded by the immature SCs making them migrate to the tubule basement membrane, where they acquire the ability to form type A spermatogonia (de Kretser *et al.*, 1998). Type A spermatogonia are of three types: As, Apr, and Aal, which all retain stem cell properties and among the As type provides the spermatogonial stem cells (SSCs). Spermatogenesis originates from the self-renewal of SSCs (Lord *et al.*, 2018). After undergoing non-mitotic differentiation, SSCs differentiate into type A1 spermatogonia, which then form A2, A3, A4, intermediate (Int), and B spermatogonia, the source of preleptotene spermatocytes and the initiation of meiosis. Subsequently, A1, A2, A3, A4, and B type spermatogonia undergo differentiation and lose their ability of self-renewal (Li *et al.*, 2019). The passage across the blood-testis barrier (BTB) is a requirement for the development process of preleptotene and leptotene spermatocytes into pachytene spermatocytes (Russell, 1977; Lui *et al.*, 2003). After the first and the second meiotic division, primary spermatocytes form secondary spermatocytes and subsequently into round spermatids. These round spermatids then transform into elongated spermatids during the process of spermiogenesis (Qian *et al.*, 2014). Finally, spermatozoa are formed and released into the tubular lumen via a process called spermiation (Qian *et al.*, 2014).

SCs are the dominant somatic cells in the seminiferous tubules that maintain and control spermatogenesis. This is mainly by providing a suitable microenvironment for GCs, ensuring the successful completion of spermatogenesis (Lim and Hwang, 1995). SCs also provide nutrients for germ cell development as they convert glucose to lactate, which then can be transported into germ cells (Rato *et al.*, 2012), where lactate dehydrogenase (LDH) plays a crucial role. In addition, SCs secrete many factors necessary for spermatogenesis (Griswold, 1998), including cytokines and hormones, like stem cell factor (SCF), glial cell line-derived neurotrophic factor (GDNF), fibroblast growth factor (FGF), and anti-Müllerian hormone (AMH) along with transport proteins such as androgen binding protein (ABP) and transferrin (Griswold, 1988). Mature SCs also establish the blood-testis barrier (BTB) structure, which divides the seminiferous tubules into basal and abluminal compartments (Dym and Fawcett, 1970) and protects GCs

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from attack by the immune system (Meng *et al*., 2011). The BTB is composed of a variety of cell junction proteins, like tight junctions (TJ), gap junctions (GJ), desmosomes, and basal ectoplasmic specialization (ES) (Wang *et al*., 2022).

Given that SCs have so many documented functions, a better knowledge about them will be important for the control of spermatogenesis. It is known there are many hormone receptors inside or on the surface of SCs. Once ligands bind to these receptors, the receptors can mediate several essential signaling pathways, such as the PI3K/AKT pathway. PI3K is composed of a regulatory subunit (p85) and a catalytic subunit (p110) and at the membrane, it converts phosphoinositide (PIP) 2 lipids to PIP3. PIP3 recruits two kinases, PDK1 and PDK2 (mTORC2), thereby phosphorylating AKT at Thr308 and Ser473, and leading to its activation. After activation, AKT mediates cell growth and cell survival through various substrates, the major of which is the mammalian target of rapamycin (mTOR) (Ersahin *et al*., 2015).

Our previous review highlighted that some hormones like follicle-stimulating hormone (FSH), estrogen 2 (E2), and thyroid hormone 3 (TH3) can regulate the PI3K/AKT signaling in SCs (Chen *et al*., 2022). However, their detailed mechanisms and effects on spermatogenesis were not completely discussed. In this review, we summarize several essential hormones which can directly regulate the PI3K/AKT signaling pathway in SCs, as well as the consequent impacts on SC development and spermatogenesis. Additionally, we reason that this review can provide clinical hints for treating male infertility.

Materials and Methods

Methodology and search strategy

We first surveyed articles in the PubMed database with the terms of "hormone including" "follicle-stimulating hormone" OR "FSH", "androgen" OR "testosterone", "estrogen" OR "17β-estradiol", "insulin", "insulin-like growth factors" OR "IGF", "relaxin", "thyroid hormone", "retinoic acid", "PI3K/AKT", "Sertoli cell", "blood-testis barrier" OR "BTB", "in testes". We then selected articles that met the criteria that hormones exert influences on SC development by regulating the PI3K/AKT signaling (Table 1). Further, we collected articles by searching terms containing "Sertoli cell secretion" (including "SCF", "GDNF", "AMH", "transferrin", "ABP", "connexin 43", "ZO-1", "occludin", "claudin", "N-cadherin", "β-catenin", "vimentin"), "germ cell development" (including "spermatogonia proliferation" OR "spermatogonia self-renewal", "spermatogonia differentiation", "spermatocyte meiosis", "spermiogenesis", "sperm maturation", "sperm release" OR "spermiation"), and "spermatogenesis". Articles that were aimed at exploring the role of SC secretions in spermatogenesis were collected (Table 2). The final screening was related to "male infertility", "male fertility diseases" OR "male reproductive diseases" where we found two notable diseases cryptorchidism and the Sertoli cell-only syndrome (SCOS). Hence, we subsequently searched for "cryptorchidism", "the Sertoli cell only syndrome" OR "SCOS" to gain insight into their mechanisms. More papers were selected from these review articles and their reference lists.

Hormone-mediated PI3K/AKT signaling directly regulates Sertoli cell proliferation, differentiation and BTB junction protein expression

In the fetal cells of mice and rats, immature SCs proliferate to increase their number (Lucas *et al*., 2014b). However, during the prepubertal period, SC proliferation stops and they start to differentiate to a mature state. During this process, SCs establish the BTB and acquire the ability to sustain spermatogenesis (Sharpe *et al*., 2003). It has been documented that several hormones participate in SC proliferation, maturation, and BTB junction protein expression or a part of these processes. We have summarized these hormones below (Fig. 1).

Follicle-stimulating hormone

Follicle-stimulating hormone (FSH) belongs to the glycoprotein hormone family (Kumar *et al*., 1997). It is a crucial hormone in the endocrine regulation of spermatogenesis (Themmen and Huhtaniemi, 2000). After binding to FSH, the follicle-stimulating hormone receptor (FSHR) is activated which stimulates the downstream signaling cascades in SCs (Walker and Cheng, 2005).

Several studies have proven that FSH is required for immature SC proliferation (Griswold *et al*., 1977; Orth, 1984; Kumar *et al*., 1997; Abel *et al*., 2000; Crépieux *et al*., 2001; Wreford *et al*., 2001; Hayes *et al*., 2001; Johnston *et al*., 2004). Additional studies suggest that the level of p-AKT significantly increases after treatment with FSH. Moreover, reports showed that concurrent treatment with the PI3K inhibitor, LY294002/wortmannin, alone or combined with the mTOR inhibitor rapamycin, significantly decreases FSH-stimulated p-AKT levels (Meroni *et al*., 2002; McDonald *et al*., 2006; Musnier *et al*., 2009; Riera *et al*., 2012; Xi *et al*., 2022) and FSH-stimulated [3H] thymidine incorporation (Riera *et al*., 2012). In another study, FSH was also found to suppress the cytoplasm-to-nucleus translocation of transcription factor EB (TFEB) via PI3K/AKT/mTORC1 in cultured goat SCs. This further lower lysosomal biogenesis, inhibits autophagy, promotes cell survival, and enhances the expression of ABP, SCF, and GDNF (Xi *et al*., 2022). Besides, FSH was also found to be related to lactate production, lactate dehydrogenase (LDH) activity, and glucose uptake in a PI3K/AKT-dependent manner to provide nutrients to SCs and GCs in another report (Meroni *et al*., 2002). Another study showed that FSH also regulates PI3K/AKT downstream target gene c-myc, an essential factor in the regulation of cellular proliferation and the SC cycle. After treating primary SCs with FSH, there was an increase of mRNA levels of c-myc (Lim and Hwang, 1995). Similarly, another regulator of cell growth, Hypoxia Inducible Factor (HIF), which is involved in the regulation of rat SC proliferation by FSH (Gorga *et al*., 2018) was also shown to be related to PI3K/AKT signaling during spermatogenesis (Wang *et al*., 2021). Another report found that while FSH stimulates aromatase activity and estradiol production, a hormone playing a key role in the

TABLE 1

The function of PI3K/AKT signaling mediated by various hormones in Sertoli cells (SCs)

Hormones	Receptor location	Species	Function	References
FSH	Cell membrane	Rat	Promotes primary SC proliferation	Meroni *et al.* (2002), McDonald *et al.* (2006), Musnier *et al.* (2009), Riera *et al.* (2012)
			Promotes lactate production, lactate dehydrogenase (LDH) activity, and glucose uptake	Meroni *et al.* (2002)
			Increases mRNA level of c-myc	Lim and Hwang (1995)
			Enhances HIF effects	Gorga *et al.* (2018), Wang *et al.* (2021)
			Stimulates aromatase activity and estradiol production	McDonald *et al.* (2006)
		Mouse	Enhances AMH expression	Lasala *et al.* (2011)
		Goat	Inhibits autophagy by reducing lysosomal biogenesis; suppression of TFEB nuclear translocation; enhances the expression of ABP, GDNF, and SCF	Xi *et al.* (2022)
Testosterone	Cytoplasm	Rat	Promotes SC proliferation	Lucas *et al.* (2012), Xu *et al.* (2014), Chen *et al.* (2021), Hu *et al.* (2021a), Hu *et al.* (2021b), Huang *et al.* (2021), Xu *et al.* (2021)
			Promote junction protein expression	Hu *et al.* (2021a), Hu *et al.* (2021b), Xu *et al.* (2021)
			Increase c-Src phosphorylation; regulates connexin 43 expression	Chojnacka *et al.* (2016a)
			Disrupts hormone receptor and SC secretion function	Huang *et al.* (2016)
17β-estradiol	Cytoplasm	Rat	Inhibits SC apoptosis	Wang *et al.* (2015)
			Regulates primary SC cell-cycle	Wang *et al.* (2018a)
			Damages the cytoskeletal structure	Zheng *et al.* (2016), Zheng *et al.* (2018)
			Affect SC secretory functions	Zheng *et al.* (2016)
			Disrupts hormone receptor and SC secretion function	Huang *et al.* (2016)
		Rat/ Boar	Promotes SC proliferation	Royer *et al.* (2012), Lucas *et al.* (2014a), Wu *et al.* (2015), Yang *et al.* (2015), Yang *et al.* (2017)
		Rat/ Mouse	Related to autophagy and ROS	Wang *et al.* (2018a), Zheng *et al.* (2018)
Insulin/IGF	Cell membrane	Rat	Promote junction protein expression	Mok *et al.* (2014)
		Rat/ Mouse	Promotes SC proliferation	Khan *et al.* (2002), Dupont *et al.* (2010), Oldknow *et al.* (2013), Pitetti *et al.* (2013), Neirijnck *et al.* (2019)
		Rat/ Boar	Regulates SC calcium uptake, amino acid and glucose transport, lactate secretion	Escott *et al.* (2013), Gan *et al.* (2022)
Relaxin	Cell membrane	Rat	Promotes SC proliferation	Nascimento *et al.* (2013), Pimenta *et al.* (2015), Nascimento *et al.* (2016)
			Upregulates the expression of connexin 43	Zhang *et al.* (2003)
TH	Cell membrane	Rat	Promotes SC differentiation	Wang *et al.* (2019)
		Calf	Inhibits SC proliferation	Sun *et al.* (2015), Wang *et al.* (2019)

proliferation of SCs (Wu *et al.*, 2015; Yang *et al.*, 2015), these stimulatory effects of FSH on aromatase and estradiol are blocked by inhibiting PI3K/AKT signaling (McDonald *et al.*, 2006). The expression of anti-Müllerian hormone (AMH), another SC-produced hormone, is triggered by SOX9 in immature SCs, and is regulated by SF1, GATA, FSH, and other factors (Rey *et al.*, 2003). It has been documented that the expression and translocation of some of these genes are

TABLE 2

The effects of Sertoli cell (SC) secretions regulated by hormone-mediated PI3K/AKT signaling on spermatogenesis

Hormones	Species	Function	References
GDNF	Mouse	Promote Spermatogonial self-renewal	Meng *et al.* (2000), Kubota *et al.* (2004), Hofmann *et al.* (2005), Wang *et al.* (2014)
SCF/KIT	Rat/ Mouse	Promotes differentiated spermatogonia proliferation	Yoshinaga *et al.* (1991), Tajima *et al.* (1994), Hakovirta *et al.* (1999), Ohta *et al.* (2000), Ohta *et al.* (2003)
	Human	Promotes SSC differentiation in cryptorchid patients	Yang *et al.* (2014)
Connexin 43	Human/ Mouse	Promotes spermatogonia differentiation	Steger *et al.* (1999), Brehm *et al.* (2007), Rode *et al.* (2018)
	Rat/ Mouse	Initiates spermatocyte meiosis	Giese *et al.* (2012), Li *et al.* (2016), Hilbold *et al.* (2020)
	Mouse	Regulates sperm formation and maturation-related genes expression	Giese *et al.* (2012)
Transferrin	Mouse	Promotes spermatocyte meiosis progression	Gao *et al.* (2021)
ABP	Rat	Increase the secretion of spermatocyte proteins	Sharpe *et al.* (1992)
		Regulates sperm fertilizing ability	Anthony *et al.* (1984a), Anthony *et al.* (1984b), Hermo *et al.* (1998)

regulated mainly through the cAMP/PKA pathway in the SC line SMAT1, which possibly also involves the role of other kinases in this process, such as PI3K/AKT (Lasala *et al.*, 2011). AMH was also shown to increase the expression of an important regulator of spermatogenesis, SCF (ur Rehman *et al.*, 2017).

Mature SCs cease to proliferate and begin to differentiate. They respond to FSH by stimulating cAMP production (Crépieux *et al.*, 2001; Dupont *et al.*, 2010; Nascimento *et al.*, 2013; Nascimento *et al.*, 2016; Bhattacharya *et al.*, 2019; Bhattacharya *et al.*, 2021) instead of PI3K/AKT. This is due to the inhibition of the PI3K/AKT pathway resulting from high level cAMP (Eskola *et al.*, 1993; Nascimento *et al.*, 2016) and enhanced PTEN activity (Dupont *et al.*, 2010).

Androgens

Testosterone is the major androgen in the testes, which is predominantly produced by Leydig cells and can pass through the plasma membrane to interact with the intracellular androgen receptor (AR) (Walker, 2021). In the testis, only Leydig cells, peritubular cells, and SCs express AR (Lyon *et al.*, 1975). There are two types of AR signaling: the classical signaling pathway and the non-classical signaling pathway. It is known the PI3K/AKT signaling is involved in the non-classical signaling pathway of AR. Here, testosterone interacts with AR to activate the PI3K/AKT pathway directly by activating the PI3K subunit p85a. Phosphorylated AKT activates Src, a component of another non-classical AR signaling pathway type, and contributes to AR translocation from the cytoplasm to the plasma membrane (Deng *et al.*, 2017).

Previous studies have reported that the androgen can regulate spermatogenesis and BTB junction proteins. This regulation can be the androgen alone (Meng *et al.*, 2005; Zhang *et al.*, 2005; Denolet *et al.*, 2006; Wang *et al.*, 2006; Kaitu'u-Lino *et al.*, 2007; Yan *et al.*, 2008; Willems *et al.*, 2010; Chakraborty *et al.*, 2014; Chojnacka *et al.*, 2016b) by working with FSH synergistically (McLachlan *et al.*, 1994; Haywood *et al.*, 2003; Johnston *et al.*, 2004; Abel *et al.*, 2008; Bhattacharya *et al.*, 2019). Other studies showed that several compounds have an impact on SC proliferation and BTB junction proteins through the AR/PI3K/AKT pathway. For example, *Lycium barbarum* polysaccharide (LBP) resists heat-stress-induced injury of SCs by increasing the expression of the androgen receptor and activates the AKT signaling pathway in rats. As a result, it restores the proliferation activity of SCs by resisting the induction of Ki67 expression. It reverses the dedifferentiation of SCs by decreasing CK-18 expression, while increasing the expression levels of junction proteins occludin and ZO-1 (Hu *et al.*, 2021a). In another report, Wuzi Yanzong Pills (WYP) improved SC viability and proliferation, ameliorated dedifferentiation and the expression of junction proteins such as ZO-1 and occludin damaged by heat stress via AKT signaling (Hu *et al.*, 2021b; Xu *et al.*, 2021), which may result from T-level restoration (Xu *et al.*, 2014; Hu *et al.*, 2021b). Ouabain is an endogenous cardiotonic steroid (Cavalcante-Silva *et al.*, 2017), and is known to participate in the non-classical AR pathway (Ni *et al.*, 2020). Treatment of the SC line 93RS2 with ouabain activated c-Src/c-Raf/Erk1/2 signaling, which is radically triggered by testosterone (Konrad *et al.*, 2011; Cavalcante-Silva *et al.*, 2017; Rajamanickam *et al.*, 2017). Moreover, another study showed that ouabain induces PI3K/AKT phosphorylation in primary rat SCs (Lucas *et al.*, 2012), but it was not clear whether PI3K/AKT signaling also was triggered by testosterone. In addition, Testosterone treatment can increase c-Src phosphorylation. However, 2-hydroxyflutamide (HF), an anti-androgen compound, inhibits the increase of c-Src activity induced by testosterone

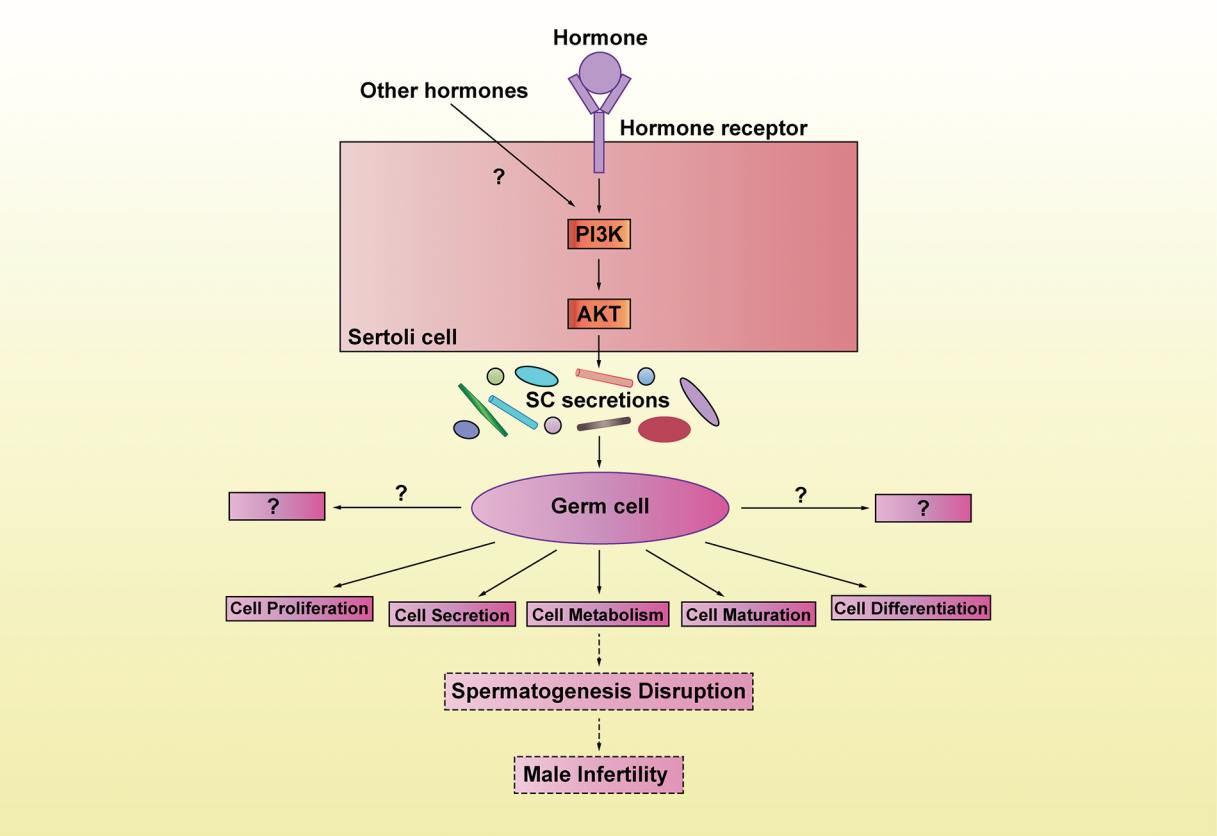

FIGURE 1. Hormones-mediated PI3K/AKT signaling pathways in Sertoli cells (SCs). The functions of different hormones are marked with different colors. FSH activates PI3K/AKT to promote activities of LDH and aromatase, the expression of SOX9, SF1, GATA4, and AMH. However, it suppresses TFEB nuclear translocation and lysosomal genes to stimulate GDNF, SCF, and ABP secretion (green). After binding to AR, testosterone (T) activates PI3K/AKT and enhances the expression of Ki67, CK-18, FSHR, WT1, SOX9 and occludin, and ZO-1. HIF and c-myc are also involved in this process (red). All the three estrogen receptors activate the PI3K/AKT pathway (orange). E2-ESR1 activates Src and HB-EGF phosphorylation to activate PI3K/AKT, which promotes NF-κB nuclear translocation and enhances CCND1 expression. E2-ESR2 directly activates the PI3K/AKT pathway to promote CREB nuclear translocation and increase the expression of CDKN1B, GATA-1, and DMRT1. Further, GPER activates Src, MMP, and HB-EGF phosphorylation successively to activate the PI3K/AKT signaling, which promotes the expression of apoptosis proteins BCL2 and BAX. Additionally, IGF/insulin stimulates calcium uptake, amino acid and glucose transport along with lactate secretion via PI3K/AKT signaling. Furthermore, the downstream signaling of AKT mTOR-p70S6K-rpS6 increases MMP9 production, which downregulates the expression of occludin, claudin, and ZO-1 (purple). By activating PI3K/AKT, relaxin promotes NF-κB nuclear translocation to promote SC proliferation (blue). RA also promotes SC proliferation and SC metabolism through PI3K/AKT (brown). However, only TH inhibits PI3K/AKT signaling to cause the upregulation of p21Cip1, p27Kip1, AR and connexin 43 while inducing the downregulation of cyclin A2, D1, E1, PCNA, Skp2 and KRT-18 (pink).

in 20-day-old rat testes. At the same time, HF augments AKT phosphorylation by upregulating PTEN phosphorylation, indicating the activation of the PI3K/AKT pathway. This is probably because testosterone is unable to exert functions properly. Besides, HF also reduces the level of SC functional marker connexin 43, which results from the activated PI3K/AKT pathway (Chojnacka *et al.*, 2016a). On the contrary, long-term exposure to phenanthrene (Phe), downregulates the GDNF/PI3K/AKT signaling pathway, reduces SC numbers, and inhibits the expression of SC markers such as FSHR, WT1, and Sox9. These observations may be related to the reduction of AR expression (Chen *et al.*, 2021; Huang *et al.*, 2021). In another study, treating TM4 cells (normal mouse testis Sertoli cell line) with just 20 μM of nonylphenol (NP) increased the p-AKT level (Liu *et al.*, 2016). However, treating prepubertal SCs with 30 μM NP, triggers apoptosis related to oxidative stress, disturbs the PI3K/AKT/mTOR pathway and the functions of the hormone receptor and SC secretory function, inducing reproductive damage (Huang *et al.*, 2016). These changes are possible because of the reduction of the androgen level or the androgen receptor (Huang *et al.*, 2016; Jambor *et al.*, 2016; Liu *et al.*, 2016).

To summarize, androgen stimulates the proliferation of SCs and regulates BTB junction protein expression via the PI3K/AKT pathways. While previous research was focused on classical pathways and the EGFR/Src/Ras/CREB non-classical pathway, additional research is needed to elucidate the function of AR/PI3K/AKT signaling.

Estrogens

In young animals, estrogens are synthesized mainly by SCs, while in adult animals, Leydig and germ cells can also synthesize these hormones (Carreau *et al.*, 2006; Carreau

and Hess, 2010). The action of certain aromatase can transform testosterone into estrogen (Walker, 2021). This then functions in male reproduction and male fertility (Lazari *et al.*, 2009). Estrogens perform their biological functions by interacting with the classical nuclear estrogen receptors (ESRs): ESR1 and ESR2 (also known as ERα and ERβ) and also the non-classical membrane receptor, a G protein-coupled estrogen receptor 1 (GPER, also known as GPR) (Aquila *et al.*, 2004; Royer *et al.*, 2012). All these three receptors have been shown to activate the PI3K/AKT pathway (Lucas *et al.*, 2014b). Once bound to estrogen, ESR1, and GPER can activate Src and EGFR, which then activates PI3K/AKT signaling. However, ESR2 activates the PI3K/AKT pathway directly after binding to estrogen.

The main physiological estrogen for male germ cell survival is 17β-estradiol (E2) (Pentikäinen *et al.*, 2000). Treatment of cultured immature boar Sertoli cell with E2 activates mTOR in a time dependent manner, whereas 10-DEBC (an inhibitor of AKT) treatment reduces E2-induced mTOR phosphorylation significantly, suggesting an activation of PI3K/AKT/mTOR signaling. Further, the mTOR inhibitor, rapamycin, reduces the mRNA levels of SKP2, CCND1, and CCNE1 mRNA and the protein levels of RB and EMI1 to suppress E2-induced SC proliferation (Yang *et al.*, 2015).

Studies show that ESR1 stimulates SC proliferation by upregulating CCND1 expression (Royer *et al.*, 2012; Lucas *et al.*, 2014a; Macheroni *et al.*, 2020). It is a vital gene of the cell cycle, and this effect depends on NF-κB activation (Lucas *et al.*, 2014a). However, a study showed that E2 and DPN (the ESR2-selective agonist) increase the expression of CDKN1B, GATA-1, and DMRT1 in a PI3K and CREB-dependent manner (Lucas *et al.*, 2014a). Similarly, E2 and G-1 (the GPER-selective agonist) rapidly activate PI3K/AKT and CREB, which may participate in the upregulation of anti-apoptotic proteins BCL2 and BCL2L2 (Royer *et al.*, 2012). Further, both G-15 (a GPR30 antagonist) and PP2 (a Src inhibitor) inhibit E2-induced activation of PI3K, indicating both GPR30 and Src participate in E2-induced PI3K/AKT phosphorylation. Besides, the activation of PI3K/AKT also enhances Skp2 expression, which contributes to SC proliferation (Yang *et al.*, 2017).

Some chemical treatments also confirm that estrogen acts through the PI3K/AKT signaling pathway. An example is zearalenone (ZEA) (Zheng *et al.*, 2016; Wang *et al.*, 2018a; Zheng *et al.*, 2018; Rogowska *et al.*, 2019), an exogenous compound that resembles the structure of natural estrogens in its chemical structure (Rogowska *et al.*, 2019). It arrests the cell cycle in the G2/M phase to inhibit SC proliferation (Wang *et al.*, 2018a) and triggers autophagy via inhibition of the PI3K/AKT/mTOR pathway (Wang *et al.*, 2018a; Zheng *et al.*, 2018). Further, studies have shown that the autophagy stimulated by ZEA leads to damage to cytoskeletal structures (F-actin, α-tubulin, and the nucleus structure) (Zheng *et al.*, 2016, 2018). Besides the damage to the cytoskeletal structure, one of these studies also shows that ZEA treatment can affect the secretion of specific proteins of SCs like ABP, transferrin, vimentin, N-cadherin, and FSHR (Zheng *et al.*, 2016). Another structural analog of estradiol, Bisphenol-A(BPA) (Chianese *et al.*, 2018), an endocrine-disrupting chemical, can also compete with E2 for ESR. BPA treatment of cultured SCs increases the protein level of PTEN while decreasing the levels of p-AKT and procaspase-3, suggesting that the PTEN/AKT pathway is involved in the apoptotic effects of BPA on SCs (Wang *et al.*, 2015). Another molecule, diosgenin induces ESR1 and ESR2 translocation from the nucleus to the plasma membrane, which is an SRC-dependent process. This process activates ERK/AKT signaling and induces the transcriptional activity of ESR, which subsequently directly regulates the cell cycle and apoptosis proteins, such as cyclin D and Bcl-2, leading to final cell proliferation (Wu *et al.*, 2015). Treating TM4 cells with just 20 μM of nonylphenol (NP) increased the p-AKT level (Liu *et al.*, 2016). However, treating prepubertal SCs with 30 μM NP, triggers apoptosis related to oxidative stress, disturbs the PI3K/AKT/mTOR pathway and induces reproductive damage (Huang *et al.*, 2016). These changes are attributed to the targeting of estrogen receptors (Huang *et al.*, 2016; Jambor *et al.*, 2016; Liu *et al.*, 2016).

Insulin family of growth factors

The insulin family of growth factors includes insulin, insulin-like growth factor I (IGF-1), insulin-like growth factor II (IGF-2), and relaxin. Though they are all structurally small polypeptides, they are key regulators of cell growth, cell metabolism, and male reproduction.

Insulin and insulin-like growth factors

Insulin and IGF1 exert biological functions by activating their receptors: the insulin receptor (IR, also known as INSR), and the IGF-1 receptor (IGF-1R), respectively which are tyrosine kinases (Meroni *et al.*, 2019). Notably, insulin could activate both INSR and IGFR (Escott *et al.*, 2014). This makes it sometimes difficult to affirm the effects induced whether by insulin or IGF-I when both receptors exist.

Both insulin and IGFs have been shown to regulate the PI3K/AKT pathway in SCs. For example, in prepubertal rat SCs, unlike FSH, insulin stimulation leads to an increase in PTEN phosphorylation, the PI3K major down-regulator. This activates the PI3K/AKT pathway to promote SC proliferation and resist the shift of SCs from proliferation to differentiation states induced by FSH (Dupont *et al.*, 2010). Similarly, IGFs also promote immature SC proliferation through the IGF/PTEN/PI3K pathway (Neirijnck *et al.*, 2019). Besides, they are also reported to regulate aspects of SC metabolism, such as calcium uptake, amino acid and glucose transport, and lactate secretion (Oliveira *et al.*, 2012; Escott *et al.*, 2013; Escott *et al.*, 2014; Faure *et al.*, 2016; Gan *et al.*, 2022). These are all important in SC survival through the PI3K/AKT pathway (Escott *et al.*, 2013; Gan *et al.*, 2022). The downstream factor p-rpS6 of mTOR was shown to disrupt IGF-1/insulin-AKT signaling that leads to an increase of MMP-9 expression. This subsequently perturbs the BTB by downregulating tight junction proteins such as claudin-11, occludin and ZO-1 (Mok *et al.*, 2014), suggesting a protective effect of IGF-1/insulin-AKT signaling on the BTB.

IGF acts synergistically with FSH to activate AKT (Froment *et al.*, 2007; Musnier *et al.*, 2009). In addition, it

was shown that IGF1 is required for the activation of AKT by FSH in immature SCs. For example, pre-treatment of these cells with NVP-AEW541, an IGF1R inhibitor, inhibits FSH-dependent AKT308 phosphorylation (Cannarella *et al.*, 2019). In SCs lacking INSR and IGF1R, The expression of the fshr gene and AKT activation are both decreased (Pitetti *et al.*, 2013). FSH was shown to stimulate immature SCs to secrete IGF1 and amplify the IGF-mediated PI3K/AKT signaling pathway in immature SCs (Khan *et al.*, 2002; Pitetti *et al.*, 2013). However, another report showed that FSH activates PI3K/AKT signaling in 20-day-old SCs independently of IGF-1 (Meroni *et al.*, 2004), implicating that the interaction between IGF and FSH depends on the developmental phase of SCs.

Interestingly, a study showed that knocking out follistatin-like 3 (FSTL3), a glycoprotein that binds and inhibits the action of TGFβ ligands increases SC numbers, which might arise from the increased IGF1/AKT signaling. This result demonstrates a cross-talk between TGF ligands and IGF/AKT signaling for cell survival (Oldknow *et al.*, 2013).

Relaxin

Relaxin (RLN/RLX) is another member of the insulin family of growth factors, the structure of which is similar to that of insulin. Relaxin binds to the relaxin family of peptide receptors (RXFP1 and RXFP2) (Hsu *et al.*, 2002), which belong to the G-protein coupled receptor (GPCR) family, and stimulate several crucial signaling pathways (Bathgate *et al.*, 2013).

Relaxin has been shown to stimulate SC proliferation (Cardoso *et al.*, 2010; Nascimento *et al.*, 2012; Pimenta *et al.*, 2013, 2015; Nascimento *et al.*, 2016). Further, relaxin increases the incorporation of [3H]-thymidine (Cardoso *et al.*, 2010; Nascimento *et al.*, 2012, 2016) and PCNA levels in SCs (Nascimento *et al.*, 2012). Other studies show that relaxin stimulates SC proliferation due to the activation of the PI3K/AKT pathways (Nascimento *et al.*, 2013). In SCs isolated from 15-day-old rats at a stage that is close to the transition between cell proliferation and cell differentiation, relaxin and FSH affect the same signaling pathways in opposite directions. While relaxin increases AKT phosphorylation, inhibits basal cAMP production, and impacts NF-κB nuclear translocation, FSH inhibits AKT phosphorylation and strongly increases cAMP production as well as CREB phosphorylation (Nascimento *et al.*, 2013, 2016). To summarize, relaxin counteracts FSH signaling but does not block its pro-differentiation function triggered by FSH in 15-day-old rats.

Thyroid hormones

Thyroid hormones (THs) are critical regulators of cell growth, cell development, and cell metabolism in almost all tissues (Oetting and Yen, 2007; Cheng *et al.*, 2010; Brent, 2012). The thyroid gland mainly secretes two types of THs, levothyroxine (T4) and triiodothyronine (T3), where the latter is identified as the more biologically active form (Sinha and Yen, 2016). The expression of the thyroid hormone receptor (TR) within the seminiferous epithelium only occurs in SCs, and the Tαreceptor is its only form expressed (Jannini *et al.*, 2000). There are two main pathways by which THs exert their functions, namely genomic and non-genomic. The former refers to the influences of gene expression, while the latter refers to the regulation of the intracellular signaling pathways, including PI3K/AKT (Geist *et al.*, 2021).

T3 has been shown to inhibit SC proliferation via the PI3K/AKT signaling pathway (Sun *et al.*, 2015; Wang *et al.*, 2019). This is accomplished by downregulating cyclinA2, cyclinD1, cyclinE1, PCNA, and Skp2 (Holsberger and Cooke, 2005; Sun *et al.*, 2015; Wang *et al.*, 2019) and upregulating the cyclin-dependent kinase inhibitors (CDKI) p21Cip1, and p27Kip1 (Buzzard *et al.*, 2003; Holsberger *et al.*, 2003, 2005; Holsberger and Cooke, 2005; Sun *et al.*, 2015; Wang *et al.*, 2019). Therefore, T3 and the above-mentioned hormones have opposite effects on SC proliferation even though they can both affect the PI3K/AKT signaling.

It is worth noting that Cx43 could be an intermediate target for T3 inhibition of neonatal SC proliferation (Gilleron *et al.*, 2006), which has been shown to be an inhibitor of Skp2 in a previous study (Zhang *et al.*, 2003). This offers a new thought that T3 is capable to upregulate connexin 43 expression, one of the main components of the BTB under the control of the PI3K/AKT signaling pathway. Further, T3 also possibly promotes SC differentiation via PI3K/AKT signaling by upregulating AR and down-regulating KRT-18 (Wang *et al.*, 2019).

Hormone-mediated PI3K/AKT signaling in Sertoli cells indirectly regulates spermatogenesis

The hormones summarized above have indirect effects on spermatogenesis by stimulating SC secretion through PI3K/AKT signaling. These secretions mainly include cytokines and hormones, like SCF, GDNF, and AMH and transport proteins, like ABP, and transferrin. They also include cell junction proteins, like ZO-1, occludin, claudin, connexin 43, β-catenin, and N-cadherin along with cellular metabolites, like amino acid, glucose, LDH, and HIF. Some SC secretions have been shown to play important roles in regulating spermatogenesis (Fig. 2).

Spermatogonia proliferation and differentiation

Spermatogenesis originates from the self-renewal of SSCs (Lord *et al.*, 2018). They then differentiate into types A_1–A_4, intermediate (Int), and B spermatogonia successively (Li *et al.*, 2019). This process is regulated by several cytokines secreted by SCs in a paracrine manner.

GDNF, produced by SCs, and its receptor GFRA1 is expressed on the surface of SSCs. The GDNF-GFRA1 complex interacts with RET tyrosine kinase, which is present on the surface of undifferentiated type A spermatogonia (Naughton *et al.*, 2006; He *et al.*, 2007). This promotes spermatogonial self-renewal in mice (Meng *et al.*, 2000; Kubota *et al.*, 2004; Hofmann *et al.*, 2005; Wang *et al.*, 2014).

SCF, specifically produced by SCs acts through its receptor KIT, which is encoded by c-kit. The stem cell factor (SCF)/KIT pathway is known to regulate type A spermatogonia proliferation and differentiation

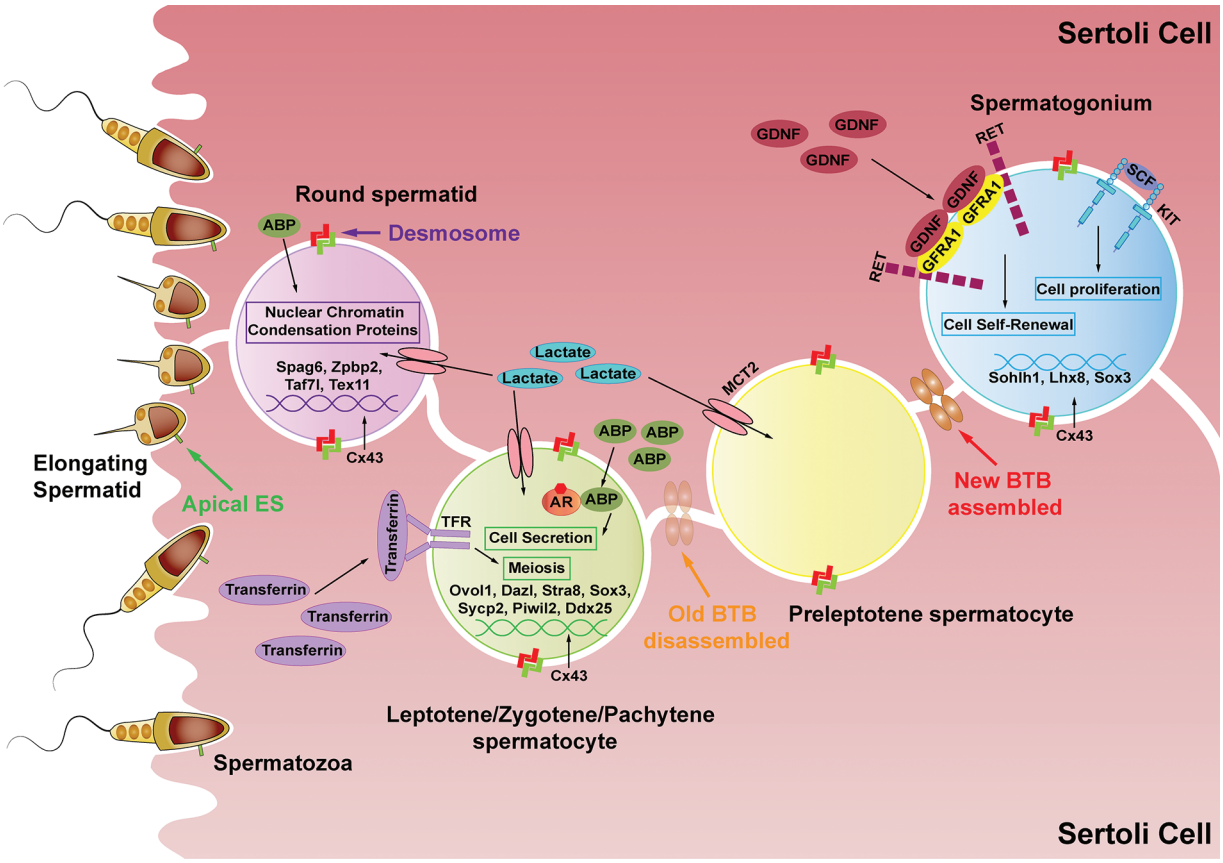

FIGURE 2. The effects of Sertoli cell secretions produced by hormone-mediated PI3K/AKT signaling on spermatogenesis. SCs (the pink cells) secrete lactate to provide energy to germ cells and secrete many kinds of proteins essential for spermatogenesis. GDNF promotes spermatogonia self-renewal after binding to its receptor GFRA1, while SCF binds to KIT to promote differentiated spermatogonia proliferation. Connexin 43 (Cx43) regulates the expression of *Sohlh1*, *Lhx8*, and *Sox3* in spermatogonia (the blue cell). Junction proteins allow preleptotene spermatocytes to go through the BTB structure while differentiating into leptotene spermatocytes. Subsequently, ABP assists AR to stimulate spermatocyte secretion. Transferrin binds to TFR to promote the meiosis process. Cx43 regulates the expression of *Ovol1*, *Dazl*, *Stra8*, *Sox3*, *Sycp2*, *Piwi2*, and *Ddx25* in spermatocytes (the green cell). ABP works with androgen to regulate sperm-fertilizing ability and reduce nuclear chromatin condensation proteins during spermiogenesis. Further, Cx43 regulates the expression of the sperm genes *Spag6*, *Zpbp2*, *Taf7l*, and *Tex11* (the purple cell).

(Blume-Jensen *et al.*, 2000; Feng *et al.*, 2000; Ohta *et al.*, 2000; Feng *et al.*, 2002). Its effects on spermatogonia proliferation are induced by type A spermatogonial DNA synthesis (Feng *et al.*, 2000). However, studies have shown that the SCF/KIT signaling also appears to be necessary for maintaining the proliferation of differentiated spermatogonia but not for the initiation of spermatogonial cell differentiation (Yoshinaga *et al.*, 1991; Tajima *et al.*, 1994; Hakovirta *et al.*, 1999; Ohta *et al.*, 2000, 2003). To elaborate, undifferentiated type A spermatogonia or SSCs are independent of KIT. Interestingly, a review states that KIT also regulates the effects of both bone morphogenetic protein 4 (BMP4) and retinoic acid (RA) on spermatogonial differentiation including SSCs (Hai *et al.*, 2014). In cryptorchid patients, SCF and RA promote human SSCs differentiating into haploid spermatids, which can be a potential approach for treating this disease (Yang *et al.*, 2014).

In SC specific connexin 43 knockout (SCCx43KO) mice, the germ cell numbers are significantly reduced, causing Sertoli-cell-only (SCO) syndrome, and spermatogenesis is arrested at the level of undifferentiated spermatogonia (Steger *et al.*, 1999; Brehm *et al.*, 2007; Rode *et al.*, 2018). This suggests a defect of spermatogonia differentiation, which may be associated with the gene expression of Sohlh1, Lhx8, and Sox3 (Giese *et al.*, 2012).

Meiosis and factors

SCs play an active role in the transfer of spermatocytes to the adluminal compartment mainly because of the BTB structure (Russell, 1977).

The expression of the major components of the BTB, ZO-1, occludin, and claudin, plays an important role in the migration of spermatocytes through the BTB. Another cell junction protein, connexin 43 restores meiosis and the BTB structure under the situation of aspermatogenesis and barrier disruption caused by toxicant, but it fails to support round spermatids to enter spermiogenesis (Li *et al.*, 2016). Further, in SCCx43KO the gene expression of Ovol1, Dazl, Stra8, Sox3, Sycp2, Piwil2, and Ddx25 are changed. These play pivotal roles in the initiation or progression of meiosis and controlling apoptosis in spermatocytes (Giese *et al.*, 2012). Additionally, Cx43 is also required for the mitosis-meiosis switch (Hilbold *et al.*, 2020).

Spermatogenesis is also a transferrin-dependent process, which is primarily secreted by SCs and can transport iron into the seminiferous tubule (Wauben-Penris *et al.*, 1986; Morales

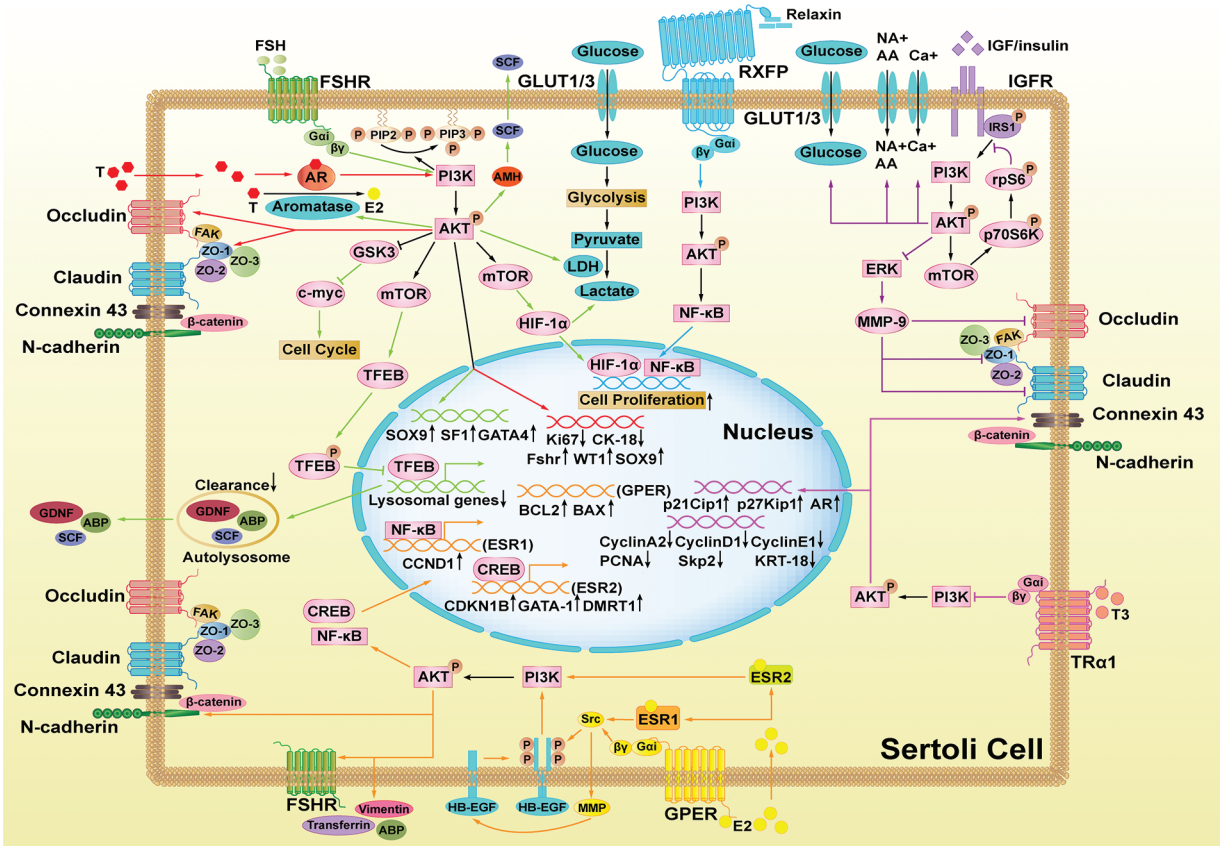

FIGURE 3. Schematic representation of the hormone-PI3K/AKT-SC secretions-spermatogenesis pathways. We pose a few questions here. Do other hormones also participate in mediating PI3K/AKT signaling? How do these hormones cooperate? What other roles may SC secretions have in regulating spermatogenesis? From the perspective of spermatogenesis, what new ideas can be provided for male fertility disease prevention and treatment?

et al., 1987). Transferrin receptors (TFRC) are localized mainly in spermatocytes and early spermatids (Sylvester and Griswold, 1984; Forti *et al.*, 1989). Further, TFRC is essential for spermatocyte meiosis progression, particularly for DNA damage repair and chromosome synapsis (Gao *et al.*, 2021).

Another SC secretion is androgen-binding protein (ABP), the receptor of which is expressed by spermatocytes (Gerard, 1995). It is also shown to increase the secretion of specific spermatocyte proteins individually (Sharpe *et al.*, 1992).

Sperm maturation and release

Round spermatids differentiate into spermatozoa, which then are released into the tubular lumen at spermiation (Qian *et al.*, 2014). SCs play an essential role during sperm maturation and release, mainly by forming Sertoli cell-spermatid adhesion. In the initial stages, round spermatids are linked to SCs by desmosomes before spermiogenesis occurs. Round spermatids then begin to elongate and apical ectoplasmic specialization (ES) replaces desmosome anchors gradually. This ES disassembles before sperm release (Wang *et al.*, 2022). Additionally, cytokines secreted by SCs (such as EGF), also regulate sperm maturation (Naz *et al.*, 1994).

SC-expressed Cx43 affects sperm development. For example, SCCx43KO changes the expression of Spag6, Zpbp2, Taf7l, and Tex11 which are essential genes for sperm count, sperm formation, and maturation (Giese *et al.*, 2012).

Sperm production and maturation are also androgen-dependent processes. ABP works with androgen to regulate the sperm-fertilizing ability (Anthony *et al.*, 1984b; Hermo *et al.*, 1998). The hypophysectomized rat model, which maintains a low testosterone level with pregnenolone, also verifies this result (Anthony *et al.*, 1984a). Further, ABP mRNA levels can be upregulated by estradiol, and this increase could upregulate the expression of transition protein 1 (TP1) involved in nuclear chromatin condensation in rodent spermatids *in vitro* (Caron *et al.*, 2001; Kierszenbaum, 2001; Della-Maria *et al.*, 2002; Aleem *et al.*, 2006).

Clinical applications

Hormones play important roles in the process of reproduction and are attracting increased attention from researchers. Studies show male infertility diseases, like cryptorchidism (Rodprasert *et al.*, 2019; Aldahhan *et al.*, 2021) and the Sertoli cell-only syndrome (SCOS) (Jain and Halder, 2012) usually document disrupted hormone levels. In cryptorchid testes, the FSH and androgen receptors of SCs are dramatically reduced (Agoulnik *et al.*, 2012). Most SCOS testes have low inhibin B but high FSH levels (Jain and Halder, 2012). Hence, detecting the relevant hormone level change can be used as a means of diagnosis, and regulation

of hormone levels. This offers a potentially feasible treatment method for male infertility diseases.

Many studies show that infertility in men is mainly (more than 90%) caused by low sperm counts, poor sperm quality, or both (Leaver, 2016). This indicates that the disruption of spermatogenesis is the major cause of male infertility. We have summarized that spermatogenesis is regulated by hormone-mediated signaling pathways, including PI3K/AKT, and its subsequent effects on SC secretions. Therefore, regulating the PI3K/AKT signaling in SCs (such as using their activators/inhibitors or changing the levels of related hormones) and SC secretions is also a good idea for regulating spermatogenesis and treating male infertility. For example, in cryptorchid testes, the ability of SCs to secrete inhibin B and AMH is affected (Cortes *et al.*, 2016). Further, spermatogenesis is arrested at the undifferentiated type A spermatogonia stage and germ cell apoptosis increases significantly (Agoulnik *et al.*, 2012). However, the SC secretions, SCF and RA promote the differentiation of human SSCs into haploid spermatids in cryptorchid patients (Yang *et al.*, 2014). SCOS, also a major cause of male infertility is characterized by a lack of germ cells, and the seminiferous tubules contain only SCs (Talati and Sheikh, 1991). Most SCs in SCOS can neither organize cell-cell junctions nor stimulate SSCs to produce normal levels of growth factors, such as GDNF, FGF8, and BMP4 (Paduch *et al.*, 2019). The failure of SCs to form normal CX43 junction protein is associated with impaired spermatogenesis in SCO testis (Defamie *et al.*, 2003), including spermatogonia differentiation, spermatocyte meiosis, and sperm maturation (Giese *et al.*, 2012). Additionally, the downregulation of GDNF is associated with reduced spermatogonial self-renewal (Meng *et al.*, 2000; Kubota *et al.*, 2004; Hofmann *et al.*, 2005; Wang *et al.*, 2014). Hence, detecting the level of SC secretions such as inhibin B, AMH, SCF, RA, GDNF, FGF8, BMP4, and CX43 can be used as a means of diagnosis. Further, the regulation of their levels may be a potential treatment method of male infertility diseases.

Therefore, the regulation of hormone levels, the PI3K/AKT signaling, SC secretions, and finally spermatogenesis may be promising methods for treating male infertility, like cryptorchidism and SCOS.

Conclusion and Future Perspectives

We discussed several hormones like FSH, androgen, estrogen, insulin/IGF, relaxin, thyroid hormone, and RA, which all directly mediate the PI3K/AKT signaling in SCs. In addition to the hormones we mentioned, studies show that prostaglandin D2 (PGD2) (Rossi *et al.*, 2016) and leptin (Wang *et al.*, 2018b) also directly stimulate PI3K/AKT signaling in SCs. However, this still requires further exploration. The cross-talk among these hormones in regulating the PI3K/AKT signaling is an interesting topic for future study.

Further, the effects of SC secretions under the control of hormone-mediated PI3K/AKT signaling on spermatogenesis have also been discussed. These include the effects on spermatogonia proliferation and differentiation, spermatocyte meiosis, sperm maturation, and release. The roles of other SC secretions during spermatogenesis however remain unclear.

Finally, we discussed two diseases-cryptorchidism and SCOS from the perspective of hormone regulation and spermatogenesis, hoping to provide clinical hints for their treatment. The deeper and broader effects of spermatogenesis and SC secretions on these diseases require further investigation (Fig. 3).

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Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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