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# The Role of Growth Factors in Ovarian Function and Development

*Shahar Kol, Richard M. Rohan, and Eli Y. Adashi*

Ovarian folliculogenesis is a dynamic process marked by exponential expansion and differentiation of the granulosa cells, maturation of the oocyte, and neovascularization. Although the central roles of gonadotropins and of gonadal steroids in this explosive agenda are well accepted, the variable fate of follicles within the same ovary suggests the existence of additional intraovarian modulatory systems.[1] Stated differently, it is presumed that gonadotropin action is "fine-tuned" in situ, thereby accounting for observed differences in the rate and extent of development of ovarian follicles. Alterations in gonadotropin secretion cannot adequately explain the initiation and arrest of meiosis within the oocyte, the acquisition of follicular dominance, or the failure of follicular development, which leads to atresia. Furthermore, it is highly likely that the earlier stages of follicular growth, generally considered to be gonadotropin-independent, may be controlled by intraovarian signalling. Interestingly, the concept of local gonadal regulators dates back to the time when the embryology of the ovary was the subject of intense scrutiny. Indeed, gonadal differentiation has been proposed by Witschi to result from the interaction of two morphogenic substances termed cortexone and medullarine, the first of which was thought of as the stimulator of ovarian development, the latter being thought of as the promoter of testicular growth.[2] Although multiple other contributors must undoubtedly be acknowledged, the notion of intraovarian regulators was promoted with special vigor by the late Cornelia Post Channing, whose pioneering experiments ushered in contemporary molecular endocrinology as it applies to ovarian physiology.[3]

Among potential novel intraovarian regulators, growth factors, cytokines, and neuropeptides have been the subject of increasingly intense investigation. Importantly, most of these agents are not expected to act in the traditional endocrine fashion given their local intraovarian generation (as opposed to circulatory-derived influences emanating from distant endocrine glands). Thus, current speculation favors the notion that a host of putative intraovarian regulators may, in fact, engage in subtle in situ modulation and coordination of growth and function of the varied follicular cell types: oocyte, granulosa, theca, and vascular epithelium. In this capacity, a given putative intraovarian regulator may modulate the replication or cyto-differentiation of a developing ovarian cell, acting either in its own right or as an amplifier-attenuator of gonadotropin action. Such putative intraovarian regulators may also be concerned with intercompartmental communication, allowing for a tighter linking of different cellular populations. For example, a growing body of evidence now suggests that granulosa cell-derived modulators may, in fact, regulate the adjacent theca-interstitial cell compartment in the interest of coordinated follicular development. In doing so, the granulosa cell may exert some control over its own destiny, in that it may regulate the very inflow of androgenic substrate from the neighboring theca. Together, gonadotropins, steroids, and locally derived peptider-

gic principles form a triad, which modulates the growth and differentiation of ovarian follicles (Fig. 1).

According to contemporary views, potential intraovarian communication is mostly paracrine or autocrine in nature. Paracrine communication involves local diffusion of regulators from producer cells to distinct target cells within the same organ. This is a heteroregulatory phenomenon that could allow for intercompartmental communication, thus providing a tighter linkage of different cellular populations. In the ovary, the ability of increasing numbers of granulosa cells to produce estrogen depends on the concomitant ability of the thecal layer to provide the proper amounts of androgenic substrate. Thus, the granulosa cell, in the interest of efficient coupling, may elaborate principles (*e.g.*, insulin-like growth factor, inhibin, activin), which could alter the function of the neighboring theca. The other type of cellular communication, autocrine regulation, involves the action of a regulator on surface receptors at its cell of origin. This is a self-regulatory phenomenon wherein a single cell type modulates its own activity. In the ovary, granulosa cells elaborate principles (*e.g.*, insulin-like growth factor, activin), which can alter granulosa cell function. Whereas steroids may be exerting intracrine (regulation within the cell of origin) effects, there is as yet no evidence for juxtacrine (contact-dependent regulation between immediately adjacent cells) effects in the ovary.

To qualify as a bona fide intraovarian regulator, the putative agent needs to meet the following minimal criteria: (1) local production, (2) local reception, and (3) local action. In addition, some evidence of indispensability to in vivo ovarian function needs to be provided. For the most part, very few of the putative intraovarian regulators currently under study (Table 1) have satisfactorily met all of the above criteria (IGF-I, activin). Accordingly, the information provided below can merely be viewed as a prelude to what the future holds. Undoubtedly, additional information will become available with respect to the putative intraovarian regulators now under consideration. It is equally certain that novel candidates will be added to this preliminary list, requiring modification of current views.

**TABLE 1. Established and Putative Intraovarian Regulators**

| |
|---|
| **Insulin-Like Growth Factor System** |
| IGF-I |
| IGF-II |
| IGF binding proteins |
| **Inhibin/Activin System** |
| Inhibin |
| Activin |
| Follistatin |
| **Interleukin-1 System** |
| Interleukin-1 |
| Interleukin-1 receptor antagonist |
| IL-1 binding protein (IL-1 receptor type II) |
| **Other Growth Factors** |
| EGF/TGF$\alpha$ |
| TGF$\beta$1, TGF$\beta$2 |
| NGF |
| aFGF, bFGF |
| VEGF |
| TNF$\alpha$ |
| **Other Peptidergic Factors** |
| Ovarian renin angiotensin system |
| VIP |
| Oxytocin |
| Endothelin |

The following is a brief listing of a select group of putative intraovarian regulators reflecting different modes of action.

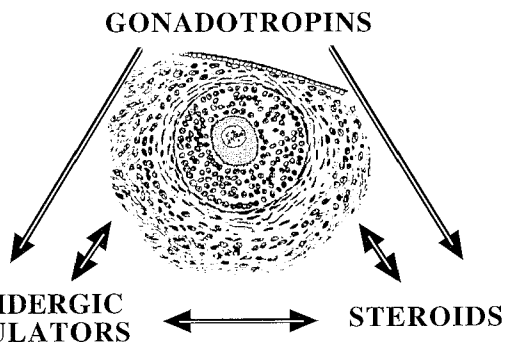


**Fig. 1.** Modulators of ovarian follicular growth and development: the regulatory triad.

## INSULIN-LIKE GROWTH FACTOR-I

A 70-amino acid polypeptide, insulin-like growth factor-I (IGF-I) plays a variety of metabolic and endocrine roles, not the least of which is the promotion of linear skeletal growth. In keeping with its ubiquitous distribution, IGF-I is also known to subserve a variety of autocrine/paracrine tissue-specific functions to suit the needs dictated by the tissues in question. In this respect, the ovary is but one example out of many exemplifying the general concept of intraorgan regulation.[4]

A large body of information now strongly supports the view that the ovary is a site of IGF-I

production, reception, and action (Fig. 2). Whereas the rat granulosa cell appears to be the only cellular site of IGF-I gene expression,[5,6] both the granulosa[7,8] and the theca-interstitial cells[9,10] possess specific receptors for this peptidergic ligand. These observations suggest that IGF-I may engage in intercompartmental communication in the interest of coordinated follicular development. Interestingly, IGF-I hormonal action appears subject to further modulation through the local elaboration of low molecular weight binding proteins (IGFBPs), the role and regulation of which are currently receiving increasing attention. The recent discovery of IGFBP-4 mRNA in early stage atretic follicles raises the intriguing possibility that depletion of IGF action may be necessary for the onset of the atretic process.[11]

Although multiple ovarian actions have been ascribed to IGF-I, its main role appears to be the amplification of gonadotropin action in both theca-interstitial and granulosa cells. All markers of follicle-stimulating hormone (FSH)-induced maturation (*e.g.*, production of progesterone, estrogen, inhibin, and luteinizing hormone binding) are enhanced by IGF-I. Indeed, optimal gonadotropin hormonal action is contingent on the prior availability of granulosa cell-derived IGF-I and the consequent amplification of the gonadotropic signal. Given a hypothetical IGF vacuum created by excess exogenous IGF binding proteins, intrinsic FSH hormonal action proves to be relatively modest (Fig 3). In contrast, given IGF replete circumstances, FSH hormonal action in toto may be comprised of a modest intrinsic component complemented by a substantial synergistic component.[12]

At the clinical level, ovarian IGF-I may have a bearing on the puberty-promoting effect of growth hormone. Indeed, an association appears to exist between isolated growth hormone deficiency and delayed puberty in both rodents and human subjects, a process reversed by systemic hormone re-

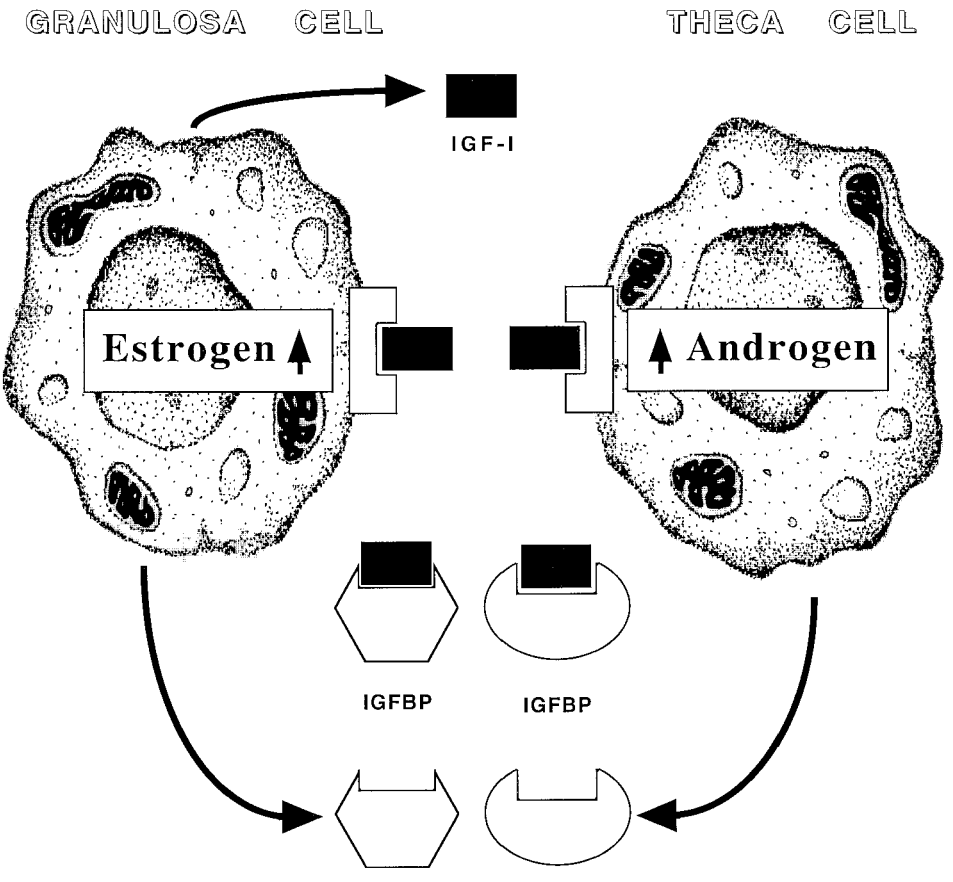


**Fig. 2.** The intraovarian insulin-like growth factor-I system.

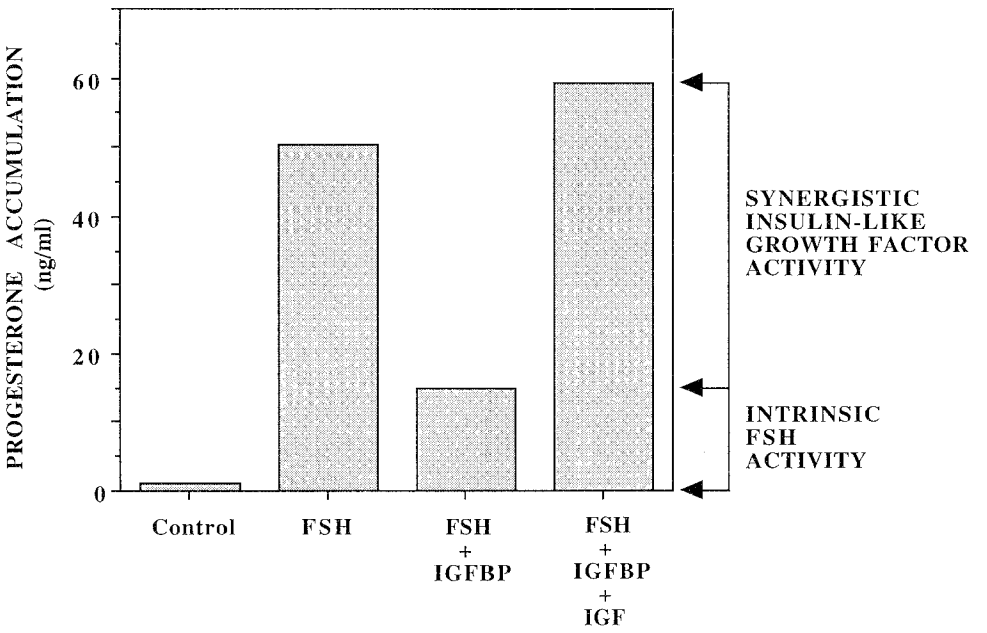


**Fig. 3.** Enhancing effect of insulin-like growth factor-I on FSH-stimulated progesterone accumulation.

placement therapy. Given that ovarian IGF-I and its receptor may be growth hormone-dependent, it is tempting to speculate that the ability of growth hormone to accelerate pubertal maturation may be due, at least in part, to the promotion of ovarian IGF-I production and reception with the consequent local potentiation of gonadotropin action.

## TRANSFORMING GROWTH FACTOR α/EPIDERMAL GROWTH FACTOR

Purified on the basis of its ability to stimulate precocious eyelid opening and tooth eruption in newborn mice, epidermal growth factor (EGF) was initially found in male mouse submaxillary glands and later in human urine as urogastrone. Mature EGF comprises a single polypeptide chain of 53 amino acids displaying three internal disulfide bonds. Originally thought to have a limited range of tissue expression, recent in situ hybridization analysis of sections of whole newborn mice indicate that RNA complementary to cloned EGF probes may be present in a large variety of tissues.

Transforming growth factor α (TGFα), a structural analog of EGF, is a single-chain, 50 amino acid polypeptide capable of binding to an apparently common EGF/TGFα receptor. Not only do EGF and TGFα both recognize the same cellular receptor, they are apparently equally potent in most systems studied. It may be the case that EGF is the adult form of the embryonic growth factor TGFα. Interestingly, TGFα is a member of a family of polypeptides best known for their ability to produce an acute, albeit reversible, phenotypic transformation of normal mammalian cells. TGFα can thus be defined operationally by its ability to stimulate anchorage-independent growth in soft agar of cells, which are otherwise anchorage-dependent.

At the level of the ovary,[13] EGF has been observed to exert potent regulatory effects on granulosa cell proliferation and differentiation.[14–17] Presumably, these effects of EGF are mediated by specific cell membrane receptors, the existence of which on bovine, ovine, and murine granulosa cells has been demonstrated.[18,19] However, the identity of the endogenous ligand occupying the receptor in question under in vivo conditions remains uncertain.

More recently, TGFα, like EGF, proved a potent inhibitor of gonadotropin-supported granulosa cell differentiation. Moreover, TGFα has been exclusively localized to the theca-interstitial cell compartment,[20] thereby raising the possibility that theca-interstitial cell-derived TGFα may exert paracrine effects at the level of the adjacent granulosa cell. Interestingly, theca-interstitial cell-derived

TGF$\alpha$ may also engage in autocrine effects.[21] Thus, it is tempting to speculate that TGF$\alpha$ of theca-interstitial cell origin may orchestrate follicular activities at both the granulosa and theca-interstitial cell level (Fig. 4). However, since TGF$\alpha$ has also been shown to suppress gonadotropin-supported theca-interstitial cell differentiation,[21] the possibility of an autocrine mode of action cannot be excluded.

## TRANSFORMING GROWTH FACTOR $\beta$1

Transforming growth factor $\beta$1 (TGF$\beta$1), a homodimeric polypeptide comprising two identical 112-amino acid chains, is now well recognized as a polyfunctional regulatory molecule.[22] Originally identified by its ability to elicit an acute reversible phenotypic transformation of normal mammalian cells, TGF$\beta$1 has now been shown to exert numerous regulatory actions in a variety of both normal and neoplastic cells. At the level of the ovary, wherein it is produced,[23–26] TGF$\beta$1 has been shown to profoundly alter the proliferation and differentiation of rat granulosa cells.[27–37] In addition, an increasing body of evidence now suggests that the ovarian theca-interstitial, as well as granulosa, cells may be sites of TGF$\beta$1 production and action.[38]

## BASIC FIBROBLAST GROWTH FACTOR

Basic fibroblast growth factor (bFGF), a 146-amino acid polypeptide, is a mitogen for a wide variety of mesoderm-derived and neuroectoderm-derived cells. Its complete isolation and characterization has thus far been accomplished from various organs, an amino terminally truncated form lacking the first 15 residues having been identified in the ovarian corpus luteum.[39] Although the physiologic relevance of bFGF to ovarian function remains under investigation,[40] several lines of evidence suggest that bFGF may play a central role in supporting the growth and development of the granulosa-luteal cell. Indeed, bFGF constitutes the main mitogenic factor isolated from crude luteal extract and has previously been shown to stimulate the replicative lifespan of cultured granulosa cells of bovine, porcine, rabbit, guinea pig, and human origin.[41–43] Since ovarian bFGF expression is not of granulosa cell origin,[44] while FSH induces functional receptors for bFGF in the granulosa cells,[45] it is tempting to speculate that locally produced bFGF[46] may play autocrine or paracrine regulatory roles at or adjacent to its sites of synthesis. In so doing, it may participate in the differentiation and replication of the developing granulosa cell.[47–53]

## ACTIVIN

Activin is a 24 kD protein with structural homology to TGF$\beta$1. It was discovered during the purification of inhibin and found to be a dimer of the beta subunits of the heterodimeric inhibin molecule.[54] Activin was concurrently discovered as a factor capable of differentiating erythroleukemia cells[55] and inducing mesoderm formation.[56] Its presence in a variety of cell types suggests that it may regulate growth and differentiation in other tissues as well.[54]

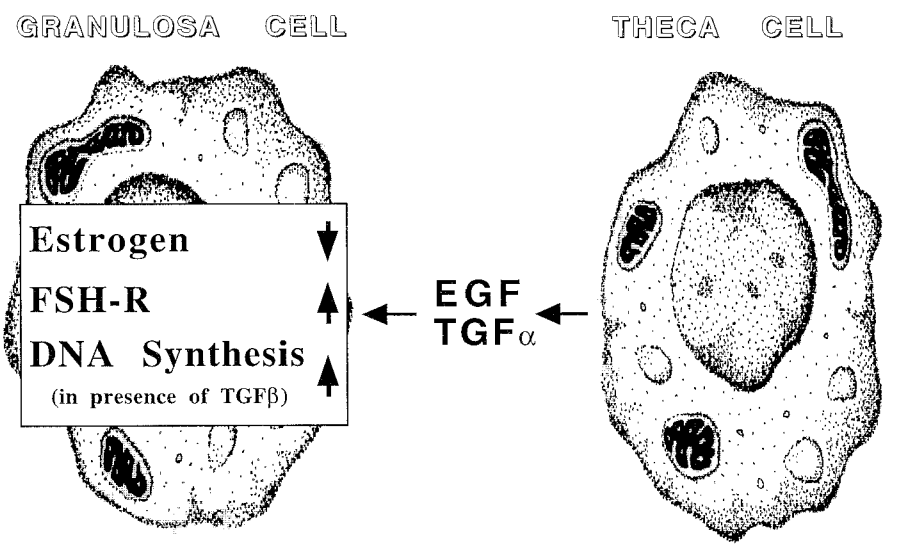


**Fig. 4.** The intraovarian EGF/TGF$\alpha$ system.

It is now clear that activins play a role in the local regulation of ovarian function. Acting through a set of recently described receptors, postulated to be membrane-bound serine/threonine kinases,[57,58] activin alters the function of both granulosa and theca-interstitial cells. For instance, activin treatment of cultured granulosa cell from immature follicles increases FSH-supported estradiol production, inhibin production, and FSH and luteinizing hormone binding. Thus, activin may maintain the immature follicle during the period of declining FSH levels, which is induced by its partner inhibin. In contrast to its action on immature granulosa cells, activin decreases progesterone production by mature granulosa cells from preovulatory follicles.[59] Based on these observations, Findlay and co-workers[60] have proposed an autocrine role for activin as a suppressor of spontaneous luteinization. Paracrine actions of activin are also a possibility since activin reduces luteinizing hormone-induced androstenedione production by cultured theca-interstitial cells.[61,62] Follistatin, a glycoprotein with isoforms of 35–40kD, was also discovered during the purification of inhibin.[54] Its ability to bind activin[63] provides a possible explanation for the observation that follistatin antagonizes the in vitro actions of activin. The presence of follistatin primarily in preovulatory follicles[64] supports the idea that blocking activin is necessary for maturation and luteinization. The study of activin action is further complicated by the ability of its component subunits to combine with the $\alpha$-subunit of inhibin to form a molecule whose action in many experimental assays is diametrically opposed to that of activin. Although autocrine actions of inhibin have not been convincingly demonstrated, granulosa cell-derived inhibin can oppose the activin blockade of thecal androgen production.[61] Thus, activin, follistatin, and inhibin form a complex mix of intraovarian regulators (Fig. 5).

## INTERLEUKIN-1

Interleukin-1 (IL-1), a polypeptide cytokine (previously referred to as lymphocyte activating factor), predominantly produced and secreted by activated macrophages, has been shown to possess a wide range of biologic functions, as well as to play a role as an immune mediator.[65–67] At the level of the ovary, IL-1 has been observed to suppress the functional and morphologic luteinization of cultured murine and porcine granulosa cells.[68–72] Exerted at "physiologic" ($10^{-9}$ M) concentrations, IL-1 action could not be attributed to altered cell viability. Rather, the antigonadotropic activity of IL-1 appeared to involve sites of action both proximal and distal to cAMP generation. More recent work by Kasson and Gorospe sheds additional light on the ovarian relevance of interleukins.[73] Indeed, both IL-1$\alpha$ and IL-1$\beta$ augmented the FSH-stimulated accumulation of 20$\alpha$-dihydroprogesterone. In all cases, less IL-1$\beta$ than IL-1$\alpha$ was required to produce a comparable effect. More recent studies in rat ovary indicate that the rat ovarian theca-interstitial cell is a site of IL-1$\beta$ gene expression,

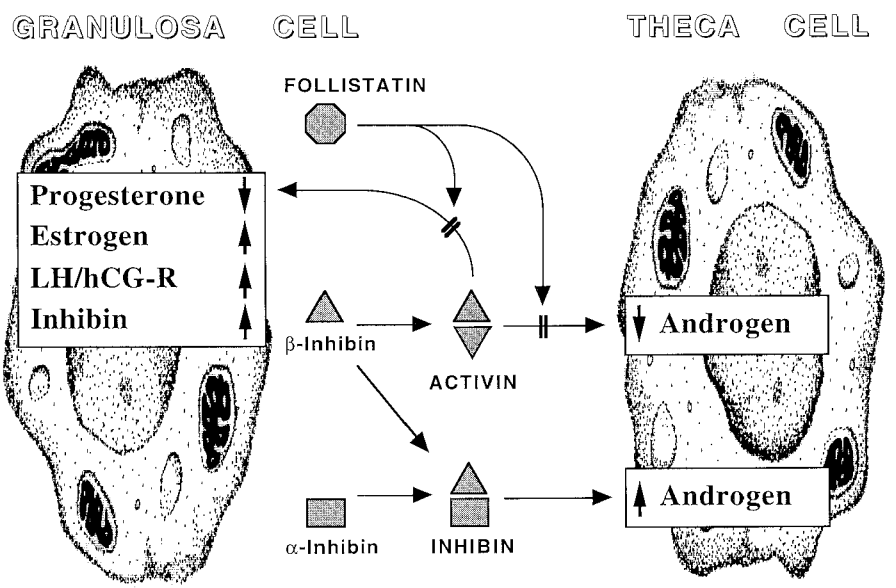


**Fig. 5.** The intraovarian activin/inhibin system.

the preovulatory acquisition of which is gonadotropin dependent.[74] However, immediately after follicle rupture, granulosa cells stain positive for IL-1$\beta$ in immunohistochemical studies in the mouse ovary.[75] Indeed, the possibility of a shift in IL-1$\beta$ origin, receptor and action to the granulosa cell compartment just before ovulation cannot be ruled out. Although the relevance of IL-1 to ovarian physiology remains a matter of study, it is tempting to speculate that IL-1 could possibly be involved in mediation of gonadotropin action and in the luteinization process (Fig. 6). Such speculation appears particularly intriguing in light of the apparent progesterone-dependence of IL-1 gene expression.[76] In contrast, higher concentrations of progesterone significantly inhibit IL-1 activity.[77,78] While much remains to be learned on the intraovarian cellular origin of IL-1, resident interstitial ovarian macrophages could well be a site of hormonally regulated IL-1 gene expression given the reported gonadotropin-dependence of their testicular counterparts.[79–81] IL-1 could also be produced by ovarian cells proper as has been shown for various other specialized cell types.[67] Interestingly, significant amounts of IL-1-like activity have been detected in follicular fluid.[82,83] The ovarian reception of IL-1 involves the type I IL-1 receptor whose transcripts have been shown in cultured human granulosa and theca cells.[84] Recently, it was shown that IL-1 signaling occurs exclusively via the type I receptor,[85] while the type II receptor inhibits IL-1 activity by acting as a "decoy" target for IL-1.[86]

## TUMOR NECROSIS FACTOR $\alpha$

The potential ovarian relevance of another macrophage product, tumor necrosis factor (TNF$\alpha$), has recently been explored.[87–89] TNF$\alpha$, a 157-amino acid polypeptide, was originally named for its oncolytic activity as displayed in the serum of bacillus Calmette-Guerin-immunized, endotoxin-challenged mice.[90,91] Indeed, TNF$\alpha$ proved capable of inducing tumor necrosis in vivo and of exerting nonspecies specific cytolytic or cytostatic effects on a broad range of transformed cell lines in vitro. Although TNF$\alpha$ was initially thought to be tumor-selective, it has become clear that certain nontumor cells possess TNF$\alpha$ receptors and that TNF$\alpha$ may be a regulatory monokine with pleiotropic noncytotoxic activities, in addition to its antitumor properties. Most importantly, TNF$\alpha$ has been shown to engage in the differentiation of a variety of cell types.

At the level of the ovary, TNF$\alpha$ was found capable of attenuating the differentiation of cultured granulosa cells from immature rats.[92] In other studies, TNF$\alpha$ has been found to effect complex dose-dependent alterations in the elaboration of progesterone and androstenedione, but not estrogen by explanted preovulatory follicles of murine origin. While the ovary contains TNF$\alpha$ mRNA,[93] its in vivo origins must be determined. In principle, two general possibilities are worthy of consideration. First, TNF$\alpha$ may be locally derived from (activated) resident ovarian macrophages as has been shown for regressing (but not young) corpora lutea.

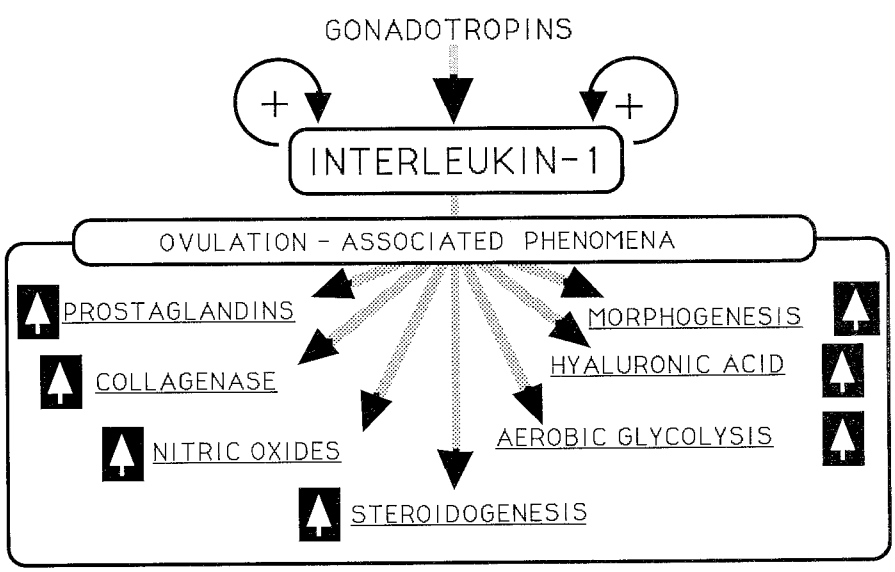


**Fig. 6.** Intraovarian interleukin-1 as a mediator of gonadotropin action.

Although basal TNFα activity was undetected in corpora lutea of both pregnancy and pseudopregnancy, TNFα activity was markedly stimulated in the presence of lipopolysaccharide.[87] However, the detection of TNFα activity in some luteal tissue on day 5 and the scarcity of macrophages at this stage raise the possibility that cells other than macrophages may also produce TNFα in the corpus luteum. Indeed, TNFα may be of granulosa cell origin, as suggested by immunohistochemical studies wherein antral or atretic granulosa cells have been implicated as a possible site of TNFα gene expression. Given such strong association between TNFα elaboration and follicular and luteal decline, it is tempting to speculate that TNFα may play a role in the still enigmatic processes of atresia or luteolysis. In this capacity, TNFα of intraovarian origin may exert its effects at or adjacent to its site of synthesis, interacting with specific granulosa/luteal cell surface receptors to modulate gonadotropin hormonal action. Undoubtedly, future studies of the regulation of the TNFα receptor and the elucidation of the in vivo source of its ligand will shed new light on the relevance of this system to the process of follicular development or demise.

## OTHER GROWTH AND PEPTIDERGIC FACTORS

There are a number of other growth and peptidergic factors that have potential physiologic relevance to folliculogenesis (see Table 1). The ovary contains a complete renin-angiotensin system that may be involved with vascularization, as well as modulation of steroidogenesis.[94] Vasoactive intestinal peptide is also produced locally in the ovary and it can enhance estrogen production by granulosa cells of prepubertal rats.[95]

Nerve growth factor is another peptidergic factor whose mRNA has been detected in the ovary[96] but its modulatory role in the ovary is unknown. Similarly, endothelin, a potent vasoconstrictor, influences ovarian progesterone production[97] but its local production in the ovary has not been documented.

## GONADOTROPIN MODULATION AND MEDIATION

If there are any lessons to be learned at this time, it is the realization that optimal gonadotropin hormonal action is highly contingent on the input of tissue-based regulatory principles. According to this view, gonadotropins may not be the omnipotent agents they were once thought to be. Rather, gonadotropins may best be viewed as "team players" and as initiators of a cascade of events facilitated, attenuated, or mediated through interaction with putative intraovarian regulators. It is the special case of IGF-I that best illustrates the role of a tissue-based modulator in that optimal gonadotropin hormonal action is clearly highly dependent on the availability of IGF-I and the consequent amplification of gonadotropin hormonal action.[12] In contrast, putative intraovarian regulators exemplified by TGFα may attenuate gonadotropin hormonal differentiation in the interest of continued proliferative ability. Yet another role for putative intraovarian regulators, exemplified by IL-1, is that of mediation of gonadotropin action. According to this view, IL-1 constitutes an extension of the gonadotropin signal, possibly one of several more distal effectors, the overall mission of which may well be the conveyance of the message (or portions thereof) imparted by the midcycle surge.

The development of the ovarian follicle is a continuum of growth and differentiation of at least three distinct cell types; thecal cells, granulosa cells, and the oocyte. Clearly then, much will depend on the localization and timing of expression of the regulatory principles. Of equal importance is the ability of the target cell to receive and respond to the regulatory signal. Activin elicits both a stimulatory and an inhibitory response, depending on the cell type being studied[61] and the developmental stage of the follicle.[59] This duality of action may be explained if the activin receptor isoforms[58] prove to have a cell-type and developmental-stage specific distribution. The action of a given regulatory factor, such as EGF/TGFα, can also be influenced by the presence or absence of other factors.[98] The ability of IL-1[99] and nerve growth factor[100] to alter EGF/TGFα binding in nonovarian cell types is an example of how one growth factor can impinge on the actions of another.

Thus, it is the net final balance representing the integration of multiple transduction pathways (Fig. 7) and often opposing signals that determines net gonadotropin hormonal action. Moreover, it is now clear that a given intraovarian growth factor may play several roles depending on its local concentration, availability of its receptors or binding proteins, the cell population with which it interacts, and the precise timing of that interaction. There is every reason to believe that future studies may reveal other modes of interaction between trophic ovarian principles and tissue-based regulatory elements. It is with a strong sense of excitement that future work in this evolving area is anticipated.

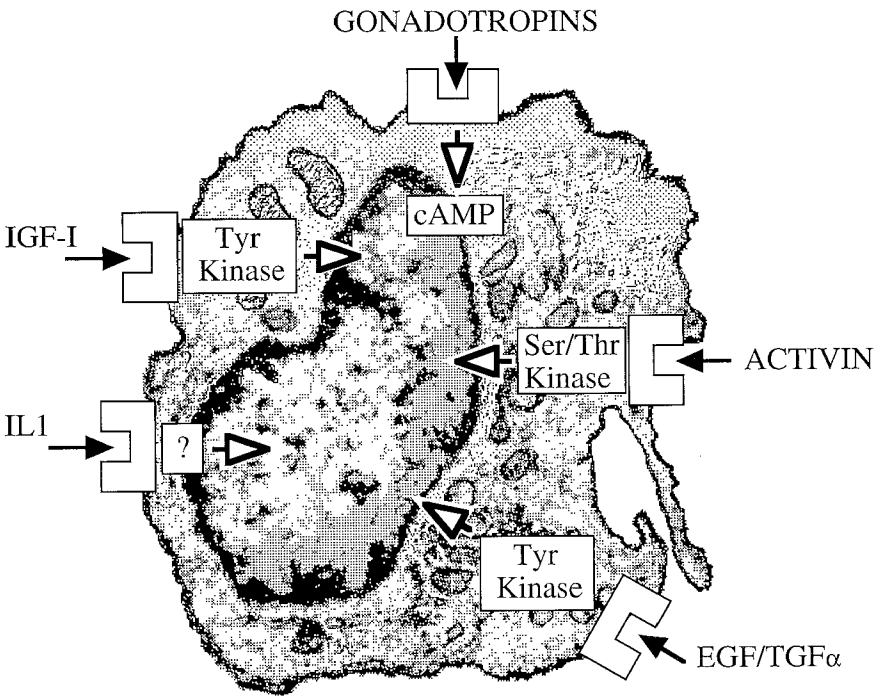


**Fig. 7.** Signal transduction pathways in the ovary.

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