

Intraovarian factors regulating ovarian function

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Ovarian folliculogenesis is a dynamic process marked by exponential expansion and differentiation of the granulosa cells, oocyte maturation, ovulation and corpus luteum formation. Although the central roles of gonadotropins and gonadal steroids in this explosive agenda are well accepted, the variable fate of follicles afforded comparable stimulation within the same ovary suggests the existence of an additional intraovarian system comprised of regulating species that 'fine-tune' the blood-borne hormones.

Current Opinion in Obstetrics and Gynecology 1995, 7:209–213

Introduction

Although the concept of local regulators was introduced nearly 50 years ago, the past 20 years have witnessed an intense investigation of potential intraovarian regulators, growth factors, cytokines and neuropeptides, most of which act in a paracrine or autocrine fashion. Thus, current speculation favors the notion that a host of intraovarian regulators may engage in subtle in-situ modulation of the growth and function of the somatic as well as germ cell compartments. Such putative regulators may also be concerned with intercompartmental communication, allowing for tighter linking of different cellular populations. Together gonadotropins, steroids and locally derived peptidergic principles form a triad which modulates the growth and differentiation of ovarian follicles (Fig. 1). Importantly, the concept of redundancy and overlapping function of these principles should be introduced. The system may cope with chronic absence of a regulator and still produce fertilizable oocytes, as recent gene 'knockout' experiments have shown.

To qualify as a bona fide intraovarian regulator, a putative agent needs to meet the following minimum criteria: local production, local reception, and local action. For the most part, very few of the putative regulators currently under study have satisfactorily met all of these criteria. This review covers a few of those agents that have been the target of recent intense investigation. 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 the list, requiring modification of current views.

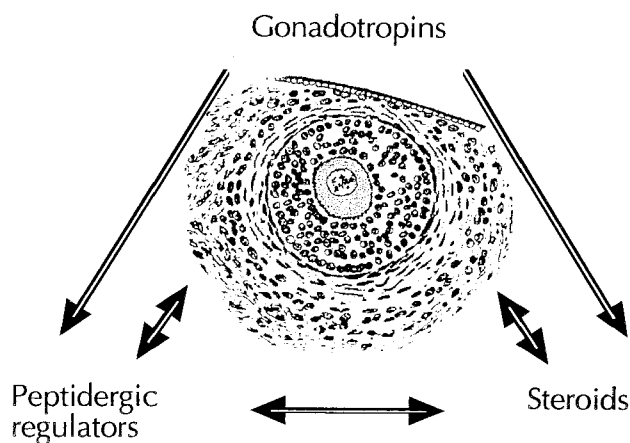


Fig. 1. Modulation of ovarian follicular growth and development: the regulatory triad. Printed by permission of the publisher from Adashi EY, Rohan RM, *Intraovarian regulation, peptidergic signaling systems*. *Trends Endocrinol Metab* 3:243–248. Copyright 1992 by Elsevier Science Inc. [1].

Interleukin-1 system

Interleukin (IL)-1, a polypeptide cytokine predominantly produced and secreted by activated macrophages, has been shown to possess a wide range of biological functions as well as playing a role as an immune mediator [2]. Although the relevance of IL-1 to ovarian physiology remains uncertain, the existence of a highly compartmentalized, hormonally dependent intraovarian IL-1 system complete with ligands, receptors, and a receptor antagonist is now well established [3]. Thus, IL-1 meets the criteria for a bona fide local regulator.

Abbreviations

FSH—follicle-stimulating hormone; **GnRH**—gonadotropin-releasing hormone; **IGF**—insulin-like growth factor; **IGFBP**—IGF-binding protein; **IL**—interleukin; **NO**—nitric oxide; **TGF**—transforming growth factor.

IL-1 interacts with two receptors: a type I receptor, which mediates all of the actions of IL-1 [4•], and a type II receptor which inhibits IL-1 activity by acting as a 'decoy' target for IL-1 [5•]. The relevance of IL-1 to follicular maturation and ovulation has been suggested by its ability to promote a host of ovulation-associated phenomena, giving rise to the speculation that locally derived IL-1 may be the center of an intraovarian regulatory loop concerned with the genesis and maintenance of the preovulatory cascade of follicular events (Fig. 2).

The ability of IL-1 to promote ovulation has been also suggested by ovarian perfusion models in the rat and rabbit. Specifically, IL-1 β has been shown to modulate prostaglandin production, whereas its natural receptor antagonist inhibits ovulation [6]. IL-1b by itself may induce ovulation and oocyte maturation [7,8]. Furthermore, midcycle increases in both ovarian IL-1 protein and peripheral blood monocyte IL-1 transcripts [9] support this notion. The specific cellular expression pattern of the different IL-1 system components is a subject of intense investigation. Clearly, this expression is not macrophage-exclusive, as new evidence points to the potential active involvement of granulosa cells and even the maturing oocyte in expression and secretion of the various IL-1 components in a time-dependent fashion [10•]. Further support for the potential role of IL-1 as an intermediary of ovulation is provided by its involvement in accumulation of gelatinase [11], in addition to its involvement in prostaglandin synthesis [12].

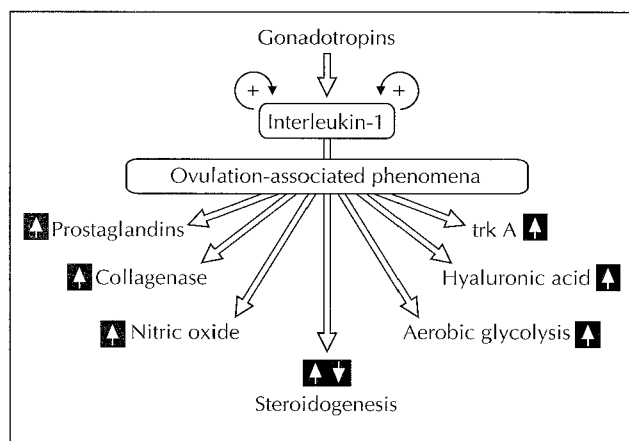


Fig. 2. Interleukin-1 mediates the action of gonadotropin. trk A, the high affinity receptor for nerve growth factor. Printed by permission of the publisher from Adashi EY, Rohan RM, **Intraovarian regulation, peptidergic signaling systems.** *Trends Endocrinol Metab* 3:243–248. Copyright 1992 by Elsevier Science Inc. [1].

The relevance of other members of the IL family to ovarian physiology is less certain, although recent evidence suggests a role for IL-6 in granulosa cell growth and differentiation [13,14].

The insulin-like growth factor system

The insulin-like growth factor (IGF) system, comprising ligands, receptors and binding proteins, plays a va-

riety of metabolic and endocrine roles, not the least of which is promotion of skeletal growth. IGF-I, a prominent member of the family, is known to serve a variety of autocrine–paracrine tissue-specific functions. A large body of information now supports the view that IGF-I may engage in intercompartmental communication in the interest of coordinated follicular development. Its main role appears to be the amplification of gonadotropin action in both theca–interstitial and granulosa cells.

IGF-I hormonal action is subjected to further modulation through the local elaboration of low molecular weight IGF-binding proteins (IGFBPs). Six mammalian IGFBPs have been identified so far, although their individual functions have not been fully elucidated, and are the subject of intense research effort. Generally, IGFBPs modulate the availability of the free ligand to interact with its receptor (Fig. 3). Consequently, follicular growth is associated with a decrease in IGFBPs' local concentrations, giving rise to intense IGF effect, whereas follicular atresia involves an increased IGFBP availability, followed by an IGF decrease [15]. Further regulation of IGFBP levels is mediated by IGF-I itself [16,17], possibly by controlling IGFBP's expression or their proteolytic degradation [18]. IGF-II hormonal action has recently received more attention, and is suggested to involve stimulation of steroidogenesis in granulosa cells [19], and cellular proliferation *in vitro* [20].

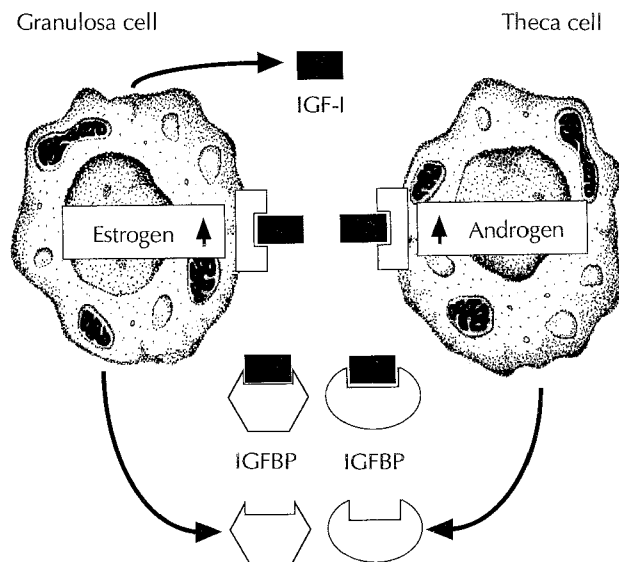


Fig. 3. The local availability of free insulin-like growth factor-I is modulated by its binding proteins. IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein. Printed by permission of the publisher from Adashi EY, Rohan RM, **Intraovarian regulation, peptidergic signaling systems.** *Trends Endocrinol Metab* 3:243–248. Copyright 1992 by Elsevier Science Inc. [1].

At the clinical level, the ovarian IGF system may have a bearing on the puberty-promoting effect of growth hormone. During the reproductive years a well coordinated IGF system appears to be critical for successful follicu-

lar growth, maturation and ovulation. Ovaries obtained from patients with polycystic ovary syndrome were compared with normal ovaries [21•]. Although no differences were found in the small antral follicle population, follicular maturation in normal ovaries was associated with IGF-II and IGFBP-1 gene expression, decreased levels of IGFBP-2, and increased levels of IGFBP-3 in granulosa cells, none of which were observed in polycystic ovaries, probably reflecting maturation arrest of these follicles [21•].

Nitric oxide

The free radical gas, nitric oxide (NO), was honored with the title 'molecule of the year' in 1992, because of its wide range of physiologic and pathologic effects [22]. A large body of information has been gathered on the role of NO in the nervous system, blood pressure regulation, immune system and even in digestion; however, information on the role of NO in ovarian physiology is scarce. Such a role seems to be plausible, as it is tempting to speculate a role for NO in regulation of blood flow to the dominant follicle and corpus luteum, or in follicular atresia. Although IL-1 was demonstrated to stimulate the ovarian inducible form of NO synthase [23], the role of NO in mediating IL-1-induced cytotoxicity is controversial [24,25]. More direct evidence for the involvement of NO in ovulation was offered by Shukovski and Tsafiriri [26] who partially inhibited ovulation by intrabursal injection of NO inhibitors.

Inhibin and activin

Inhibin and activin are glycoprotein members of the transforming growth factor (TGF)- β superfamily, which regulates growth and differentiation, and in that capacity probably plays a significant role in embryonic development. Inhibin is a heterodimer composed of a common α -subunit and one of two β -subunits: inhibin-A (α , β A), and inhibin-B (α , β B). Similarly, activin is a dimer of the β -subunits only: activin-A (β A, β A) and activin-AB (β A, β B). Classically, inhibin is characterized by its inhibitory effect on follicle-stimulating hormone (FSH) secretion, whereas activin exerts an opposite effect; both serve as non-steroidal gonadal input at the pituitary level.

In addition to these effects, a large body of evidence shows their role in mediating local ovarian events. Binding sites for activin-A were found on rat granulosa cells of all follicular sizes, whereas binding of inhibin-A was restricted to the antral granulosa cells of Graafian follicles [27]. A role for both species in ovarian steroidogenesis has been suggested [28], underscoring activin as a promoter of estrogen synthesis and suppressor of progesterone synthesis during folliculogenesis. Inhibin probably plays a secondary role in this regard. During the luteal phase, inhibin suppresses FSH levels in primates, whereas activin is a potent luteolytic agent, probably through suppression of luteinizing hormone levels [29]. To further dissect the role of activin and inhibin in reproduction, use was made of a gene 'knockout' ex-

periment: mouse strains carrying mutations in the gene encoding the β B subunit were generated [30••]. Mutant males bred normally, and mutant females were able to become pregnant, probably reflecting the principle of redundancy and overlapping roles for the numerous intraovarian mediators and regulators, especially when a mediator is chronically absent from the reproductive arena. Interestingly, mutant females failed to raise their offspring normally, probably because of a defect in the delivery process or in maternal nursing [30••].

Gonadotropin-releasing hormone

A growing body of evidence now supports the possibility that gonadotropin-releasing hormone (GnRH) may also qualify as an intraovarian regulator. GnRH or GnRH-like peptides are produced in the ovary [31], and GnRH receptors that are identical in primary structure to the well characterized pituitary GnRH receptors have been identified in the ovary [32]. The full scope of GnRH action on the ovary is yet to be elucidated, but current information suggests that GnRH may play a role in ovarian steroidogenesis [33], and may antagonize gonadotropin stimulation of follicle development, possibly by inducing apoptotic cell death [34]. GnRH involvement in apoptosis could be mediated by stimulating the expression of IGFBP-4, and abolishing the ability of FSH to inhibit IGFBP-4 expression and action [35], an effect that was blocked by GnRH antagonist. In addition, a mechanism for a rapid termination of the effect of GnRH on granulosa cells has been suggested [36].

Transforming growth factors

TGF α and TGF β are intra-ovarian regulators that have been associated with a host of follicular functions. TGF β 1 and TGF α have been detected in theca-interstitial cells, whereas TGF β 2 is expressed by granulosa cells and in the developing oocyte [37]. TGF α has been observed to exert potent regulatory effects on granulosa cell proliferation and differentiation. Specifically, TGF α proved to be a potent inhibitor of gonadotropin-supported granulosa cell differentiation. This effect is illustrated by direct inhibition of aromatization and inhibin production by the granulosa cell [38], and by a decrease in the basal FSH receptor messenger-RNA levels in the granulosa cells. In contrast, both TGF β 1 and TGF β 2 were shown to increase FSH receptor messenger-RNA levels [39]. The differentiation effect of TGF α is further suggested by its prevalence both at all stages of fetal ovarian development [40], and in surface epithelium and ovarian carcinoma cells [41].

Sex steroids

Generally, estrogen secretion is associated with healthy follicles, whereas decreased estrogen and increased an-

drogen production are associated with a follicle that is destined to atresia. DNA fragmentation, the molecular presentation of atresia, is evident in estrogen-depleted rats [42]. In-situ analysis showed that the apoptotic process is confined to antral and preantral follicles, whereas primordial follicles and theca-interstitial cells are spared. The antiatretogenic effect of estrogen was blocked by androgens [42].

Further insight into the role of estrogens in the ovary is gained by insertional disruption of the mouse estrogen receptor gene. Because no human estrogen receptor gene mutation is known, in contrast to androgen receptor (testicular feminization), it is indeed surprising that mutant mice lacking estradiol receptors have survived to adulthood with normal external phenotype. Mutant female mice were infertile, but had a uterus, albeit hypoplastic, and ovaries with a few primary follicles [43]. Although this experiment may lead us to question the crucial role of estrogens in the embryonic development of the reproductive tract, it is possible that the residual function, as illustrated by hypoplastic ovaries and uterus, is a product of a parallel regulatory system that can take over some of the lost function, again underscoring the principle of redundancy and overlapping function between the many regulatory systems that are operational in the ovary.

Conclusion

If there are any lessons to be learned at this time, it is the realization that optimal gonadotropin hormonal action is highly contingent upon the input of tissue-based regulatory principles. Gonadotropins may be viewed as 'team players' and as initiators of a cascade of events facilitated, attenuated, or mediated through a well orchestrated interaction with intraovarian regulators. The development of the ovarian follicle is a continuum of growth and differentiation of at least three distinct cell types; theca cells, granulosa cells and the oocyte. Clearly then, much will depend on the localization as well as 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. Thus, it is the net final balance representing the integration of multiple transduction pathways, and often opposing signals, that determines the fate of a given follicle. As only 0.1% of follicles will ovulate, much effort is invested not only in the process of selecting the dominant follicle, but also in a controlled process of atresia. There is every reason to believe that the huge puzzle of ovarian regulators is far from being solved, and that future studies may reveal other modes of interaction in the ovary.

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