Published December 11, 2014

Growth Factors in Gonadal Development

Michael K. Skinner

Reproductive Endocrinology Center, University of California, San Francisco 94143-0556

ABSTRACT: Growth factor-mediated cell-cell interactions are required for both testicular and ovarian cell growth during gonadal development. A number of different growth factors have been shown to be produced locally in the testis and ovary that can regulate cellular proliferation. The specific growth factors potentially involved in gonadal cell-cell interactions are briefly reviewed. Combined observations imply that the hormonal and developmental regulation of gonadal growth is indirectly regulated through the production and action of locally produced growth factors.

Key Words: Testis, Ovary, Growth Factors and Gonadal Development

J. Anim. Sci. 1992. 70(Suppl. 2):30–41

Introduction

The growth and development of a tissue requires the local production and integrated actions of specific growth factors. These growth factors mediate critical cell-cell interactions that control cell proliferation and organ development. The specific growth factors identified have increased dramatically in number and often exist in families. Growth factor families are composed of unique gene products that have similarities in structure and function but often differ in the manner in which gene expression is controlled. This allows for unique sites of expression and more precise developmental and hormonal regulation of growth factor production. A partial list of several of the major types of growth factors is shown in Table 1 to provide the nomenclature and major functions attributed to common growth factors.

Gonadal development also requires growth factor-mediated cell-cell interactions as a general mechanism to control cellular proliferation. Concerning the testis the majority of research and information available involves pubertal development and adult testis function. For the ovary the majority of information involves the process of follicular development and adult ovarian function. These will be the primary stages of development discussed in the current review; however, many of these same cell-cell interactions and growth factors will also likely be important for other stages of gonadal development. Research has also primarily been focused on the identification of the sites of production and actions of specific growth factors. Therefore, the current review will emphasize the specific growth factors potentially involved in gonadal cell-cell interactions, whereas the specific functions these growth factors have during development largely remain to be elucidated.

Growth Factors in the Testis

Testis physiology requires a complex network of cell-cell interactions (Skinner, 1991), and one class of regulatory agents involved in these interactions are growth factors (Bellve and Zheng, 1989). The primary physiological process controlled, spermatogenesis, occurs within the seminiferous tubules, which are composed of a variety of cell types. Sertoli cells form the tubule, create the blood-testis barrier, and provide the proper structural support and microenvironment for germinal cell development. The mesenchymal (i.e., stromal) derived peritubular myoid cells surround the tubule and are separated from the epithelial Sertoli cells by a complex extracellular matrix. Leydig cells in the interstitium produce androgens necessary for testis function. Other cell types present in the interstitial tissue include macrophages, fibroblasts, and lymphatic endothelial cells. Precise growth regulation is necessary for the development of the testis and maintenance of spermatogenesis (Clermont and Perey, 1957). During fetal development all testicular cell types proliferate. The Leydig, peritubular, and Sertoli cells continue to grow actively in the prepubertal

Table 1. Properties and nomenclature of several common growth factors

Growth factor		Approximate size, kDa	Examples of physiological action
Insulin-like growth factor-I	IGF-I	7.5	Skeletal growth
Insulin-like growth factor-II	IGF-II	7.5	Fetal development
Epidermal growth factor	EGF	6	Tissue growth
Transforming growth factor- alpha	TGF-α	5	Tissue growth
Transforming growth factor-beta	TGF-β	25/dimer	Growth inhibition and tissue repair
Fibroblast growth factor	FGF	17	Angiogenesis and tissue growth
Nerve growth factor	NGF	13	Neuronal development
Interleukin-1	IL-1	17	Immune response and inflammation
Platelet-derived growth factor	PDGF	30/dimer	Tissue growth

testis. Sertoli cells terminally differentiate and cease to divide at an early stage in pubertal development (Orth, 1982). Peritubular-myoid cells first appear in late fetal development, and the majority of peritubular proliferation may occur during formation of tubules. Peritubular cells continue to proliferate slowly in the adult and have a defined turnover rate (Teerds et al., 1989). Leydig cells appear in late fetal development and continue to grow and differentiate before puberty (Lording and deKretser, 1987). Leydig cell growth is slowed but continues in the adult (Hardy et al., 1989; Teerds et al., 1989). Compared with the somatic cells of the testis, germinal cells exhibit a delayed growth pattern. Some germinal cell development begins shortly after birth, when gonocytes mitotically divide to form spermatogonia. At the onset of puberty, germinal cells mitosis and meiosis begins, initiating "waves" of spermatogenic cell proliferation. The control of cell proliferation of these various cell types throughout testis development requires the local production and action of various growth factors. Several of the major growth factors identified to be produced and(or) to act in the testis are summarized in Table 2.

Insulin-Like Growth Factor

The insulin-like growth factors (IGF) derive their name from their structural similarity to insulin (Froesch et al., 1985). Insulin-like growth factor I (previously termed somatomedin C) is considered an essential factor for cellular replication and is a progression factor for cell growth and DNA synthesis. Liver production and secretion of IGF-I accounts for the high levels of IGF-I in serum and interstitial fluid (Daughaday and Rotwein, 1989). Insulin-like growth factor II is another member of this family and may act as a growth factor during fetal development.

Insulin-like growth factor I mRNA was originally identified in whole testis (Casella et al., 1987), and subsequently all the somatic cells have been shown to express and produce this factor (Chatelain et al., 1987; Smith et al., 1987; Closset et al., 1989; Cailleau et al., 1990; Naville et al., 1990). All the cell types also seem to respond to IGF. Both Sertoli and germinal cells contain receptors for IGF-I (Handelsman et al., 1985; Hansson et al., 1989; Oonk et al., 1988, 1989). Insulin-like growth factor I stimulates DNA synthesis (Borland et al., 1984), increases transferrin and lactate production in immature Sertoli cells (Skinner and Griswold, 1983; Oonk et al., 1989), and stimulates Leydig cell steroidogenesis (Kasson and Hsueh, 1987; Perrard-Sapori et al., 1987). Luteinizing hormone (LH) upregulates IGF-I receptors on Leydig cells (Lin et al., 1986; Kasson and Hsueh, 1987; Lin et al., 1987b).

Table 2. Growth factors in the testis

Growth factor	Proposed site of production	Proposed site of action	Proposed function[a]
IGF-I	Leydig	Leydig	+Steroidogenesis
	Peritubular	Peritubular	+Growth
	Sertoli	Sertoli	+Growth/Differentiation
		Germinal	?
TGF-α	Peritubular	Leydig	-Steroidogenesis
	Sertoli	Peritubular	+Growth
	Leydig	Sertoli	± Differentiation
		Germinal	?
TGF-β	Peritubular	Leydig	-Steriodogenesis
	Sertoli	Peritubular	-Growth/ +Differentiation
		Sertoli	+Differentiation
		Germinal	?
IL-1	Sertoli	Leydig	-Steroidogenesis
		Germinal	?
FGF	Sertoli	Leydig	± Steroidogenesis
		Sertoli	+Growth
NGF	Germinal	Sertoli	?

[a]A (+) denotes an increase, (-) indicates a decrease, and (?) represents an unknown function.

The presence of the blood-testis barrier prevents interstitial fluid-derived IGF-I from directly affecting sequestered germ cells. Thus, Sertoli cell production of this essential factor may allow for paracrine control of germinal cell proliferation. This is further suggested by the presence of IGF-I receptors and immunoreactivity in spermatocyctes and spermatids (Tres et al., 1986). Local production of IGF-I binding protein may act as a mechanism to concentrate local levels of this factor (Cailleau et al., 1990). Although IGF-I may influence cell function, speculated cell-cell interactions involving IGF-I need to be questioned because all the somatic cell types are exposed to high levels of liver-derived IGF-I present in interstitial fluid.

Another member of the IGF family, IGF-II, has also been suggested to be involved in local interactions. Both Sertoli and germinal cells contain IGF-II receptors (O'Brien et al., 1989), but IGF-II does not seem to be expressed locally (Murphy et al., 1987). Insulin-like growth factor II seems to stimulate Sertoli cell differentiation, perhaps by cross-reacting with IGF-I receptors (Borland, 1984). Neither IGF-I nor IGF-II has been demonstrated to act directly on germ cells, and further study is necessary to understand the physiological importance of these factors.

Transforming Growth Factor-α/Epidermal Growth Factor

Transforming growth factor-alpha (**TGF-α**) is one of the structurally related peptides belonging to the epidermal growth factor (**EGF**) family (Derynck, 1988; Carpenter and Cohen, 1990). Due to similar protein structure, these factors act at the same receptor to stimulate cell growth (Carpenter, 1987). Transforming growth factor-alpha is synthesized as a transmembrane precursor, which may activate EGF receptors on neighboring cells or be proteolitically cleaved to release mature peptide. Transforming growth factor-alpha seems to be produced by non-transformed cells and may play an important role as a growth regulator in normal tissues.

Epidermal growth factor has been implicated in the maintenance of spermatogenesis (Stastny and Cohen, 1972). Removal of the salivary glands from mice resulted in a 50% reduction of mature sperm, whereas EGF replacement returned spermatogenesis to normal levels (Tsutsumi et al., 1986). Circulating concentrations of EGF, however, are considered too low to mediate endocrine action (Carpenter and Zendegui, 1986). Epidermal growth factor does not seem to be expressed in the testis (Skinner et al., 1989), and EGF actions may be mediated by a locally produced EGF-like factor that blocks EGF from binding to its receptor (Holmes et al., 1986). Transforming growth factor-alpha is an EGF-like factor that may mediate these effects, and both peritubular cells and Sertoli cells, but not germinal cells, express the gene for TGF-α and produce this factor (Skinner et al., 1989). Transforming growth factor-alpha also has been immunohistochemically detected in Leydig cells (Teerds et al., 1990). Leydig cell production of TGF-α has not been demonstrated, and this observation may be due to membrane-bound TGF-α precursor or endocytosis of paracrine-derived TGF-α. Scatchard analysis indicates that high-affinity EGF receptors are present on peritubular cells (Skinner et al., 1989). Transforming growth factor-alpha stimulates peritubular cell DNA synthesis and cell division (Skinner et al., 1989). Both peritubular and Sertoli cell production of TGF-α may contribute to peritubular cell growth. At present it is unclear whether Sertoli or germinal cells contain receptors for EGF. Scatchard analysis and histochemistry do not indicate the presence of receptors on differentiated Sertoli cells (Skinner et al., 1989; Stubbs et al., 1990). However, another report presents immunological evidence that Sertoli cells may contain EGF receptors (Suarez-Quian et al., 1989). Further examination using molecular probes for the receptor are necessary. Recent literature suggests that EGF may alter Sertoli cell function, including stimulation of lactate and inhibin production (Mallea et al., 1986; Welsh and Hsueh, 1982). Due to potential peritubular-Sertoli interactions, analysis of Sertoli cell function requires pure preparations of Sertoli cells. Some actions of TGF-α/EGF may be mediated indirectly by peritubular cell production of other factors. Leydig cells contain EGF receptors but do not proliferate in response to EGF (Ascoli, 1981). Epidermal growth factor/TGF-α inhibits LH-induced steroidogenesis and decreases LH receptor binding to Leydig cells (Welsh and Hsueh, 1982). Sertoli and peritubular cell production of TGF-α may regulate interstitial cell growth during development. The role of TGF-α in Sertoli cell-germ cell interactions is also not clear. Developing spermatogonia might respond to TGF-α; this could provide an appropriate mechanism for Sertoli cells to influence spermatogonial growth. Two models using TGF-α transgenic mice, which overexpress TGF-α in the testis, show no abnormal features in this tissue (Jhappan et al., 1990; Matsui et al., 1990).

Transforming Growth Factor-β

Transforming growth factor-beta (**TGF-β**) is a multifunctional regulatory molecule that can stimulate or inhibit aspects of cellular growth and differentiation (Roberts and Sporn, 1988). Transforming growth factor-beta acts as a growth inhibitor by inhibiting the actions of growth factors such as EGF/TGF-α. It can also promote

cellular differentiation, extracellular matrix production, and chemotaxis. Different subtypes of TGF-β are produced as latent secreted precursors. Most cell types contain receptors for this ubiquitous factor.

Transforming growth factor-beta may also act as a multifunctional agent in the seminiferous tubule. Growth inhibitors may be necessary to prevent spermatogonial growth before puberty and to terminate growth of the maturing Sertoli cell. Studies suggest that TGF-β is produced by Sertoli cells and may be modulated by gonadotrophins (Skinner and Moses, 1989; B. P. Mullaney and M. K. Skinner, unpublished results). Interestingly, the testis seems to be one of the few tissues in which TGF-β_3 is predominantly expressed (Miller et al., 1989). Transforming growth factor-beta does not seem to affect immature Sertoli cell growth or cellular differentiation dramatically (Skinner and Moses, 1989). However, TGF-β may be important in regulating environmental interactions necessary for spermatogenesis. Transforming growth factor-beta increases Sertoli cell plasminogen activator production, perhaps involved in tissue remodeling for germ cell development (Nargolwalla et al., 1990). Due to the antagonistic growth regulation of TGF-α by TGF-β, the local production of TGF-β may act to limit TGF-α action in the tubule.

Peritubular cells also express and produce TGF-β (Skinner and Moses, 1989). Transforming growth factor-beta acts as a growth inhibitor for peritubular cells and blocks TGF-α-induced peritubular proliferation (B. P. Mullaney and M. K. Skinner, unpublished results). A number of observations suggest that TGF-β may be important in peritubular cell differentiation. Transforming growth factor-beta may regulate the production of extracellular matrix components by peritubular cells (Skinner and Moses, 1989) and increase production of plasminogen activator inhibitor type 1 (**PAI-1**) by peritubular cells. Transforming growth factor-beta induces peritubular cell contractility potentially required for sperm transport in the tubule (Ailenberg et al., 1990) and migration and colony formation of peritubular cells in culture (Skinner and Moses, 1989). Chemotaxis stimulated by TGF-β may be a mechanism to recruit undifferentiated fibroblasts to the exterior of the tubule during development. Therefore, TGF-β may influence morphogenesis and structural formation of the seminiferous tubule.

Transforming growth factor-beta also may regulate Leydig cell growth and differentiation. Similar to TGF-α, TGF-β inhibits LH-induced steroidogenesis, possibly by decreasing LH receptor binding (Avallet et al., 1987; Lin et al., 1987a). During development, the growth of maturing Leydig cells slows and may require a growth inhibitor such as TGF-β. Transforming growth factor-beta decreased DNA synthesis in a transformed Leydig cell line; however, TGF-β had little effect on Leydig growth in primary culture (Benahmed et al., 1989; Gonzalez-Manchon and Vale, 1989). The local production of TGF-β in the interstitium needs to be determined to elucidate the importance of TGF-β-mediated cell-cell interactions.

Fibroblast Growth Factor

Fibroblast growth factor (**FGF**) can influence aspects of both cellular growth and differentiation (Gospodarowicz et al., 1987). Aside from growth stimulation, recent studies indicate that FGF may play a critical role in angiogenesis and tissue repair. Fibroblast growth factor has many cellular targets and widespread tissue distribution and is important in many organ systems, including the testis (Gospodarowicz and Ferrara, 1989).

Basic FGF (**bFGF**) has been isolated from bovine and human testis (Ueno et al., 1987; Story et al., 1988). Sertoli cells seem to produce this factor, although localization of FGF gene expression has not been demonstrated (Smith et al., 1989). The angiogenic properties of FGF suggest that this factor may be involved in vascularization of this tissue during development. Fibroblast growth factor is mitogenic for immature Sertoli cells (Jaillard et al., 1987; Smith et al., 1989). Basic FGF may also be important in tissue remodeling for spermatogenesis in its ability to stimulate Sertoli cell plasminogen activator activity (Jaillard et al., 1987). Fibroblast growth factor is reported to both stimulate and inhibit Leydig steroidogenesis in different species (Fauser et al., 1988; Raeside et al., 1988; Sordoillet et al., 1988; Murono and Washburn, 1990). The action of FGF on germ cells has not been demonstrated. Further molecular studies are necessary to localize cellular expression of FGF and its receptor to determine its role in specific testicular cell-cell interactions.

Nerve Growth Factor

Nerve growth factor (**NGF**) is another mitogen that may mediate intercellular interactions involving growth (Yanker and Shooter, 1982). Nerve growth factor is important for the development and maintenance of sympathetic neurones in the peripheral nervous system and cholinergic neurones in the central nervous system. The expression of NGF typically correlates with the amount of sympathetic innervation. Surprisingly, NGF is expressed at higher levels than expected in testosterone-dependent organs, including the testis.

Nerve growth factor mRNA is present in spermatocytes and early spermatids of the adult

mouse (Olson et al., 1987; Ayer-LeLievre et al., 1988), and Sertoli cells express NGF receptor (Persson et al., 1990). Hypophysectomy increases NGF receptor mRNA in whole testis, and replacement of LH, but not of follicle-stimulating hormone (FSH), returns expression to basal levels. This observation suggests that testosterone down-regulates the receptor and may be an example of a gene that is negatively regulated by androgens. Interestingly, levels of NGF receptor may also correlate with stage VI–VIII of the seminiferous cycle, perhaps stimulating the Sertoli cell for later steps in germ cell maturation. The function of NGF in the testis is not known and requires further study.

Interleukin-1

The interleukins (IL) are a family of cytokines produced by activated lymphocytes and macrophages. One of these factors, IL-1, may play an important role in mediating cellular activation during inflammation and infection (Durum et al., 1985). The β form of IL-1 is typically secreted by lymphocytes; however, IL-1α is produced by nonimmune tissues. The mitogenic properties of these factors suggest that IL-1 may mediate growth regulation.

Interleukin-1α-like activity was isolated from cultures of mature Sertoli cells, but it was absent from cultures of other testicular cell types (Gustafsson et al., 1985; Khan et al., 1988). Activity of IL-1 in conditioned media increases at puberty, coinciding with the onset of spermatogenesis (Syed et al., 1988). Interleukin-1 is mitogenic for a variety of cell types; thus, the Sertoli cell might directly stimulate germ cell development through production of IL-1. One study indicated that intratesticular injection of IL-1 into hypophysectomized rats stimulated [^{3}H]thymidine incorporation in spermatogonia (Pollanen et al., 1989). Presently, it is not known which cells contain IL-1 receptors; however, IL-1 can inhibit Leydig steroidogenesis (Calkins et al., 1988). Another potential role for this cytokine may be to mediate immune suppression.

Additional Testicular Growth Factors

Other mitogenic factors found in the testis include seminiferous growth factor (**SGF**) and Sertoli cell-secreted growth factor (**SCSGF**). Seminiferous growth factor was the first mitogenic factor identified in the tubule (Feig et al., 1980). This 16-kDA mitogen was isolated from Sertoli cell based on its affinity for heparin and seems to be immunologically distinct from FGF (Feig et al., 1983; Bellve and Zheng, 1989). Seminiferous growth factor stimulated growth in transformed TM4 Sertoli, TM3 Leydig cells, and 6-d-old mouse Sertoli cells (Bellve and Feig, 1984). The activity of SGF has been detected in many species and is predominant during prepubertal development (Feig et al., 1980). Another Sertoli cell-secreted growth factor, SCSGF, has also been partially purified and seems to be mitogenic for a number of cell lines (Buch et al., 1988). Sertoli cell-secreted growth factor has some similarities to TGF-α, including its apparent molecular weight of 8 kDa and its ability to displace radiolabeled EGF from binding its receptor (Buch et al., 1988). Neither SGF nor SCSGF has been fully characterized, and whether these factors are previously identified growth factor remains to be thoroughly investigated.

A number of additional growth factors have been shown to act on specific cell types or to be localized in interstitial fluid. The site of production and specific functions of these agents remains to be investigated. Factors such as activin have been shown to influence germ cell proliferation in vivo (Mather et al., 1990). Whether the actions of such agents are direct or indirectly mediated through alterations in the production of the growth factors previously discussed remains to be elucidated.

Growth Factors in the Ovary

Ovarian physiology requires rapid and continuous growth regulation associated with the process of folliculogenesis. Growth factor-mediated interactions between theca cells, granulosa cells, and the oocyte are required for the maintenance of ovarian function and the process of oogenesis. Granulosa cells provide the cytoarchitectural support for the developing oocyte and also help to form the follicle and antrum. Theca cells surround and form the exterior wall of the follicle. The interactions between theca cells and granulosa cells provide an example of a mesenchymal (stromal)-epithelial cell interaction. The effects of this cellular interaction on oocyte development and the influence of the endocrine system on this cellular interaction are essential for female reproductive biology. The theca cells and granulosa cells of the pre-antral and small antral follicles must undergo an extensive proliferation and functional differentiation prior to ovulation (Hsueh et al., 1984). In most large animals, follicle size increases from millimeters to centimeters. The primary cell expansion required is associated with the granulosa and theca cells. In addition to the cell proliferation required during follicle development, follicles of various stages of development become atretic and cell growth is arrested. Therefore, regulation of cell proliferation in the follicle will require stimulatory and inhibitory growth factors.

Table 3. Growth factors in the ovary

Growth factor	Proposed site of synthesis	Proposed site of action	Proposed function[a]
IGF-I	Granulosa	Granulosa	+Growth/+Differentiation
		Theca	+Growth/+Differentiation
FGF	Granulosa	Granulosa	+Growth
		Endothelium	Angiogenesis
TGF-β	Theca	Granulosa	–Growth/+Differentiation
	Granulosa	Theca	–Growth/+Differentiation
TGF-α	Theca	Granulosa	+Growth/–Differentiation
		Theca	+Growth

[a]A (+) denotes an increase, and (–) indicates a decrease.

Administration of FSH and estrogen has been shown to stimulate follicle cell proliferation in vivo (Goldenberg et al., 1972; Louvet and Vaitukaitis, 1976; Richards, 1979). These hormones, however, have negligible effects on cell growth in vitro. These observations imply that the in vivo hormone actions are likely indirectly mediated through the local production of growth factors. The mechanisms and specific growth factors involved in the control of ovarian cell proliferations remain to be fully elucidated (Carson et al., 1989). Several of the major growth factors identified to be produced and(or) to act in the ovary are summarized in Table 3.

Insulin-Like Growth Factor

Insulin-like growth factor I has been shown to be produced by granulosa cells under the control of growth hormone (Davoren and Hsueh, 1986), FSH, and estradiol (Hammond et al., 1985; Hsu and Hammond, 1987). The gene expression of IGF-I has also been localized in granulosa cells (Hernandez et al., 1989; Oliver et al., 1989) but not in theca cells. The receptors for IGF-I have been localized to granulosa cells (Baranao and Hammond, 1984; Davoren et al., 1986; Adashi et al., 1988a) and are affected by the actions of FSH (Adashi et al., 1986, 1988b,c). Insulin-like growth factor I has been shown to stimulate granulosa cell oxytocin production (Schams et al., 1988), P450-side chain cleavage enzyme (Veldhuis et al., 1986), lipoprotein metabolism (Veldhuis et al., 1987), adenylate cyclase activity (Adashi et al., 1986), and LH receptor induction (Adashi et al., 1985b). In addition to effects on cellular function, IGF has been shown to stimulate the proliferation of bovine (Savion et al., 1981) and porcine (Baranao and Hammond, 1984) granulosa cells, but not rat granulosa cells, in vitro (Adashi et al., 1984). Although theca cells do not seem to produce IGF-I, IGF-II gene expression has been localized to theca cells and not to granulosa cells (Hernandez et al., 1990b). Theca cells also contain IGF receptors and respond to IGF through an alteration in steroidogenesis (Hernandez et al., 1988). Potential interactions between granulosa cells and theca cells through the local production and action of IGF have previously been suggested (Adashi et al., 1985a; Geisthovel et al., 1990). A physiological parameter to consider, however, is the high circulatory levels of liver-derived IGF-I available to both cell types. This is an additional source of IGF-I that needs to be considered in understanding IGF-mediated cell-cell interactions. Insulin-like growth factor binding proteins have also been shown to be produced by ovarian cell types and are present in the follicle. These binding proteins can reduce the effective concentration and modulate the actions of IGF. Therefore, IGF will likely be an important growth factor in the control of ovarian cell proliferation and function; however, the role and specific mechanisms involved remain to be fully elucidated.

Transforming Growth Factor-α/Epidermal Growth Factor

Epidermal growth factor was not found to be produced in the ovary; however, an EGF-like substance was found to be produced by theca cells (Skinner et al., 1987b) and was identified as TGFα (Kudlow et al., 1987; Skinner and Coffey, 1988). Granulosa cells were not found to express TGFα (Skinner and Coffey, 1988; Lobb et al., 1989). The granulosa cell has been shown to contain the EGF receptor (Mondschein and Schomberg, 1981; Chabot et al., 1985; Feng et al., 1986), and EGF generally has inhibitory actions on adenylate cyclase (Dodson and Schomberg, 1987), LH receptor activity (Mondschein and Schomberg, 1981; Knecht and Catt, 1983a; May et al., 1987), and FSH-induced aromatase activity (Hsueh et al., 1981; May et al., 1982). Transforming growth factor-alpha has inhibitory actions similar to those of EGF on granulosa cells (Adashi et al., 1987). Theca cells have also been shown to contain the EGF

receptor (Skinner and Coffey, 1988) and influence steroidogenesis (Erickson and Case, 1983). One of the initial observations on growth regulation in the ovary was the ability of EGF to stimulate granulosa cell proliferation (Gospodarowicz et al., 1977). Theca cell growth can also be stimulated by TGFα/EGF (Skinner and Coffey, 1988). The actions of TGFα/EGF and the potential presence of EGF in the ovaries of various species can vary and remain to be fully elucidated.

The circulatory levels of EGF/TGFα are negligible and require local production within a specific organ. The ability of the theca cell to produce TGFα that can stimulate the growth of both granulosa and theca cells implies that TGFα may have an important role in promoting cell expansion during follicle development. This seems to be a mesenchymal/stromal-controlled growth process (Skinner and Coffey, 1989). The ability of hormones to influence TGFα production may provide a mechanism for the endocrine regulation of ovarian follicle cell growth.

Transforming Growth Factor-β

Ovarian theca cells have been shown to express and produce TGFβ (Skinner et al., 1987a), and in vivo immunocytochemical localization of TGFβ is primarily confined to the theca (Thompson et al., 1989). Although freshly isolated bovine granulosa cells do not seem to express TGFβ (Skinner et al., 1987a), cultured rat granulosa cells have been shown to produce TGFβ, which can be suppressed by FSH (Kim and Schomberg, 1989; Mulheron and Schomberg, 1990). Therefore, theca cells seem to be a predominant source of ovarian TGFβ, but granulosa cells also have the capacity to express TGFβ. The specific types of TGFβ expressed and their hormonal and developmental regulation remain to be fully elucidated (Mulheron et al., 1991).

The local production of TGFβ allows for subsequent actions on various ovarian cell types. Transforming growth factor-beta has been shown to stimulate a number of granulosa cell functions, including FSH-induced LH receptors (Knecht et al., 1986; Dodson and Schomberg, 1987), EGF actions (Feng et al., 1986), FSH-induced aromatase activity (Ying et al., 1986; Hutchinson et al., 1987), IGF-I production (Mondschein et al., 1988), and inhibin production (Zhiwen et al., 1988). Transforming growth factor-beta also can influence theca cell function and steroidogenesis (Caubo et al., 1989; Magoffin et al., 1989; Hernandez et al., 1990a). Oocyte maturation is also influenced by TGFβ (Feng et al., 1988; Tsafrirri et al., 1989), although the direct vs indirect actions on oocytes remain to be investigated. In addition to effects on cellular differentiation, TGFβ can also influence ovarian cell growth. Transforming growth factor-beta has been shown to inhibit TGFα/EGF-induced bovine and porcine granulosa cell growth (Skinner et al., 1987a; Mondschein et al., 1988). Conflicting data were found with rat granulosa cells (Dorrington et al., 1988). Transforming growth factor-beta can also inhibit TGFα/EGF-induced theca cell growth (Roberts and Skinner, 1991). Observations suggest that TGFβ may have an important role as a growth inhibitor in the ovary. The ability of TGFβ to inhibit cell growth will allow for a more differentiated state of the cell, reflected in the generally stimulatory effects of TGFβ observed on cell function. Therefore, the influence of TGFβ on cell function may be indirectly mediated through the inhibition of cellular proliferation. Growth inhibition may be important to prevent premature cell growth of the pre-antral follicle, arrest cell growth during atresia, and control cell growth during follicle cell expansion.

Fibroblast Growth Factor

Basic FGF has been shown to be produced by granulosa cells (Neufeld et al., 1987; Koos and Olson, 1989), and the angiogenic factor in the ovary and corpus luteum has been identified as FGF (Gospodarowicz et al., 1985). Fibroblast growth factor can act on granulosa cells to alter the steroidogenic capacity of the cell (Baird and Hsueh, 1986; Adashi et al., 1988), gonadotropin receptors (Mondschein and Schomberg, 1981), and plasminogen activator expression (LaPolt et al., 1990). In addition to these effects on cell function, FGF can stimulate granulosa cell proliferation (Gospodarowicz et al., 1977; Gospodarowicz and Bialecki, 1979) and may indirectly cause many of the effects observed on cell function. An additional role for FGF production in the ovary would be to act as an angiogenic factor and promote vascularization of the developing follicle and corpus luteum.

Additional Growth Factors

Several additional types of growth factors have also been shown to act on and(or) to be produced by ovarian cells. Platelet-derived growth factor (**PDGF**) acts on granulosa cells to enhance FSH-induced progesterone secretion, adenylate cyclase activity (Knecht and Catt, 1983b), and LH receptor induction (Knecht and Catt, 1983b; Mondschein and Schomberg, 1984). The effects of PDGF on ovarian cell growth are uncertain; however, action as a potential competence factor for cell proliferation is a plausible activity. A factor that is structurally related to FGF has recently been identified as vascular endothelial growth factor (**VEGF**). This growth factor has been shown to be expressed in the ovary, in particular in luteal

tissue, and is postulated to have a role in angiogenesis of the follicle (Phillips et al., 1990). Nerve growth factor has also been recently shown to be expressed in the ovary and is affected by ovarian innervation (Lara et al., 1990). Production of NGF in the ovary will likely have actions on ovarian sympathetic neurons.

Summary

Clearly, a large number of growth factors have been shown to be produced and to act in the testis and ovary. The majority of research has focused on the effects of specific growth factors on differentiated functions of gonadal cell types. Factors that promote cell growth generally have suppressive effects and attenuate hormone responsiveness. Factors that inhibit growth generally enhance and increase hormone responsiveness. In considering the function and physiology of locally produced growth factors, a distinction needs to be made between growth and differentiation. A factor that promotes cell proliferation and the cell cycle will indirectly reduce the differentiated state of the cell, whereas the inverse is true of a factor that arrests cell proliferation and inhibits the cell cycle. Although specific growth factors may have a role in the regulation of differentiated functions, the possibility that many of the actions observed may be the indirect result of effects on cell growth needs to be considered. Therefore, the physiological importance of growth factor regulation of differentiated function remains to be elucidated. The control of cell growth, however, will be a major function of the specific growth factors. The integrated actions of various factors such as transforming growth factors-α and -β could provide an efficient mechanism to regulate cell proliferation required in gonadal development. Further investigation of the developmental regulation of the expression, production, and action of individual growth factors will provide insight into the potential physiological roles for the various growth factors. Observations obtained imply that growth factors will likely be critical regulatory agents involved in gonadal cell-cell communication.

The endocrine regulation of gonadal cell growth has been well documented in vivo. The actions of gonadotropins and reproductive steroids, however, are distinct from the pharmacology of most growth factors. In addition, in vitro these hormones often have negligible effects on cell proliferation. The possibility that the actions of reproductive hormones on gonadal cell growth are indirectly mediated through alterations in the expression of locally produced growth factors needs to be seriously considered. Several current observations imply that hormones may regulate growth factor production. Further investigation of the hormonal regulation of the production and actions of growth factors will help elucidate the mechanisms involved in the endocrine regulation of gonadal development.

Literature Cited

Adashi, E. Y., C. E. Resnick, M. E. Svoboda, and J. J. Van Wyk. 1984. A novel role for somatomedin-C in the cytodifferentiation of ovarian granulosa cells. Endocrinology 155:1227.

Adashi, E. Y., C. E. Resnick, C. S. Croft, J. V. May, and D. Gospodarowicz. 1988. Basic fibroblast growth factor as a regulatory of ovarian granulosa cell differentiation: a novel non-mitogenic role. Mol. Cell. Endocrinol. 55:7.

Adashi, E. Y., C. E. Resnick, A. J. D'Ercole, M. E. Svoboda, and J. J. Van Wyk. 1985a. Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. Endocr. Rev. 6:400.

Adashi, E. Y., C. E. Resnick, E. R. Hernandez, J. V. May, M. Knecht, M. E. Svoboda, and J. J. Van Wyk. 1988c. Insulin-like growth factor-I as an amplifier of follicle-stimulating hormone action: studies on mechanism(s) and site(s) of action in cultured rat granulosa cells. Endocrinology 122:1583.

Adashi, E. Y., C. E. Resnick, E. R. Hernandez, M. E. Svoboda, and J. J. Van Wyk. 1988a. Characterization and regulation of a specific cell membrane receptor for somatomedin-C/insulin-like growth factor I in cultured rat granulosa cells. Endocrinology 122:194.

Adashi, E. Y., C. E. Resnick, E. R. Hernandez, M. E. Svoboda, and J. J. Van Wyk. 1988b. In vivo regulation of granulosa cell somatomedin-C/insulin-like growth factor I receptors. Endocrinology 122:1383.

Adashi, E. Y., C. E. Resnick, and D. R. Twardzik. 1987. Transforming growth factor-α attenuates the acquisition of aromatase activity by cultured rat granulosa cells. J. Cell. Biochem. 33:1.

Adashi, E. Y., C. E. Resnick, M. E. Svoboda, and J. J. Van Wyk. 1985b. Somatomedin-C enhances induction of luteinizing hormone receptors by follicle-stimulating hormone in cultured rat granulosa cells. Endocrinology 116:2369.

Adashi, E. Y., C. E. Resnick, M. E. Svoboda, and J. J. Van Wyk. 1986. Somatomedin-C as an amplifier of follicle-stimulating hormone action: enhanced accumulation of adenosine 3′,5′-monophosphate. Endocrinology 118:149.

Ailenberg, M., P. S. Tung, and I. B. Fritz. 1990. Transforming growth factor beta elicits shape changes and increases contractility of testicular peritubular cells. Biol. Reprod. 42: 499.

Ascoli, M. 1981. Regulation of gonadotropin receptors responses in a clonal strain of Leydig tumor cells by epidermal growth factor. J. Biol. Chem. 25:179.

Avallet, D., M. Vigier, M. H. Perrard-Sapori, and J. M. Saez. 1987. Transforming growth factor beta inhibits Leydig cell functions. Biochem. Biophys. Res. Commun. 146:575.

Ayer-LeLievre, C., L. Olson, T. Ebendal, F. Hallbrook, and H. Persson. 1988. Nerve growth factor mRNA and protein in the testis and epididymis of mouse and rat. Proc. Natl. Acad. Sci. USA 85:2628.

Baird, A., and A.J.W. Hsueh. 1986. Fibroblast growth factor as an intraovarian hormone: differential regulation of steroidogenesis by an angiogenic factor. Regul. Pept. 16:243.

Baranao, J. L., and J. M. Hammond. 1984. Comparative effects of insulin and insulin-like growth factors on DNA synthesis and differentiation of porcine granulosa cells. Biochem.

Biophys. Res. Commun. 124:484.
Bellve, A. R., and L. A. Feig. 1984. Cell proliferation in the mammalian testis: biology of the seminiferous growth factor. Recent Prog. Horm. Res. 40:531.
Bellve, A. R., and W. Zheng. 1989. Growth factors as autocrine and paracrine modulators of male gonadal functions. J. Reprod. Fertil. 85:771.
Benahmed, M., G. Esposito, C. Sordoillet, E. dePeretti, M. A. Chauvin, C. Ghiglieri, A. Revol, and A. M. Morera. 1989. Transforming growth factor beta and its related peptides in the testis: an intragonadal polypeptide control system. Perspect. Androl. 53:191.
Borland, K., M. Mita, C. L. Oppenheimer, L. A. Blinderman, J. Massague, P. F. Hall, and M. P. Czech. 1984. The actions of insulin-like growth factors I and II on cultured Sertoli cells. Endocrinology 114:240.
Buch, J. P., D. L. Lamb, L. I., Lipschultz, and R. G. Smith. 1988. Partial characterization of a unique growth factor secreted by human Sertoli cells. Fertil. Steril. 49:658.
Cailleau, J., S. Vermeire, and G. Verhoeven. 1990. Independent control of the production of insulin-like growth factor I and its binding protein by cultured testicular cells. Mol. Cell. Endocrinol. 69:79.
Calkins, J. H., M. M. Sigel, H. R. Nankin, and T. Lin. 1988. Interleukin-1 inhibits Leydig cell steroidogenesis in primary culture. Endocrinology 123:1605.
Carpenter, G. 1987. Receptors for epidermal growth factor and other polypeptide mitogens. Annual Review of Biochemistry 56:881-914.
Carpenter, G., and S. Cohen. 1990. Epidermal growth factor. J. Biol. Chem. 265:7709.
Carpenter, G., and J. Zendegui. 1986. A biological assay for epidermal growth factor and related polypeptides. Anal. Biochem. 153:279.
Carson, R. S., Z. Zhang, L. A. Hutchinson, A. C. Herington, and J. K. Findlay. 1989. Growth factors in ovarian function. J. Reprod. Fertil. 85:735.
Casella, S. J., E. P. Smith, J. J. Van Wyk, D. R. Joseph, M. A. Hynes, E. C. Hoyt, and P. K. Lund. 1987. Isolation of a rat testis cDNA encoding an insulin-like growth factor I precursor. DNA 6:325.
Caubo, B., R. S. De Vinna, and S. A. Tenetta. 1989. Regulation of steroidogenesis in cultured porcine theca cells. Endocrinology 125:321.
Chabot, J. G., R. St-Arnaud, P. Walker, and G. Pelletier. 1985. Distribution of epidermal growth factor receptors in the rat ovary. Mol. Cell. Endocrinol. 44:99.
Chatelain, P. G., D. Naville, and J. M. Saez. 1987. Somatomedin-c/insulin-like growth factor I-like material secreted by porcine Sertoli cells in vitro: characterization and regulation. Biochem. Biophys. Res. Commun. 146:1009.
Clermont, Y., and B. Perey. 1957. Quantitative study of the cell population of the seminiferous tubules in immature rats. Am. J. Anat. 100:241.
Closset, J., A. Gothot, B. Sente, M. L. Scippo, A. Igout, M. Vandebroeck, D. Dombrowicz, and G. Hennen. 1989. Pituitary hormone dependent expression of insulin-like growth factors I and II in the immature hypophysectomized rat testis. Mol. Endocrinol. 3:1125.
Daughaday, W. H., and P. Rotwein. 1989. Insulin-like growth factors I and II: peptide, messenger RNA and gene structure, serum, and tissue concentrations. Endocr. Rev. 10:68.
Davoren, J. B., and A.J.W. Hsueh. 1986. Growth hormone increases ovarian levels of immunoreactive somatomedin c/insulin-like growth factor I *in vivo*. Endocrinology 119:2155.
Davoren, J. B., B. G. Kasson, C. H. Li, and A.J.W. Hsueh. 1986. Specific insulin-like growth factor (IGF) I- and II-binding sites on rat granulosa cells: relation to IGF action. Endocrinology 119:2155.
Derynck, R. 1988. Transforming growth factor alpha. Cell 54: 593.
Dodson, W. C., and D. W. Schomberg. 1987. The effect of transforming growth factor-beta on follicle-stimulating hormone-induced differentiation of cultured rat granulosa. Endocrinology 120:512.
Dorrington, J. H., A. V. Chuma, and J. J. Bendall. 1988. Transforming growth factor-β and follicle-stimulating hormone promote rat granulosa cell proliferation. Endocrinology 123:352.
Durum, S. K., J. A. Schmidt, and J. Oppenheim. 1985. Interleukin-1: an immunological perspective. Annu. Rev. Immunol. 3:263.
Erickson, G. F., and E. Case. 1983. Epidermal growth factor antagonizes ovarian theca-interstitial cytodifferentiation. Mol. Cell. Endocrinol. 31:71.
Fauser, B.C.J.M., A. Baird, and A.J.W. Hsueh. 1988. Fibroblast growth factor inhibits luteinizing hormone- stimulating androgen production by cultured rat testicular cells. Endocrinology 123:2935.
Feig, L. A., A. R. Bellve, N. H. Erickson, and M. Klagsbrun. 1980. Sertoli cells contain a mitogenic polypeptide. Proc. Natl. Acad. Sci. USA 77:4774.
Feig, L. A., M. Klagsbrun, and A. R. Bellve. 1983. Mitogenic polypeptide of the mammalian seminiferous epithelium: biochemical characterization and partial purification. J. Cell Biol. 97:1435.
Feng, P., K. J. Catt, and M. Knecht. 1986. Transforming growth factor beta regulates the inhibitory actions of epidermal growth factor during granulosa cell differentiation. J. Biol. Chem. 261:14167.
Feng, P., K. J. Catt, and M. Knecht. 1988. Transforming growth factor-β stimulated meiotic maturation of the rat oocyte. Endocrinology 122:181.
Froesch, E. R., C. Schmid, J. Schwander, and J. Zapf. 1985. Actions of insulin-like growth factors. Annu. Rev. Physiol. 47:443.
Geisthovel, F., I. Moretti-Rojas, F. J. Rojas, and R. H. Asch. 1990. Insulin-like growth factors and thecal-granulosa-cell function. Hum. Reprod. 5:785.
Goldenberg, R. L., J. L. Vaitukaitis, and G. T. Ross. 1972. Estrogen and follicle-stimulating hormone interactions on follicle growth in rats. Endocrinology 90:1492.
Gonzalez-Manchon, C., and W. Vale. 1989. Activin-A, inhibin and transforming growth factor beta modulate growth of two gonadal cell lines. Endocrinology 125:1662.
Gospodarowicz, D., and H. Bialecki. 1979. Fibroblast and epidermal growth factors are mitogenic agents for cultured granulosa cells of rodent, porcine, and human origin. Endocrinology 104:757.
Gospodarowicz, D., J. Cheng, G.-M. Lui, and P. Bohlen. 1985. Corpus luteum angiogenic factor is related to fibroblast growth factor. Endocrinology 117:201.
Gospodarowicz, D., and N. Ferrara. 1989. Fibroblast growth factor and the control of pituitary and gonad development and function. J. Steroid Biochem. 32:183.
Gospodarowicz, D., N. Ferrara, L. Schweigerer, and G. Neufeld. 1987. Structural characterization and biological functions of fibroblast growth factor. Endocr. Rev. 8:95.
Gospodarowicz, D., C. R. Ill, and C. R. Birdwell. 1977. Effects of fibroblast and epidermal growth factors on ovarian cell proliferation *in vitro*. 1. Characterization of the response of granulosa cells to FGF and EGF. Endocrinology 100:1108.
Gustafsson, K., O. Soder, P. Pollanen, and E. M. Ritzen. 1985. Isolation and partial characterization of an interleukin-1-like factor from rat testis interstitial fluid. J. Reprod. Immunol. 14:139.
Hammond, J. M., J.L.S. Baranoa, D. Skaleris, A. B. Knight, J. A. Ronanus, and M. M. Rechler. 1985. Production of insulin like growth factors by ovarian granulosa cells. Endocrinology 117:2553.

Handelsman, D. J., J. A. Spaliviero, C. D. Scott, and R. C. Baxter. 1985. Identification of insulin-like growth factor-I and its receptor in the rat testis. Acta Endocrinol. 109:543.

Hansson, H. A., H. Billig, and J. Isgaard. 1989. Insulin-like growth factor I in the developing and mature rat testis: immunohistochemical aspects. Biol. Reprod. 40:1321.

Hardy, M. P., B. R. Zirkin, and L. L. Ewing. 1989. Kinetic studies on the development of the adult population of Leydig cells in testes of the pubertal rat. Endocrinology 124:762.

Hernandez, E. R., A. Hurwitz, D. W. Payne, A. M. Dharmarajan, A. F. Purchio, and E. Y. Adashi. 1990a. Transforming growth factor-β1 inhibits ovarian androgen production: gene expression, cellular localization, mechanism(s), and site(s) of action. Endocrinology 127:2804.

Hernandez, E. R., C. E. Resnick, M. E. Svoboda, J. J. Van Wyk, D. W. Payne, and E. Y. Adashi. 1988. Somatomedin-C/insulin-like growth factor I as an enhancer of androgen biosynthesis by cultured rat ovarian cells. Endocrinology 122:1603.

Hernandez, E. R., C. T. Roberts, Jr., A. Hurwitz, D. LeRoith, and E. Y. Adashi. 1990b. Rat ovarian insulin-like growth factor II gene expression is theca-interstitial cell-exclusive: Hormonal regulation and receptor distribution. Endocrinology 127:3249.

Hernandez, E. R., C. T. Roberts, Jr., D. LeRoith, and E. Y. Adashi. 1989. Rat ovarian insulin-like growth factor I (IGF-I) gene expression is granulosa cell-selective: 5′-untranslated mRNA variant representation and hormonal regulation. Endocrinology 125:572.

Holmes, S. D., G. Spotts, and R. G. Smith. 1986. Rat Sertoli cells secrete a growth factor that blocks epidermal growth factor binding to its receptor. J. Biol. Chem. 261:4076.

Hsu, C., and J. M. Hammond. 1987. Gondotropins and estradiol stimulate immunoreactive insulin-like growth factor-I production by porcine cells *in vitro*. Endocrinology 120:198.

Hsueh, A.J.W., E. Y. Adashi, P.B.C. Jones, and T. H. Welsh, Jr. 1984. Hormonal regulation of the differentiation of cultured ovarian granulosa cells. Endocr. Rev. 5:76.

Hsueh, A.J.W., T. H. Welsh, and P.B.C. Jones. 1981. Inhibition of ovarian and testicular steroidogenesis by epidermal growth factor. Endocrinology 108:2002.

Hutchinson, L. A., J. K. Findlay, F. L. de Vos, and D. M. Robertson. 1987. Effects of bovine inhibin, transforming growth factor-beta and bovine Activin-A on granulosa cell differentiation. Biochem. Biophys. Res. Commun. 146:1405.

Jaillard, C., P. G. Chatelain, and J. M. Saez. 1987. In vitro regulation of pig Sertoli cell growth and function: effects of fibroblast growth factors and somatomedin-c. Biol. Reprod. 37:665.

Jhappan, C., C. Stahle, R. N. Harkins, N. Fausto, G. H. Smith, and G. T. Merlino. 1990. TGF-alpha overexpression in transgenic mice induces liver neoplasia and abnormal development of the mammary gland and pancreas. Cell 61:1137.

Kasson, B. G., and A. J. Hsueh. 1987. Insulin-like growth factor I augments gonadotropin-stimulated androgen biosynthesis by cultured rat testicular cells. Mol. Cell. Endocrinol. 52:27.

Khan, S. A., K. Schmidt, P. Hallin, R. DiPauli, C. DeGeyter, and E. Nieschlag. 1988. Human testis cytosol and ovarian follicular fluid contain high amounts of interleukin-1-like factor(s). Mol. Cell. Endocrinol. 58:221.

Kim, I.-C., and D. W. Schomberg. 1989. The production of transforming growth factor-β activity by the rat granulosa cells. Endocrinology 124:1345.

Knecht, M., K. Catt. 1983a. Epidermal growth factor and gonadotropin-releasing hormone inhibit cyclic AMP-dependent luteinizing hormone receptor formation in ovarian granulosa cells. J. Cell Biol. 21:209.

Knecht, M., and K. J. Catt. 1983b. Modulation of cAMP-mediated differentiation in ovarian granulosa cells by epidermal growth factor and platelet-derived growth factor. J. Biol. Chem. 258:2789.

Knecht, M., P. Feng, and K.J. Catt. 1986. Transforming growth factor-beta regulates the expression of luteinizing hormone receptors in ovarian granulosa cells. Biochem. Biophys. Res. Commun. 139:800.

Koos, R. D., and C. E. Olson. 1989. Expression of basic fibroblast growth factor in the rat ovary: detection of mRNA using reverse transcription-polymerase chain reaction amplification. Mol. Endocrinol. 3:2041.

Kudlow, J. E., M. S. Kobrin, A. F. Purchio, D. R. Twardzik, E. R. Hernandez, S. L. Asa, and E. Y. Adashi. 1987. Ovarian transforming growth factor-α gene expression: immunohistochemical localization to the theca-interstitial cells. Endocrinology 121:1577.

LaPolt, P. S., M. Yamoto, M. Veljkovic, C. Sincich, N. Y. Tor, A. Tsafrirri, and A.J.W. Hsueh. 1990. Basic fibroblast growth factor induction of granulosa cell tissue-type plasminogen activator expression and oocyte maturation: potential role as a paracrine ovarian hormone. Endocrinology 127:2357.

Lara, H. E., D. F. Hill, K. H. Katz, and S. R. Ojeda. 1990. The gene encoding nerve growth factor is expressed in the immature rat ovary: effect of denervation and hormonal treatment. Endocrinology 126:357.

Lin, T., J. Blaisdell, and J. F. Haskell. 1987a. Transforming growth factor beta inhibits Leydig cell steroidogenesis in primary culture. Biochem. Biophys. Res. Commun. 137:387.

Lin, T., J. Blaisdell, and J. F. Haskell. 1987b. Type I IGF receptors on Leydig cells are upregulated by human chorionic gonadotropin. Biochem. Biophys. Res. Commun. 149:852.

Lin, T., J. Haskell, and L. Terracio. 1986. Characterization of insulin and insulin-like growth factor I receptors of purified Leydig cells and their role in steroidogenesis in primary culture: a comparative study. Endocrinology 119:1641.

Lobb, D. K., M. S. Kobrin, J. E. Kudlow, and J. H. Dorrington. 1989. Transforming growth factor-alpha in the adult bovine ovary: identification in growing ovarian follicles. Biol. Reprod. 40:1087.

Lording, D. W., and D. M. deKretser. 1972. Comparative ultra structural and histochemical studies of the interstitial studies of the rat testis during fetal and postnatal development. J. Reprod. Fertil. 29:261.

Louvet, J.-P., and J. L. Vaitukaitis. 1976. Induction of follicle-stimulating hormone (FSH) receptors in rat ovaries by estrogen priming. Endocrinology 99:758.

Magoffin, D. A., B. Gancedo, and G. F. Erickson. 1989. Transforming growth factor-β promotes differentiation of ovarian thecal-interstitial cells but inhibits androgen production. Endocrinology 125:1951.

Mallea, L. E., A. J. Machado, F. Navoroli, and F.F.G. Rommerts. 1986. Epidermal growth factor stimulates lactate production and inhibits aromatization in cultured Sertoli cells from immature rats. Int. J. Androl. 9:201.

Mather, J. P., K. M. Attie, T. K. Woodruff, G. C. Rice, and D. M. Phillips. 1990. Activin stimulates spermatogonial proliferation in germ-Sertoli cell cocultures from immature rat testis. Endocrinology 127:3206.

Matsui, Y., S. A. Halter, J. T. Holt, B.L.M. Gogan, and R. J. Coffey. 1990. Development of mammary hyperplasia and neoplasia in MMTV-TGF-alpha transgenic mice. Cell 61: 1147.

May, J. V., P. A. Buck, and D. W. Schomberg. 1987. Epidermal growth factor enhances [125Iiodo-follicle-stimulating hormone binding by cultured porcine granulosa cells. Endocrinology 120:2413.

May, J. V., F. R. Gilliam, M. S. Rein, J. S. Mondschein, and D. W. Schomberg. 1982. Growth and differentiated functions of porcine and rat granulosa cells following cryopreservation. Biol. Reprod. 27:641.

Miller, D. A., A. Lee, Y. Matsui, E. Y. Chen, H. L. Moses, and R. Derynck. 1989. Complementary DNA cloning of the murine

transforming growth factor-β_3 (TGF_3) precursor and the comparative expression of TGF_3 and $TGF\beta_1$, messenger RNA in murine embryos and tissues. Mol. Endocrinol. 3: 1926.

Mondschein, J. S., S. F. Canning, and J. M. Hammond. 1988. Effects of transforming growth factor-β on the production of immunoreactive insulin-like growth factor I and progesterone and on [^{3}H]thymidine incorporation in porcine granulosa cell cultures. Endocrinology 123:1970.

Mondschein, J. S., and D. W. Schomberg. 1984. Effects of partially and highly purified platelet-derived growth factor preparations in luteinizing hormone receptor induction in granulosa cell cultures. Biol. Reprod. 30:603.

Mondschein, J. S., and D. W. Schomberg. 1981. Growth factors modulate gonadotropin receptor induction in granulosa cell cultures. Science 211:1179.

Mulheron, G. W., D. Danielpour, and D. W. Schomberg. 1991. Rat thecal/interstitial cells express transforming growth factor-β type 1 and 2, but only type 2 is regulated by gonadotropin *in vitro*. Endocrinology 129:368.

Mulheron, G. W., and D. W. Schomberg. 1990. Rat granulosa cells express transforming growth factor-β type 2 messenger ribonucleic acid which is regulatable by follicle-stimulating hormone *in vitro*. Endocrinology 126:1777.

Murono, E. P., and A. L. Washburn. 1990. Basic fibroblast growth factor inhibits 3-beta-hydroxysteroid dehydrogenase-isomerase activities in cultured immature Leydig cells. Biochem. Biophys. Res. Commun. 168:248.

Murphy, L. J., G. I. Bell, and H. G. Friesen. 1987. Tissue distribution of insulin-like growth factor I and II messenger ribonucleic acid in the adult rat. Endocrinology 120:1279.

Nargolwalla, C., D. McCabe, and I. B. Fritz. 1990. Modulation of levels of messenger RNA for tissue-type plasminogen activator in rat Sertoli cells, and levels of messenger RNA for plasminogen activator inhibitor in testis peritubular cells. Mol. Cell. Endocrinol. 70:73.

Naville, D., P. C. Chatelain, O. Avallet, and J. M. Saez. 1990. Control of production of insulin-like growth factor I by pig Leydig and Sertoli cells cultured alone or together: cell-cell interactions. Mol. Cell. Endocrinol. 70:217.

Neufeld, G., N. Ferrara, R. Mitchell, L. Schweigerer, and D. Gospodarowicz. 1987. Granulosa cells produce basic fibroblast growth factor. Endocrinology 121:597.

O'Brien, D. A., C. A. Gabel, D. L. Rockett, and E. M. Eddy. 1989. Receptor-mediated endocytosis and differential synthesis of mannose 6-phosphate receptors in isolated spermatogenic and Sertoli cells. Endocrinology 125:2973.

Oliver, J. E., T. J. Aitman, J. R. Powell, C. A. Wilson, and R. N. Clayton. 1989. Insulin-like growth factor I gene expression in the rat ovary is confined to the granulosa cells of developing follicles. Endocrinology 124:2671.

Olson, L., C. Ayer-LeLievre, T. Ebendal, and A. Sieger. 1987. Nerve growth factor-like immuno-reactivities in rodent salivary glands and testis. Cell Tissue Res. 248:275.

Oonk, R. B., R. Jansen, and J. A. Grootegoed. 1988. Insulin-like growth factor I receptors on Sertoli cells from immature rat and age dependent testicular binding of IGF-I and insulin. Mol. Cell. Endocrinol. 55:33.

Oonk, R. B., R. Jansen, and J. A. Grootegoed. 1989. Differential effects of FSH, insulin and insulin-like growth factor-I on hexose uptake and lactate production by rat Sertoli cells. J. Cell Physiol. 139:210.

Orth, J. 1982. Proliferation of Sertoli cells in fetal and postnatal rats: a quantitative autoradiographic study. Anat. Rec. 203: 485.

Perrard-Sapori, M. H., P. C. Chatelain, N. Rogemond, and J. M. Saez. 1987. Modulation of Leydig cell functions by culture with Sertoli cells or with Sertoli cell-conditioned medium: effect of insulin, somatomedin-c and FSH. Mol. Cell. Endocrinol. 50:193.

Persson, H., C. Ayer-LeLievre, O. Soder, M. J. Villar, M. Metsis, L. Olson, M. Ritzen, and T. Hokfelt. 1990. Expression of beta-nerve growth factor receptor mRNA in Sertoli cells down-regulated by testosterone. Science 247:704.

Phillips, H. S., J. Hains, D. W. Leung, and N. Ferrara. 1990. Vascular endothelial growth factor is expressed in rat corpus luteum. Endocrinology 127:965.

Pollanen, P., O. Soder, and M. Parvinen. 1989. Interleukin-1-alpha stimulation of spermatogonial proliferation in vivo. Reprod. Fertil. Dev. 1:85.

Raeside, J. I., M. C. Berthelon, P. Sanchez, and J. M. Saez. 1988. Stimulation of aromatase activity in immature porcine Leydig cells by fibroblast growth factor. Biochem. Biophys. Res. Commun. 151:163.

Richards, J. S. 1979. Hormonal control of ovarian follicular development: a 1978 perspective. Recent Prog. Horm. Res. 35:343.

Roberts, A. B., and M. B. Sporn. 1988. Transforming growth factor beta. Advances Cancer Res. 51:107.

Roberts, A. J., and M. K. Skinner. 1991. Transforming growth factors-α and -β differentially regulate growth and steroidogenesis of bovine thecal cells during antral follicle development. Endocrinology 129:2041.

Savion, N., G. Lui, R. Laherty, and D. Gospodarowicz. 1981. Factors controlling proliferation and progesterone production by bovine granulosa cells in serum-free medium. Endocrinology 109:409.

Schams, D., R. Koll, and C. H. Li. 1988. Insulin-like growth factor-I stimulates oxytocin and progesterone production by bovine granulosa cells in culture. J. Endocrinol. 116:97.

Skinner, M. K. 1991. Cell-cell interactions in the testis. Endocr. Rev. 12:45.

Skinner, M. K., and R. J. Coffey. 1988. Regulation of ovarian cell growth through the local production of transforming growth factor-α by theca cells. Endocrinology 123:2632.

Skinner, M. K., and M. D. Griswold. 1983. Multiplication stimulating activity (MSA) can substitute for insulin to stimulate the secretion of testicular transferrin by cultured Sertoli cells. Cell Biol. Int. Rep. 7:441.

Skinner, M. K., J. Keski-Oja, K. G. Osteen, and H. L. Moses. 1987a. Ovarian thecal cells produce transforming growth factor-β which can regulate granulosa cell growth. Endocrinology 121:786.

Skinner, M. K., and H. L. Moses. 1989. Transforming growth factor beta gene expression and action in the seminiferous tubule: peritubular-Sertoli cell interactions. Mol. Endocrinol. 3:625.

Skinner, M. K., D. Lobb, and J. H. Dorrington. 1987b. Ovarian thecal/interstitial cells produce an epidermal growth factor-like substance. Endocrinology 121:1892.

Skinner, M. K., K. Takacs, and R. J. Coffey. 1989. Transforming growth factor-alpha gene expression action in the seminiferous tubule: peritubular cell-Sertoli cell interactions. Endocrinology 124:845.

Smith, E. P., S. H. Hall, L. Monaco, F. S. French, M. W. Wilson, and M. Conti. 1989. A rat Sertoli cell factor similar to basic fibroblast growth factor increases c-fos messenger ribonucleic acid in cultured Sertoli cells. Mol. Endocrinol. 3: 954.

Smith, E. P., M. E. Svoboda, J. J. Van Wyk, A. L. Kierszenbaum, and L. T. Tres. 1987. Partial characterization of a somatomedin-like peptide from the medium of cultured rat Sertoli cells. Endocrinology 120:186.

Sordoillet, C., M. A. Chauvin, A. Revol, A. M. Morera, and M. Benahmed. 1988. Fibroblast growth factor is a regulator of testosterone secretion in cultured immature Leydig cells. Mol. Cell. Endocrinol. 58:283.

Stastny, M., and S. Cohen. 1972. The stimulation of ornithine decarboxylase activity in testes of the neonatal mouse. Biochim. Biophys. Acta 261:177.

Story, M. T., J. Sasse, D. Kakuska, S. C. Jacobs, and R. K. Lawson. 1988. A growth factor in bovine and human testes structurally related to basic fibroblast growth factor. J. Urol. 140:422.

Stubbs, S. C., T. B. Hargreave, and F. K. Habib. 1990. Localization and characterization of epidermal growth factor receptors on human testicular tissue by biochemical and immunohistochemical techniques. J. Endocrinol. 125:485.

Suarez-Quian, C. A., M. Dai, M. Onada, R. M. Kriss, and M. Dym. 1989. Epidermal growth factor receptor localization in the rat and monkey testis. Biol. Reprod. 41:921.

Syed, V., O. Soder, S. Arver, M. Lindh, S. Khan, and E. M. Ritzen. 1988. Ontogeny and cellular origin of an interleukin-1-like factor in the reproductive tract of the male rat. Int. J. Androl. 11:437.

Teerds, K. J., D. G. DeRooij, F.F.G. Rommerts, I. Van Der Tweel, and C.J.G. Wensing. 1989. Turnover time of Leydig cells and other interstitial cells in the testis of the adult rat. Arch. Androl. 23:105.

Teerds, K. J., F.F.G. Rommerts, and J. H. Dorrington. 1990. Immunohistochemical detection of transforming growth factor-alpha in Leydig cells during development of the rat testis. Mol. Cell. Endocrinol. 69:R1.

Thompson, N. L., K. C. Flanders, J. M. Smith, L. R. Ellingsworth, A. B. Roberts, and M. B. Sporn. 1989. Expression of transforming growth factor-β1 in specific cells and tissues of adult and neonatal mice. J. Cell Biol. 108:661.

Tres, L. L., E. P. Smith, J. J. Van Wyk, and A. L. Kierszebaum. 1986. Immunoreactive sites and accumulation of somatomedin-c in rat Sertoli-spermatogenic cell cocultures. Exp. Cell Res. 162:33.

Tsafrirri, A., W. Vale, and A.J.W. Hsueh. 1989. Effects of transforming growth factors and inhibin-related proteins on rat preovulatory Graafian follicles *in vitro*. Endocrinology 125: 1857a.

Tsutsumi, O., H. Kurachi, and T. Oka. 1986. A physiological role of epidermal growth factor in male reproductive function. Science 233:975.

Ueno, N., A. Naird, F. Esch, N. Ling, and R. Guillemin. 1987. Isolation and partial characterization of basic fibroblast growth factor from bovine testis. Mol. Cell. Endocrinol. 49: 189.

Veldhuis, J. D., J. E. Nestler, and J. F. Strauss. 1987. The insulin-like growth factor, somatomedin-C, modulates low density lipoprotein metabolism by swine granulosa cells. Endocrinology 121:340.

Veldhuis, J. D., R. J. Rodgers, A. Dee, and E. R. Simpson. 1986. The insulin-like growth factor, somatomedin C, induces the synthesis of cholesterol side-chain cleavage cytochrome P-450 and adrenodoxin in ovarian cells. J. Biol. Chem. 261: 2499.

Welsh, T. H., and A.J.W. Hsueh. 1982. Mechanisms of the inhibitory action of epidermal growth factor on testicular androgen biosynthesis. Endocrinology 11:1498.

Yanker, B. A., and E. M. Shooter. 1982. The biology and mechanism of action of nerve growth factor. Annu. Rev. Biochem. 51:845.

Ying, S. T., A. Becker, N. Ling, N. Ueno, and R. Guillemin. 1986. Inhibin and beta type transforming growth factor (TGFβ) have opposite modulating effects on the follicle stimulating hormone (FSH)-induced aromatase activity of cultured rat granulosa cells. Biochem. Biophys. Res. Commun. 136:969.

Zhiwen, Z., J. K. Findlay, R. S. Carson, A. C. Herington, and H. G. Burger. 1988. Transforming growth factor β enhances basal and FSH-stimulated inhibin production by rat granulosa cells in vitro. Mol. Cell. Endocrinol. 58:161.