

# Cell–Cell Signaling in the Testis and Ovary

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## INTRODUCTION

The evolution of multicellular organisms was facilitated by the ability of different cells to communicate and interact. This cell–cell signaling generates a higher order functional state than possible with individual cell types. Cell–cell interactions have become an essential requirement for the physiology of any organ or tissue, and are critical in the regulation of any cell's biology. For this reason, elaborate networks of cell–cell interactions have evolved to control the development and maintenance of tissue functions. The focus of the current chapter will be on the regulatory signals that mediate cell–cell interactions in the testis and ovary.

Several previous reviews have discussed the cell–cell interactions in the testis [1–4] and ovary [5–7]. These include a focus on secretory products of the various cell types, and actions of individual regulatory molecules. The current chapter will briefly discuss the advances in cell–cell signaling in these organs.

Many different types of cell–cell interactions are required for the control of tissue physiology and cellular functions. These have been previously categorized into regulatory, nutritional, and environmental classifications [4]. Regulatory interactions are generally mediated by extracellular factors that, through receptor-mediated actions, cause a signaling event to modulate cell functions. Nutritional interactions generally involve the transport of nutritional substances, energy metabolites, or metabolic substrates between cells. Environmental interactions involve extracellular environmental factors that affect cell contacts and cytoarchitecture. The focus of the current chapter will be primarily on regulatory-type interactions that involve a receptor-mediated signaling event. It is this type of cellular signaling that actively regulates a cell's function on a molecular level. The factors involved are generally paracrine and autocrine agents such as growth factors and cytokines.

Both the testis and ovary are endocrine organs. Endocrine hormones from the pituitary (i.e., gonadotropins,

follicle stimulating hormone (FSH), and luteinizing hormone (LH)) act on various cell types to influence cellular functions and cell–cell interactions. The influence these endocrine hormones have on cell–cell signaling events is in part how hormones regulate gonadal function. The testis and ovary are also sites for the production of hormones. These gonadal hormones have an endocrine role in regulating a wide variety of tissues in the body, but also can act in a paracrine manner within the gonads to influence cell–cell signaling and cellular functions. Again, the role these gonadal steroids and peptide hormones play in the regulation of cell–cell signaling within the gonad will be discussed.

## CELL–CELL SIGNALING IN THE TESTIS

### Testis Cell Biology

The adult testis is a complex organ that is composed of seminiferous tubules which are enclosed by a surrounding interstitium. The seminiferous tubules are the site of spermatogenesis where germ cells develop into spermatozoa in close interaction with Sertoli cells (Figure 314.1). The Sertoli cell is an important testicular somatic cell which controls the germ cell environment by the secretion and transport of nutrients and regulatory factors. The Sertoli cells [8] form the basal and apical surface of the seminiferous tubule, and provide the cytoarchitectural framework for the developing germinal cells [3, 9]. Tight junctional complexes between the Sertoli cells contribute to the maintenance of a blood–testis barrier [10], and create a unique environment within the tubule [3, 11]. The structure of the Sertoli cell has been reviewed by several investigators [9], and a three-dimensional reconstruction has increased appreciation for the complexity of the structural relationships between cells within the seminiferous tubule [12]. The biochemical analysis of the Sertoli cell has primarily

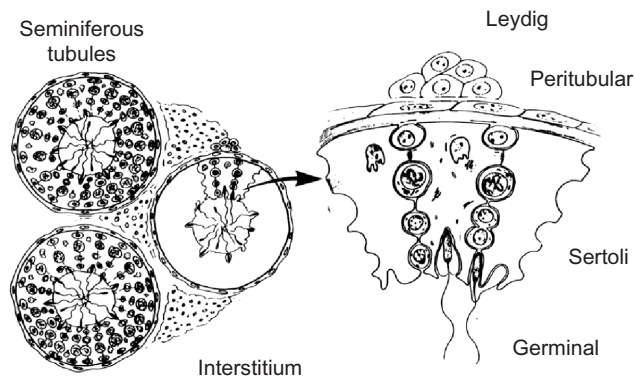


FIGURE 314.1 Testis cell biology.

focused on an examination of the components synthesized and secreted by the cell. The list of products includes steroids such as estradiol [13], metabolites such as lactate [14], and various proteins such as plasminogen activator [15], testicular transferrin [16], testicular ceruloplasmin [17], inhibin [2], and others [2]. The majority of the secretory products are hormonally regulated and provide useful markers of Sertoli cell differentiation.

Surrounding the basal surface of the Sertoli cells is a layer of peritubular myoid cells (Figure 314.1), which function in contraction of the tubule. The peritubular cells surround and form the exterior wall of the seminiferous tubule. Peritubular cells are mesenchymally derived cells that secrete fibronectin [18] and several extracellular matrix components [19]. Both the peritubular and the Sertoli cells form the basement membrane surrounding the seminiferous tubule, and their interactions are important in germ cell development.

The interstitial space around the seminiferous tubules contains another somatic cell type, the Leydig cell (Figure 314.1), which is responsible for testosterone production. Leydig cells have a major influence on spermatogenesis through the actions of testosterone on both the seminiferous tubule and the pituitary. Although the Leydig cell has numerous secretory products [4], testosterone is the most significant secretory product of these cells. Thus, interactions of all three somatic cells, Sertoli, peritubular, and Leydig, are important for regulation of normal spermatogenic function in the testis (for review, see [4]).

## Testis Development

The process of fetal testis formation occurs late in embryonic development (embryonic day 13 where plug date = E0 (E13) in the rat) and is initiated by migration of primordial germ cells, first from the yolk sac to the hindgut, and then from the hindgut to the genital ridge. The first phase of migration is proposed to occur through a mechanism where transient interactions between fibronectin molecules on the extracellular matrix and corresponding receptors on the primordial germ cells cause movement of the germ cells. The second

migration is thought to occur by the release of chemoattractant factors from the genital ridge. Kit ligand and its receptor c-kit appear to be involved first in the migration to the genital ridge, and later in the proliferation of germ cells after colonization of the genital ridge. Expression of kit ligand has been localized to cells along the migratory pathway, and c-kit is expressed by primordial germ cells at this time in development (for review, see [20]). After migration, germ cell differentiation in the gonad is dependent on locally produced factors such as prostaglandins [21], growth factors [22], and the induction of specific transcription factors [23]. It is a complex network of cellular interactions that controls testis and germ cell development.

The gonad has bipotential after germ cell migration, and can be distinguished morphologically from the adjoining mesonephros (E12 in rat), but cannot yet be identified as an ovary or a testis. A variety of genes, such as SRY, SOX-9, SF1, and DMRT1, are involved in the transcriptional induction of sex determination and testis development [24–33]. Two morphological events occur early on embryonic day 13 (E13) to alter the bipotential gonad. First, Sertoli cells, which are proposed to be the first cell in the testis to differentiate, aggregate around primordial germ cells [34, 35]. Secondly, migration of mesenchymal cells occurs from the adjoining mesonephros into the developing gonad to surround the Sertoli cell–germinal cell aggregates. The migrating population of cells has been speculated to be pre-peritubular cells [36–38]. The mechanism for this migration appears to involve chemotactic factors from the Sertoli cell, such as NT3 [39] and FGF9 [22], that cause cell migration. This is postulated due to the observation that ovarian mesonephros can also be stimulated to initiate cell migration after close interaction with a developing testis [40]. In addition, using an organ culture system in which mesonephros and embryonic testis were separated by an embryonic ovary, mesonephros cells migrated through the ovary to the testis [36]. Several growth factors appear to be involved in this initial testis morphogenesis, including interactions between FGF9 and Wnt 4 [22, 41], Wnt(s) [42, 43], and Notch regulators [43]. Therefore, during early testis development Sertoli–peritubular cell interactions may allow for cord formation to occur. The cords develop neonatally into seminiferous cords and, at the onset of puberty, develop into the seminiferous tubules. Sertoli cells have been postulated to originate from stem cells in the coelomic epithelium at an early stage in gonadal development. Other cells which may potentially originate from the coelomic epithelium are interstitial or Leydig cells [44].

Seminiferous cords, precursors of adult seminiferous tubules, form as the Sertoli cell–primordial germ cell aggregates become more organized and are fully surrounded by mesenchymal cells. The formation of the seminiferous cords (E14 in rat) is a critical event in the morphogenesis of the testis, since this is the first indication of male sex differentiation [27]. During the process of cord formation Sertoli

cells undergo a number of morphological changes, including: a change in expression of mesenchymal to epithelial cell markers (vimentin to cytokeratin [45]), a change in expression of cytokeratin 19 to cytokeratin 18 (cytokeratin 21 expressed in ovary [46]), and expression of Müllerian inhibiting substance (MIS), which inhibits the development of the Müllerian duct—the precursor of the female uterus, cervix, fallopian tubes and upper vagina [47, 48]. Vascular endothelial growth factor (VEGF) appears to mediate cell–cell interactions and migrations required for vascularization of the gonad [49].

Outside of the seminiferous cords, the peritubular layer of cells becomes identifiable from the interstitium or Leydig cells at E15 [50], and  $3\beta$ -hydroxysteroid dehydrogenase ( $3\beta$ HSD) production is detected after E15 [48]. Leydig cells have been hypothesized to differentiate after cord formation and Sertoli cell differentiation is completed [51, 52]. This is important, since the production of testosterone and other androgens by the Leydig cells has been demonstrated to stabilize the Wolffian duct derivatives for normal male duct development [53, 54]. Therefore, appropriate differentiation of somatic cell types in the testis around the time of cord formation is crucial not only to the normal development of the testis, but also for the continued presence of the Wolffian duct and normal male reproductive tract development.

### Testis Cell–Cell Interactions

Table 314.1 outlines a number of the factors produced locally in the testis that mediate cell–cell signaling events in the control of spermatogenesis and testis function. Several reviews address the topic of cell–cell interactions in the testis and the control of spermatogenesis [2, 4, 55, 56]. Recent observations are cited below.

Transforming growth factor- $\alpha$  (TGF $\alpha$ ) is an epidermal growth factor (EGF) superfamily member, and is produced by Sertoli, peritubular, and Leydig cells. TGF $\alpha$  can act as a growth stimulator on all the major cell types in the testis [57–59]. In contrast, transforming growth factor-beta (TGF $\beta$ ) is also produced by Sertoli, peritubular, and Leydig cells, and can act on all the major cells in the testis [60–64]. TGF $\beta$  primarily acts as a growth inhibitor, and can stimulate a variety of functions of differentiated cell types. A number of other TGF $\beta$  superfamily members have also been shown to regulate testis function and development [65], including bone morphogenic proteins (BMPs), activins, and growth differentiation factors (GDFs) [65]. Another example of a factor that is produced by all the testis cells and acts on all major cell types in the testis is insulin-like growth factor-1 (IGF-1) [66–68]. IGF-1 plays a general role in regulation of the growth cycle and homeostasis of the testis. A related family member, IGF-2, mediates paracrine interactions between Sertoli cells and germ

cells [69]. These are examples of regulatory factors that mediate cell–cell signaling events between the majority of the cell types in the testis.

Several interleukins (IL-1 $\alpha$ , IL-1 $\beta$ , IL-6) are produced in the testis by Sertoli cells and Leydig cells. These interleukins can regulate Sertoli, Leydig, and germ cell growth and differentiation functions [70–77]. Nitric oxide may be a mediator of interleukin actions [78]. Although further analysis is needed, interleukins appear to mediate primarily Sertoli–germ cell and Leydig–Sertoli cell interactions, as well as having autocrine roles for these factors.

Several hormonal factors produced in the testis also act locally within the testis as paracrine factors. One example is inhibin and its related peptide, activin [79–81]. Inhibin is primarily produced by Sertoli cells, and can act on germ cells and Leydig cells. Further investigation of the actions of inhibin and related compounds within the testis is needed. Another major endocrine factor produced in the testis is testosterone, which is generated by Leydig cells and can in turn act on Sertoli, peritubular, and Leydig cells [82]. Androgens have a major role in the maintenance of testis function by inducing cellular differentiated functions. The specific mechanism of action and gene products influenced by androgens remain to be elucidated. Early in prepubertal development, testosterone can also be metabolized by Sertoli cells to produce estrogen [13]. The ability of Sertoli cells to produce estrogen declines as the cells differentiate during puberty, and the role of estrogen in the testis is unclear.

Fibroblast growth factor (FGF) family members have been shown to be expressed in the testis, and regulate the growth and differentiation of a variety of cells [83–87]. FGF receptors are predominant in germ cells and Leydig cells, but are also present in others [84]. FGF-14 has recently been shown to be expressed in spermatocytes, and may influence adjacent Sertoli or peritubular cells. FGF-9 null mutants also suggest a role for FGF9 in early testis development, but this remains to be investigated in the adult [85]. Basic-FGF (bFGF) is produced by Sertoli cells and also can act on the other cells [86, 88], and appears to be influenced by androgens [88]. The role of the various FGF ligands and receptors in testis function remains to be elucidated.

Platelet-derived growth factor (PDGF) has been shown to be produced by Sertoli cells and to influence peritubular cells and Leydig cells [89–91]. Although PDGF in the adult may also be produced by the Leydig cell [92], it appears to be a factor produced within the seminiferous tubules that acts on adjacent peritubular and Leydig cells. Another factor that is only produced by Sertoli cells is stem cell factor (SCF)/kit ligand (KL), which has a direct role in regulating spermatogonial cell proliferation [93–96]. Mutations in SCF/KL block the process of spermatogenesis. A growth factor with similar activity is glial cell derived neurotrophic factor (GDNF), which is produced by Sertoli cells and acts on spermatogonial stem cells [97]. These are the best examples of somatic–germ cell interactions.

**TABLE 314.1** Cell–cell signaling factors in the testis

Signaling factor	Site production	Site action	Functions	Ref(s)
Transforming growth factor $\alpha$ (TGF $\alpha$ )	Sertoli	Sertoli	Growth stimulation	[57–59]
	Peritubular	Peritubular		
	Leydig	Leydig		
Transforming growth factor $\beta$ (TGF $\beta$ )	Sertoli	Sertoli	Growth inhibition	[60–64]
	Peritubular	Peritubular	Differentiation, stimulation	
	Leydig	Leydig		
		Germ		
Insulin-like growth factor (IGF1)	Sertoli	Sertoli	Homeostasis and DNA synthesis	[66–68]
	Peritubular	Peritubular		
	Leydig	Leydig		
Interleukin-s	Sertoli	Sertoli	Growth regulation	[70–77]
	Leydig	Leydig	Cellular differentiation	
		Germ		
Inhibin	Sertoli	Germ	Cellular differentiation	[79–81]
		Leydig		
Androgen	Leydig	Sertoli	Cellular differentiation	[82]
		Peritubular		
		Leydig		
Fibroblast growth factors	Sertoli	Germ	Growth stimulation	[83–87, 239]
	Germ	Peritubular		
	Leydig	Sertoli		
Platelet-derived growth factor (PDGF)	Sertoli	Peritubular	Growth stimulation	[89–92]
		Leydig	Cellular differentiation	
Stem cell factor/kit ligand (SCF/KL)	Sertoli	Germ	Growth stimulation	[93–96]
Leukemia inhibitory factor (LIF)	Peritubular	Germ	Growth stimulation	[98, 99]
	Sertoli		Cell survival	
	Leydig			
Tumor necrosis factors	Germ	Sertoli	Cellular apoptosis	[100–102]
	Leydig	Germ	Cellular differentiation	
Hepatocyte growth factor (HGF)	Peritubular	Leydig	Growth stimulation	[105–107]
		Peritubular	Tubule formation	
		Sertoli		
Neurotrophins	Germ	Sertoli	Growth stimulation	[4, 109]
	Sertoli	Peritubular	Cell migration	
Glial cell derived neurotropic factor (GDNF)	Sertoli	Spermatogonia	Growth stimulation	[97]
			Cellular differentiation	

Leukemia inhibitory factor (LIF) is a pleiotropic cytokine that influences stem cell growth and survival. LIF is predominantly produced by peritubular cells, but also by Sertoli cells and Leydig cells [98]. Although LIF has been shown to influence germ cell growth and survival [99], other functions remain to be elucidated.

Tumor necrosis factors (TNF $\alpha$ ) and related ligands (TRAIL) are produced in the testis by germ cells and Leydig cells. Both TNF and TRAIL have a role in regulating germ cells and Sertoli cells [100–103]. Germ cell apoptosis in response to hormone deficiency or environmental compound exposure is mediated in part through TNF $\alpha$  and TNF $\beta$  involving Sertoli cell and germ cell interactions [103, 104]. These regulatory factors for the germ cells may be more involved in apoptosis regulation, unlike in Sertoli cells, in which they may be more involved in cellular differentiated functions.

Hepatocyte growth factor (HGF) is generally a mesenchymal-derived factor that acts on adjacent epithelial cells. HGF was found to be expressed by the mesenchymal-derived peritubular cells, and its receptor (cmet) was found on both Sertoli cells and Leydig cells [105–107]. Interestingly, cmet was also found in the peritubular cells. HGF also may have a role in seminiferous tubule formation [107].

Several neurotrophins have been shown to be expressed in the testis. Nerve growth factor (NGF) is produced by germ cells in the adult, and appears to act on the Sertoli cells [4, 108]. NGF can act as both an autocrine and a paracrine factor to regulate spermatogenesis [108]. In embryonic development, neurotrophin-3 is expressed by Sertoli cells and acts on the migrating mesonephros cells to promote seminiferous cord formation [39, 109]. Further investigations are needed to elucidate the roles of these and other neurotrophins in the testis.

Additional factors are anticipated to be identified and have critical roles in testis development. Newly identified factors such as erythropoietin (found to be expressed by Sertoli cells and peritubular cells [110]), hedgehog factors (found to affect spermatogenesis [111]), ghrelin [112] and interferon-gamma (found to act on Sertoli cells [113]), and relaxin-like factor (RLF) (expressed by Leydig cells [114]) will all likely have roles in cell–cell signaling in the testis. These and other factors [115] need to be further investigated to determine roles in testis cell biology. Clearly, a complex network of cell–cell signaling events and factors regulates testis function and spermatogenesis.

## CELL–CELL SIGNALING IN THE OVARY

### Ovarian Cell Biology

The ability of somatic cells in the gonad to control and maintain the process of gametogenesis is an essential requirement for reproduction. The basic functional unit

in the ovary is the ovarian follicle, which is composed of somatic cells and the developing oocyte (Figure 314.2). The two primary somatic cell types in the ovarian follicle are the theca cells and granulosa cells. These two somatic cell types are the site of action and synthesis of a number of hormones which promote a complex regulation of follicular development. The proliferation of these two cell types is in part responsible for the growth of the ovarian follicle. The elucidation of factors that control ovarian somatic cell growth and development is critical to understanding ovarian physiology.

Granulosa cells are the primary cell type in the ovary that provide the physical support and microenvironment required for the developing oocyte (Figure 314.2). Granulosa cells are actively differentiating cell with several distinct populations. Alteration and progression of cellular differentiation is required during folliculogenesis from the arrested primordial stage of development through ovulation to the luteal stage of development. Regulation of granulosa cell cytodifferentiation requires the actions of a number of hormones and growth factors. Specific receptors have been demonstrated on granulosa cells for the gonadotropins FSH [116] and LH [117]. In addition, receptors have been found for factors such as EGF [118, 119], insulin-like growth factor [120], and anti-Müllerian hormone [121]. The actions of these hormones and growth factors on granulosa cells vary with the functional marker being examined and the stage of differentiation. The biosynthesis of two important ovarian steroids, estradiol (Figure 314.2) and progesterone, is a primary function of the granulosa cells in species such as cattle, humans, and rodents. Estrogen biosynthesis is controlled by the enzyme aromatase, which requires androgen (Figure 314.2) produced by the theca cells as a substrate. As the follicle develops, granulosa cells differentiate and estrogen biosynthesis increases. FSH promotes this follicular development via the actions of cAMP. As the follicle reaches the stages before ovulation, the granulosa cells develop an increased capacity to synthesize and secrete progestins under the control of LH. In contrast, the early follicle stage (e.g., primordial) granulosa cells appear to be hormone-independent and are non-steroidogenic.

Another important cell type in the ovary is the ovarian theca cell (Figure 314.2). These are differentiated stromal cells that surround the follicle and have also been termed theca interstitial cells [122]. The inner layer of cells, the theca interna, has a basement membrane separating it from the outermost layer of mural granulosa cells. One of the major functions of theca cells in species such as cattle, humans, and rodents is the secretion of androgens which are used by granulosa cells to produce estrogen [123]. Theca cells respond to LH by increasing the production of androgens from cholesterol [124] (Figure 314.2). Theca cells also produce progestins under gonadotropin control [125–128]. Other secretory products of theca cells have not been thoroughly investigated. At the primordial

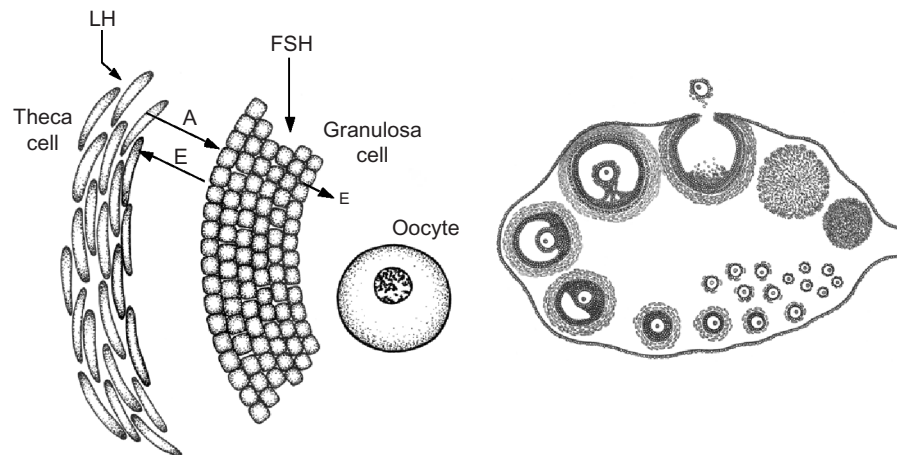


FIGURE 314.2 Ovary cell biology.

stage no theca cells are present; however, during transition to the primary stage theca cells (i.e., pre-cursor cells) are recruited to the follicle [7].

### Follicle Growth and Differentiation (i.e., Folliculogenesis)

The control of ovarian follicle development is complex, and involves multiple waves of growth [129]. In the initial stage of follicle development, arrested primordial follicles undergo primordial follicle transition to begin follicle growth [5]. In both the human and bovine ovary, two or three waves of follicles are initiated to develop in a single ovarian cycle [129, 130]. For both these species, follicles expand to up to 2cm in diameter during this process. A combination of granulosa cell growth, theca cell growth, and antrum formation (i.e., formation of fluid-filled space in the developing follicle) results in the expansion of the ovarian follicle (Figure 314.2). Although a rapid stimulation of cell growth is required for the ovulatory follicle to develop, the vast majority of follicles undergo atresia, in which cell growth is arrested at various stages of follicle development. Hormones such as estrogen and FSH have been shown to promote follicle cell growth *in vivo*; however, these hormones alone do not stimulate growth of ovarian cells *in vitro* [131]. The possibility that these hormones may act indirectly through the local production of growth factors is proposed for later stages of development. Therefore, the regulation of ovarian cell growth is a complex process that requires an array of externally and locally derived regulatory agents. Interactions between theca cells, granulosa cells, and oocytes are required for follicular maturation [132]. The individual processes, such as dominant follicle selection [133] and follicle cell apoptosis/atresia [134, 135], also require integrated cell–cell interactions. A variety of specific growth factors produced in the follicle appear to mediate many of these cellular interactions in later stages of follicle development.

### Ovarian Cell–Cell Interactions

Table 314.2 outlines a number of the factors produced locally in the ovary that mediate cell–cell signaling events in the control of follicle development and ovarian function. Several reviews address the topic of cell–cell interactions in the ovary and the control of follicle development [5, 7, 136–139]. Recent observations are cited below.

The epidermal growth factor (EGF) family of growth factors regulates cell–cell interactions in the ovary. TGF $\alpha$  has been shown to be produced by theca cells [140–143] and influence the growth of both theca and granulosa cells [143, 144]. Several *in vivo* experiments have shown that TGF $\alpha$  can influence follicle development [145, 146]. TGF $\alpha$  appears to be important for follicle development, and involves theca cell–granulosa cell interactions. TGF $\alpha$  has also been localized to isolated granulosa cells, but appears predominately in theca cells [147]. The primary function of TGF $\alpha$  is growth stimulation. Several other members of the EGF family are also involved including amphiregulin (AR), beta cellulin (Bt), and epiregulin (Ep) for granulosa cells [148, 149]. The EGF receptor is also expressed and can be regulated by hormones such as LH and GnRH [150, 151]. The EGF family also has a role in the ovarian surface epithelial cell biology [152]. Therefore, the EGF family members mediate cell–cell interactions in the ovarian follicle, with autocrine granulosa interactions being predominant.

HGF is produced by theca cells, and acts on granulosa cells to promote cell proliferation and function [153, 154]. This is an excellent example of the role HGF plays in mediating mesenchymal–epithelial interactions in tissues. Interestingly, SCF/KL produced by the granulosa cells can provide feedback to the theca cells to stimulate HGF production [155, 156]. In a similar manner, keratinocyte growth factor (KGF) is produced by theca cells and acts on granulosa cells to regulate cell growth [157, 158]. KGF can promote primordial follicle transition [159], and is also

**TABLE 314.2** Cell–cell signaling factors in the ovary

Signaling factor	Site production	Site action	Functions	Ref(s)
Transforming growth factor $\alpha$ (TGF $\alpha$ )	Theca	Granulosa Theca	Growth stimulation	[144–147, 166]
Transforming growth factor $\beta$ (TGF $\beta$ )	Theca Granulosa	Granulosa Theca	Growth inhibition Cellular differentiation	[186–190]
Hepatocyte growth factor (HGF)	Theca	Granulosa	Growth stimulation	[154–156, 158]
Keratocyte growth factor (KGF)	Theca	Granulosa	Growth stimulation	[153, 157, 160]
Colony stimulating factor (CSF)	Theca	Granulosa Theca	Growth regulation	[161, 162]
Tumor necrosis factor (TNF)	Granulosa Theca Oocyte	Oocyte Granulosa Theca	Apoptosis Growth regulation	[170–173]
Fas ligand	Granulosa Theca Oocyte	Oocyte Granulosa Theca	Apoptosis	[176, 177, 240, 241]
Nerve growth factor (NGF)	Theca	Granulosa Theca	Growth stimulation Ovulation	[180]
Fibroblast growth factor (bFGF)	Granulosa Theca Oocyte	Granulosa Theca	Growth stimulation	[182–184]
Growth differentiation factor-9 (GDF-9)	Oocyte	Granulosa Theca	Cellular differentiation	[192–196]
Bone morphogenic proteins (BMP)	Oocyte Theca	Granulosa Theca	Cellular differentiation	[200, 201, 203–205, 207, 108]
Kit ligand/stem cell factor (KL)	Granulosa	Oocyte Theca	Growth stimulation	[209–213]
Leukemia inhibitory factor (LIF)	Granulosa	Oocyte Theca	Growth stimulation Cellular differentiation	[215, 216]
Vascular endothelial factor (VEGF)	Theca Granulosa	Edothelium Granulosa	Angiogenesis	[217, 221]
Interleukins	Granulosa Theca	Granulosa Theca	Cellular differentiation	[164–167]
Insulin-like growth factor (IGF-1)	Granulosa Theca	Oocyte Granulosa Theca	Growth stimulation Cellular differentiation	[222–224]
Inhibin	Granulosa	Oocyte Theca Granulosa	Cellular differentiation	[226, 227]
Anti-Müllerian hormone (AMH)	Granulosa	Oocyte Granulosa	Cellular differentiation	[228]

expressed in the corpus luteum [160]. As was the case for HGF, SCF/KL was found to stimulate KGF expression by theca cells [156]. These factors reflect the importance of the theca cell in the regulation of follicle growth.

A number of immune-related cytokines have a potential role in the ovary. Granulocyte-macrophage colony-stimulating factor (GM-CSF) was found to be expressed primarily by theca cells in the ovary [161, 162]. The GM-CSF can influence granulosa cell growth and function. Null mice had abnormal follicle development, suggesting effects on the local cell–cell interactions [161]. Cytokines, as seen with the testis, also influence ovary function [163]. The interleukins -1, -6, and -8 have all been shown to regulate follicle development. IL-1 is expressed by the granulosa, and affects granulosa function [164, 165]. IL-8 is primarily expressed by the theca, and to a lesser extent by granulosa, and influences cellular function [166]. IL-6 is also expressed by granulosa cells and acts on various cells, including granulosa [167]. Further investigation of the specific roles of these and other members of the interleukin family is needed. Recent analyses of the granulosa cell transcriptome revealed that a number of immune-related cytokines are expressed, suggesting roles for these secreted factors in local cell–cell interactions that also require further investigation [168, 169].

Apoptosis is an essential aspect of follicle development and ovarian function. The vast majority of follicles undergo atresia and apoptosis. TNF has been shown to be produced by most cell types in the ovary associated with apoptosis [170–175]. TNF can act on all the cell types, and induce apoptosis or growth regulation. Another death ligand that binds death receptors to induce apoptosis is Fas ligand. Fas is also produced by all the cells associated with apoptosis, and acts to promote apoptosis in the atretic follicles [176–178]. The endocrine system can regulate the expression and action of these factors to subsequently regulate apoptosis [175, 178]. These signaling molecules are essential for ovarian function in promoting follicle atresia during folliculogenesis.

Nerve growth factor (NGF) was found to be expressed by theca cells and act on theca and granulosa cells [150]. NGF promotes the early stage of follicle growth [179]. The localization and actions suggest a potentially important role at the time of ovulation [180]. Other neurotrophins (e.g., NT4) are also expressed at various stages of ovary development [181] and require further investigation.

Basic fibroblast growth factor (bFGF) has been shown to be expressed by granulosa cells, and to a lesser extent by theca cells [182]. bFGF can regulate both granulosa cell and theca cell growth and differentiated functions [183, 184]. During follicle development the expression of bFGF changes, being in the oocyte at the primordial stage and then in the granulosa at the primary stage [7]. FGF9 has been shown also to mediate ovarian cell–cell interactions, being produced by theca cells, stroma, and the CL, and acting on granulosa,

the oocyte, theca cells, and CL [185]. The role of other FGF family members has not been rigorously addressed.

The TGF $\beta$  superfamily of growth factors also has a critical role in regulating ovarian function [138]. Members of the family involved include TGF $\beta$ , GDF9, BMPs, and AMH. TGF $\beta$  is predominately produced by theca cells [186], but is also produced by isolated granulosa in selected follicle stages [187]. TGF $\alpha$  and TGF $\beta$  differentially regulate granulosa and theca cell differentiated functions and growth [188–190]. Although TGF $\beta$  inhibits TGF $\alpha$  growth stimulation, TGF $\beta$  also can influence cell functions [191].

Growth differentiation factor-9 (GDF-9) is a member of the TGF $\beta$  superfamily, and is specifically localized to the oocyte. GDF-9 can act on both granulosa cells and theca cells to regulate steroidogenesis and differentiated functions [191–197]. The actions of GDF-9 are follicle stage-specific, and appear to be expressed in a variety of species. In early follicle development in the rat, GDF9 promotes primary follicle progression [198], while in pigs GDF9 promotes primordial to primary follicle transition [199]. GDF-9 regulates the expression of other paracrine factors such as SCF/KL in the developing follicle [139, 194]. This is one of the few oocyte-specific products identified to be involved in cell–cell signaling in the ovary.

Another factor specifically expressed in the oocyte that appears to regulate granulosa cell function is BMP-15 [197, 200–202]. BMP-15 and GDF-9 may act synergistically during follicle development. Other BMP family members include BMP4 and -7, which are primarily localized in the theca cells and appear to act on the granulosa cells [203]; BMP2, which acts on granulosa cells [202, 204]; and BMP6, which also is expressed in the oocyte and acts on the granulosa cells [202, 205]. In early follicles, BMP4 promotes oocyte survival and primordial follicle transition [206]. The BMP family of growth factors are members of the TGF $\beta$  superfamily, and appear to be critical to follicle development [197, 202, 207, 208].

Stem cell factor/kit ligand (SCF/KL) is produced by the granulosa cell, and acts on the oocyte and theca cells [139, 209–213]. The null mutant suggests a critical role in oocyte viability and recruitment of primordial follicles. This role in promoting primordial follicle transition was confirmed in organ culture experiments [214]. In addition to the role in granulosa–oocyte interactions, granulosa KL also influences theca cell function and development [212]. Oocytes appear to have a regulatory role in influencing the expression of KL by granulosa cells [139, 210]. As found in the testis, this is a critical somatic–germ cell interaction. Another factor found to be expressed by granulosa cells and that regulates oocytes is LIF [215, 216]. LIF can promote primordial follicle transition [198], and is also produced by stromal cells in the ovary. This action of LIF in mediating granulosa–oocyte interactions is supported by levels of LIF that increase in follicular fluid as the follicle develops [215, 216].



Vascular endothelial factor (VEGF) has a critical role in angiogenesis. This process is important for developing follicles past the primary stage of development. VEGF is primarily expressed in theca cells, and to a reduced level by granulosa cells [217–221]. VEGF has a major role in acting on endothelial cells to promote angiogenesis, but also can influence granulosa cell functions [220]. This cell–cell signaling event controlled by VEGF is critical for follicle development.

IGF-1 also has a role in the ovarian follicle [222]. IGF-1 is expressed by granulosa and theca cells, and acts on the oocyte, granulosa, and theca cells [222–224]. Mice with null mutations in IGF-1 have impaired follicle development [224]. IGF-2 and the IGF-binding proteins also have a critical role in follicle development [223]. A related family member, relaxin, also integrates with the insulin family and may have a role in the ovary [225].

Inhibin also has a paracrine role in the developing follicle. Inhibin is primarily produced by the granulosa cells, and acts on the oocyte, theca, and granulosa cells [226, 227]. Related family members, such as the activins, are also anticipated to have similar roles. This is distinct from the roles these factors have in the endocrine system.

Additional signaling factors are anticipated to be essential for ovarian function and follicle development. One example is anti-Müllerian hormone (AMH), which is expressed by the granulosa cells [228] and may have a role as a negative regulator of oocyte viability and/or primordial follicle development [229, 230]. Local steroid production is also expected to influence the network of local cell–cell signaling events. This includes both androgen and estrogen production [231]. Newly identified developmental factors, such as Nodal, affect granulosa cell apoptosis [232, 233]; the Notch ligands (e.g., Delta) mediate oocyte and somatic cell interactions [234]; and endothelin 2 has effects on granulosa cells [235]. Platelet-derived growth factor (PDGF) also has a role in primordial follicle transition in the adult follicle and in the CL [175, 236, 237].

## SUMMARY

The above descriptive discussion of cell–cell signaling in the testis and ovary demonstrates a growing complexity in the networks of cellular interactions and factors. It is anticipated some of these factors will have compensatory roles to assure growth and differentiation of the tissues. The list of factors provided is likely only partially complete, and will have more added as investigation of cell–cell interactions in the gonads expands. The advent of microarray procedures and analysis of the ovarian transcriptome have expedited this research [238]. Currently, we are primarily in the research phase of identifying the sites of production and action for these factors. The functions of some individual factors are also being analyzed. However, the next research

phase of cell–cell signaling will involve a more systems biology approach to tie together all the potential interactions and gain more insight into the regulation of testis and ovary function.

The specific cell–cell signaling events identified are shown in most cases to change during development. The requirements and physiology of the embryonic testis and ovary are very different from the adult. Another research area to expand is the elucidation of cell–cell signaling at these different stages of development.

A comparison of the cell–cell signaling events between the testis and ovary is very useful. Some signaling events are the same. For example, the role SCF has in mediating direct somatic–germ cell interaction and the role HGF and KGF play in mesenchymal–epithelial cell interactions is similar. A direct correlation of the cell–cell interactions of the testis and ovary will be invaluable in elucidating the systems biology approach to understanding gonadal function.

Elucidation of cell–cell signaling events is required for the future development of therapeutic agents to control fertility and treat reproductive diseases. Through understanding the signaling events involved in testis and ovary function, basic information is provided to design more effective therapeutics. Significant advances are anticipated to be in the area of contraceptive and fertility agent development, and treatment of diseases such as polycystic ovarian disease or premature ovarian failure. Although an understanding of the intracellular signaling events is essential for understanding how a factor acts, the elucidation of the network of extracellular signaling molecules that regulates a cell's function is essential to understand how a whole tissue or organism functions.

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