

Growth factors and the control of folliculogenesis

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There is increasing evidence that growth factors modulate folliculogenesis. However their precise role in the processes of follicular growth, differentiation and atresia is still unknown. Growth factors belong to complex systems, including all the factors structurally and functionally related, their receptor(s) and, in most cases, binding proteins or proteoglycans. In this review, the insulin-like growth factor (IGF) system is presented as a paradigm for the study of other growth factor systems in the ovary. IGFs are probably positive regulators of follicular development, as they enhance both proliferation and differentiation and amplify the action of gonadotrophins on follicular cells. In the ovaries of most mammals, there is little or no change in intrafollicular IGF concentrations during terminal follicular growth and atresia. By contrast, the concentrations of IGF-binding proteins < 40 kDa (mainly IGFBP-2, -4 and -5) decrease markedly during terminal follicular growth, but increase in atretic follicles. These variations could result from both changes in expression of these IGFbps by follicular cells and in local degradation by gonadotrophin-induced specific protease(s). Experiments *in vitro* showed that these IGFbps, present in a soluble form in follicular fluid, could play an important role by sequestering the IGFs and decreasing their bioavailability. More generally, biological actions of growth factors critically depend on their bioavailability in the vicinity of the cell, resulting from the concentrations of the factors themselves, their binding proteins and the ability of specific proteases to degrade binding proteins or to cleave latent forms of growth factors. These mechanisms and their control by gonadotrophins throughout follicular development have now to be investigated for the different growth factor systems.

Introduction

Follicular development involves important morphological and functional changes in thecal and granulosa cells (proliferation, steroidogenesis, gonadotrophin sensitivity) and in the oocyte (cytoplasmic and nuclear maturation). There is substantial evidence that gonadotrophins play a key role in controlling folliculogenesis. However, follicular development is also controlled by various other factors (steroids, growth factors) of endocrine and paracrine origin. In this review, attention will be directed towards the importance of growth factors in the control of folliculogenesis.

Growth factors are directly involved in regulation of cell proliferation and differentiation. Thereby, they probably play an essential role in all processes of morphogenesis, cell growth and differentiation, and maintenance of tissue homeostasis. The different growth factors have been classed into separate families, based on their structure and biological activity: the epidermal growth factor (EGF) family, the fibroblast growth factor (FGF) family, the platelet-derived growth factor (PDGF) family, the insulin-like growth factor (IGF) family, the transforming growth factor β (TGF- β) family and the haemopoietic growth factors (cytokines). Our knowledge of their exact role in folliculogenesis is still limited for two reasons.

Table 1. Growth factors, receptors and binding compounds in four families of growth factors

Family	Growth factors	Receptors	Binding compounds
EGF	EGF TGF- α HBEGF Amphiregulin	EGF receptor (<i>c-erb B</i>)	Heparin HSPG
FGF	FGF-1 (acidic FGF, aFGF) FGF-2 (basic FGF, bFGF) FGF-3 (<i>int-2</i>) FGF-4 (<i>Hst-1/K-FGF</i>) FGF-5 FGF-6 (<i>Hst-2</i>) FGF-7 (keratinocyte growth factor)	FGF receptor 1 (<i>flg</i>) FGF receptor 2 (<i>bek</i>) FGF receptor 3 FGF receptor 4 <i>flg-2</i>	Heparin HSPG ^a α_2 -Macroglobulin
TGF- β	TGF- β 1 TGF- β 2 TGF- β 3 TGF- β 5	Type I receptor Type II receptor	Latent TGF- β binding protein Proteoglycans ^b Type IV collagen Fibronectin Thrombospondin α_2 -Macroglobulin
IGF	IGF-I IGF-II	IGF-I receptor IGF-II-M6P receptor	6 Binding proteins (IGFBPs)

Other nomenclatures for factors are indicated in parentheses. The name of the proto-oncogenes, when they are known, are indicated in italics. EGF: epidermal growth factor; FGF: fibroblast growth factor; TGF: transforming growth factor; IGF: insulin-like growth factor; HBEGF: heparin-binding EGF-like growth factor; HSPG: heparan sulfate proteoglycans. ^aParticularly syndecan and betaglycan; ^bparticularly betaglycan, endoglin and decorin.

First, growth factors are ubiquitous; they are secreted by various types of cell and act locally in a paracrine or autocrine way. Some of them, such as factors of the EGF and the PDGF families and various cytokines, are expressed in a transmembrane form and can activate receptors on adjacent cells, in a so-called juxtacrine way (Wong *et al.*, 1989). In addition, a particular intracellular mode of action (intracrine action) was described for factors of the FGF family (Logan, 1990). However, growth factors can also act on target cells in an endocrine way, like hormones. This is of particular importance in the ovary, since proteins with molecular masses of less than 10^3 kDa, and presumably growth factors, cross the basal membrane of the follicle (Shalgi *et al.*, 1973). Consequently, intrafollicular growth factors may have both a local and an endocrine origin.

Second, growth factors belong to complex systems, including all the factors structurally and functionally related, their receptor(s), and, in most cases, binding proteins or proteoglycans. In this review, we will present some of the general features of these growth factor systems.

Main Characteristics of Growth Factor Systems

Each system of growth factors includes three different types of elements: the growth factors, their signalling receptors and binding proteins and proteoglycans (Table 1). Four factors are known in the EGF system, at least seven in the FGF system, four in the TGF- β system (not including TGF- β -related peptides, such as activin, inhibin and the Müllerian inhibitory substance) and two in the IGF system.

Growth factors bind and activate specific signalling receptors. Only one tyrosine kinase receptor is known in the EGF system. In the FGF system, five different tyrosine kinase receptors have been described. Moreover, a number of variants of these receptors can be generated by alternative splicing of mRNAs (Turnbull and Gallagher, 1993). In the TGF- β system, two different types of serine/threonine

kinase receptors are known; interaction of both receptors is required to achieve cellular signalling. The same type I receptor binds either activin or TGF- β , depending on the presence of either the activin or TGF- β type II receptor (Attisano *et al.*, 1993). In this system, interactions between receptor types would control different cellular responses to TGF- β -like molecules. The IGF system is characterized by two types of receptor, which are structurally and functionally different (see below).

Binding proteins and proteoglycans act both as carriers in biological fluids and as reservoirs of growth factors in the extracellular matrix (ECM) and at the cell surface, modulating bioavailability of their ligands. In the EGF family, heparin and heparan sulfate proteoglycans (HSPG) bind amphiregulin and heparin-binding EGF-like growth factor (HBEGF), but not EGF and TGF- α (Higashiyama *et al.*, 1993). In the FGF family, HSPG, by interacting with bFGF and aFGF, protects them from proteolytic cleavage and concentrates these factors in the pericellular space in the vicinity of the receptors. In addition, it was shown that bFGF and aFGF cannot bind their receptors or activate a cellular response unless heparin or HSPG molecules are present (Yayon *et al.*, 1991). In the TGF- β system, various types of binding compound are known. First, the latent TGF- β binding protein (LTBP) binds the propeptide TGF- β latency associated protein in a latent complex bound to the ECM (Taipale *et al.*, 1994). Second, various other ECM compounds interact with TGF- β and can mask or enhance the action of TGF- β . Among them, β -glycan, a cell-surface proteoglycan, can bind the mature form of TGF- β and plays an important role in facilitating its interaction with the signalling receptors. In the IGF system, six specific binding proteins (IGFBPs) are known to bind IGFs with high affinity. Recent studies also suggest that IGFBPs on cell surfaces can play a local determinant role in modulating the bioavailability of IGFs for their receptors (see below).

To complete this rapid description of the growth factor systems, it is essential to mention also the proteases responsible for growth factor release by cleavage of precursors (TGF- α , TGF- β), or by proteolysis of binding proteins (TGF- β , FGF, IGF). Additional studies are now necessary to identify these proteases and to assess how they are implicated in the modulation of growth factor bioavailability.

To understand the role of a growth factor in a physiological process like folliculogenesis, it is necessary to take into account all the elements of the system that is studied. The main questions are then the following: What is the origin (local, endocrine or both) of these elements in the ovary? Which elements are involved in the control of follicular development? How do these elements interact with gonadotrophins and other factors in the regulation of folliculogenesis? Our knowledge of the growth factor systems is too limited to answer all of these questions. In our laboratory, we are attempting to understand the role of the IGF system in sheep folliculogenesis, as a paradigm for the study of other growth factor systems.

Role of the IGF System in Folliculogenesis

As mentioned above, the IGF system is composed of different elements (Fig. 1): two ligands (IGF-I and IGF-II), two receptors and six IGF-binding proteins.

The type I receptor mediates most of the somatomedin-like actions of both IGF-I and -II. It is a α 2 β 2 tetramer structurally and functionally related to the insulin receptor. The affinity of this receptor for IGF-I is slightly higher than for IGF-II, and much higher than for insulin. However, the existence of insulin/IGF-I hybrid receptors, and of 'atypic' type I receptors leads to some frequent exceptions to this rule. The type II receptor, or IGF-II-mannose-6-phosphate (IGF-II-M6P) receptor, binds IGF-II and molecules that possess a mannose-6-phosphate residue such as lysosomal enzymes and TGF- β 1 (Nissley and Kiess, 1991). This receptor does not bind insulin, and binds IGF-I with very low affinity. It is therefore unlikely that IGF-II-M6P receptor mediates some IGF-I actions *in vivo*. An important function of type II receptor is to mediate the turnover of lysosomal enzymes. This receptor would also play a role in degradation of IGF-II after internalization (Nolan *et al.*, 1990). Moreover, experiments using IGF-II mutants have recently shown that the IGF-II-M6P receptor could mediate some unexpected actions of IGF-II, such as cell motility, but not 'classical' somatomedin-like actions such as mitogenesis.

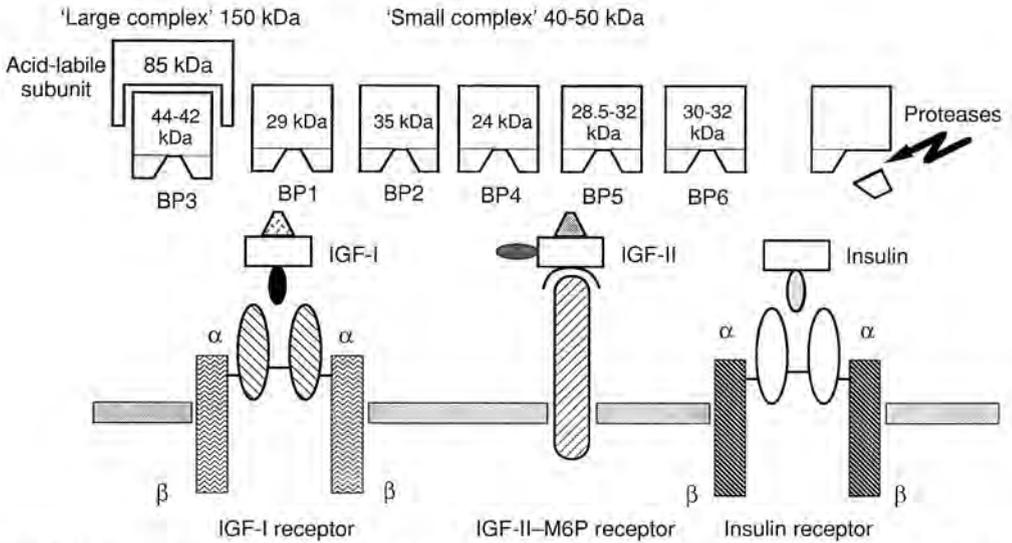


Fig. 1. Schematic representation of the IGF system. The IGF system is composed of IGF-I and IGF-II, which are structurally related to insulin, two receptors (IGF-I receptor and IGF-II-M6P receptor), six binding proteins (IGFBPs) and proteases. The IGF-I, or type I, receptor is structurally and functionally related to the insulin receptor; it binds IGF-I, IGF-II and pharmacological concentrations of insulin. The IGF-II-mannose-6-phosphate (IGF-II-M6P), or type II, receptor binds only IGF-II. The six IGFBPs (BP1 to BP6) bind IGF-I and IGF-II, but not insulin. Proteases can degrade IGFBPs and decrease their affinity for IGFs.

The six IGF-binding proteins bind IGF-I and -II with high affinity (Rechler, 1993). IGFBPs are present in all biological fluids. They can be arbitrarily classified into two groups.

IGFBP-1, -2, -4, -5 and -6 are present in serum and other fluids in the so called 'small complex'. When visualized by western-ligand blotting, their apparent molecular masses range between approximately 24 kDa and 35 kDa. In this review, they will be called small molecular weight IGFBPs, or IGFBPs < 40 kDa. In serum, their concentrations are either negatively (IGFBP-1 and -2) or not regulated by growth hormone (GH).

IGFBP-3 is the most predominant IGFBP in serum. In this fluid, it is mostly present in a 150 kDa form ('large complex'), composed of IGF-I or IGF-II, and an acid-labile 85 kDa subunit. Native IGFBP-3 is visualized as a 42-44 kDa doublet by western-ligand blotting. The concentration of IGFBP-3 is positively regulated by GH and IGF-I.

IGFBPs, by increasing the half-life of IGFs, constitute a large pool of IGFs in all the compartments of the organism. Furthermore, IGFBPs can both inhibit and potentiate IGF action at target cells. They can inhibit IGF action by sequestration, since the affinity of IGFBPs for IGF-I and -II is of the same order of magnitude as the affinity of the type I receptor. However, the affinity of IGFBPs for IGF-I and -II can be modulated by post-translational changes. In particular, the affinity of IGFBP-1 for IGFs can be increased by phosphorylation as reported for human amniotic fluid at the end of pregnancy by Koistinen *et al.* (1993). Moreover, the affinity of IGFBPs for IGFs can be reduced when IGFBPs are bound to the ECM (IGFBP-5; Jones *et al.*, 1993), or when they are proteolysed (IGFBP-3, IGFBP-5 and IGFBP-4). Such proteolysis was first described in the serum of female rodents and humans during pregnancy (Hossenlopp *et al.*, 1990), after nutritional fasting, during severe illness or following severe trauma (Frost *et al.*, 1993). Limited proteolysis of IGFBPs has also been described in serum and lymph of normal human adults (Lalou and Binoux, 1993), and in culture medium of osteoblastic cells, granulosa cells (Liu *et al.*, 1993) and tumour cells (Frost *et al.*, 1993). It is therefore highly likely that such a phenomenon occurs *in vivo* in most tissues. Moreover, it is important to note that this proteolysis can occur at the cell membrane (Conover, 1992). Such decreases in the affinity of IGFBPs for IGFs, by increasing bioavailability of IGFs, can lead to potentiation rather than inhibition of the action of the ligands.

Actions of IGF-I on follicular cells

The actions of IGF-I on follicular cells are mediated on granulosa and thecal cells by type I receptors (reviewed by Adashi *et al.*, 1992a and Giudice, 1992). Expression of this receptor in granulosa cells is increased by oestrogens and gonadotrophins in rats. In cows, expression of the IGF-I receptor increases in small antral follicles (Wandji *et al.*, 1992). In sheep, no change in follicular IGF-I receptor concentrations was observed during growth of follicles from 0.8 to 8.0 mm diameter (Monget *et al.*, 1989).

In sheep as in rats, pigs and humans, IGF-I stimulates both proliferation and differentiation of granulosa cells. IGF-I also stimulates steroidogenesis on thecal cells (reviewed by Adashi *et al.*, 1992a and Giudice, 1992). Furthermore, in our laboratory we have clearly established that, in sheep, IGF-I stimulates mainly proliferation of granulosa cells from small follicles (1–3 mm diameter). By contrast, IGF-I stimulates secretion of progesterone by granulosa cells from large (> 5 mm diameter), but not small, follicles (Fig. 2, Monniaux and Pisselet, 1992). It then seems that IGF-I stimulates either proliferation or differentiation of granulosa cells depending on the stage of development of the follicle.

Two non-exclusive hypotheses can be advanced to explain this phenomenon. First, other factors (such as growth factors and IGF-BPs) may modulate the action of IGF-I in some way. For example, the presence of high concentrations of EGF (or TGF- α) in small follicles (Hsu *et al.*, 1987) may orientate IGF-I action towards stimulation of proliferation rather than differentiation. By contrast, the decrease in intrafollicular EGF concentrations (and possibly some changes in TGF- β concentrations or bioavailability) in large follicles may allow the enhancement of cell differentiation in response to IGF-I. Second, the intrinsic capacity of granulosa cells to respond to IGF-I stimulation may change during growth of the follicle. We showed recently that *in vitro*, the percentage of proliferating cells was about 70% in granulosa cells from small follicles and less than 10% in cells from large follicles (Monniaux *et al.*, 1994). This difference in proliferation rate might be associated with a difference in intracellular concentrations of specific transcription factors. For example, *c-jun* is selectively expressed in mitotically active, but not in differentiating granulosa cells in rats (Delidow *et al.*, 1992). Similarly in humans, *c-myc* was detected in granulosa from preantral rather than from large antral follicles (Li *et al.*, 1994). It can then be hypothesized that high concentrations of intracellular factors like *c-jun* and *c-myc* may promote proliferation rather than differentiation of granulosa cells in response to IGF-I.

In rats (Adashi *et al.*, 1990), IGF-II seems to play similar roles to IGF-I *in vitro*; these actions are mediated by type I receptors.

Changes in intrafollicular IGF-I and IGF-II concentrations during growth and atresia of ovarian follicles

There is a heterogeneity in the localization of IGF expression in the ovary. In humans, IGF-I is expressed in thecal cells from small antral (5–7 mm diameter) follicles, whereas IGF-II is highly expressed in granulosa cells from preovulatory follicles (El-Roeiy *et al.*, 1993). By contrast, in rats, IGF-II is exclusively expressed in thecal cells (Hernandez *et al.*, 1990), but IGF-I is expressed in granulosa cells from growing antral follicles (Zhou *et al.*, 1991). In pigs, IGF-I mRNAs are found mainly in granulosa cells of growing antral follicles (Hammond *et al.*, 1993). In humans (Voutilainen and Miller, 1987) and in pigs (Hatey *et al.*, 1992), gonadotrophins stimulate synthesis of IGF-II and IGF-I by granulosa cells *in vitro*, respectively.

In follicular fluid, some authors have reported an absence of correlation between the size or stage of atresia of the follicles and their IGF-I concentrations (pigs: Veldhuis *et al.*, 1986; cattle: Rutter and Manns, 1991; humans: Geisthovel *et al.*, 1989; sheep: Monget *et al.*, 1993), whereas others have found positive (pigs: Hammond *et al.*, 1993) or negative correlations (pigs: Howard and Ford, 1992) between IGF-I concentrations and follicle size. Two main explanations can be proposed to explain the discrepancies between these results. First they may reflect interspecies differences in the localization of IGF synthesis in the ovary. Second, these discrepancies could be due to variations in the efficiency of the techniques of extraction of IGF-BPs from small and large follicles (which contain different amounts of IGF-BPs) before the IGF assays. Indeed some techniques have a poor efficacy of extraction, particularly concerning IGF-BPs < 40 kDa (Holly and Cwyfan Hughes, 1994). Hence, when such techniques are used, variations of IGF-BP concentrations as high as observed between follicles of different sizes and qualities (see below) might probably not permit an accurate estimation of IGF concentrations in follicular fluid.

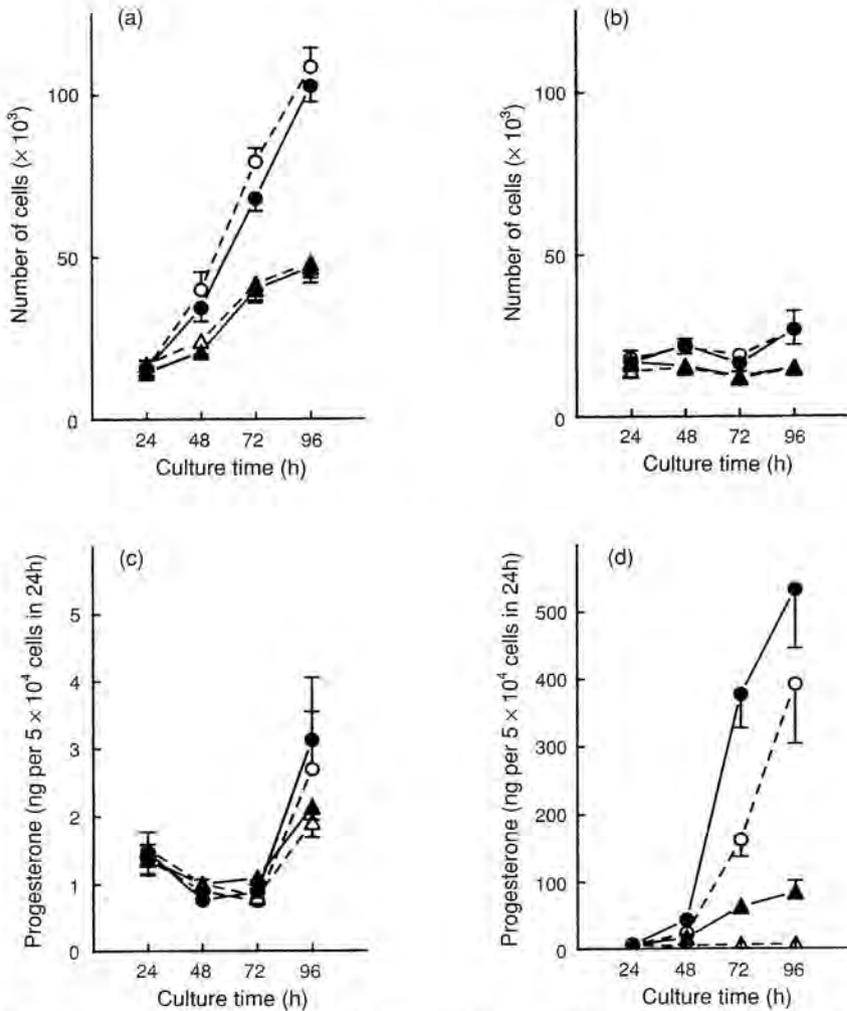


Fig. 2. Actions of insulin-like growth factor I (IGF-I) and FSH on ovine granulosa cells *in vitro*: (a) and (b) effects on proliferation and (c) and (d) progesterone synthesis. (a) and (c) Granulosa cells from small (1–3 mm diameter) (b) and (d) large (5–7 mm diameter) follicles were cultured for 96 h with and without FSH (100 ng ml^{-2}) in the presence or absence of IGF-I (100 ng ml^{-2}). (Δ) Without FSH and IGF-I; (\blacktriangle) with FSH only; (\circ) with IGF-I only; (\bullet) with FSH and IGF-I. Data represent (a) and (b) changes in the number of cells per culture well and (c) and (d) in the amount of progesterone secreted by granulosa cells with culture time. Redrawn from Monniaux *et al.* (1994) with permission of the Journal of Endocrinology Ltd.

Nevertheless, in all cases, the differences between IGF-I concentrations in the various classes of follicular fluid are small, and the follicular fluid IGF-I concentrations measured are always within the range of those shown to be effective in stimulating proliferation and steroidogenesis in granulosa cells *in vitro*. In fact, it seems that IGF bioavailability rather than IGF concentrations change markedly during growth and atresia of ovarian follicles (see below).

Finally, in women (Geisthovel *et al.*, 1989), cows (Echternkamp *et al.*, 1990), ewes (Monget *et al.*, 1993) and mares (Spicer *et al.*, 1991), the concentrations of IGF-I in large healthy follicles are positively correlated with serum concentrations. In women, significant concentrations of IGF-I are found in

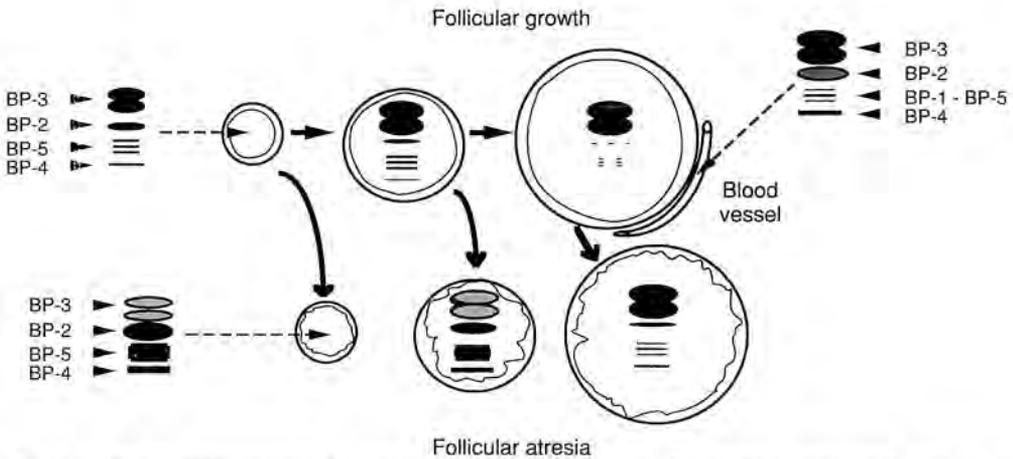


Fig. 3. Schematic representation of changes in intrafollicular insulin-like growth factor binding proteins (IGFBPs) during growth and atresia of antral follicles in sheep. IGFBPs were assessed by western-ligand blotting on follicular fluid from follicles with various sizes (2–8 mm diameter) and qualities. Follicular growth is characterized by a decrease and an increase in IGFBP-2, -4 and -5 (IGFBPs < 40 kDa) during follicular growth and atresia, respectively. Note that poorly vascularized small atretic follicles contain greater amounts of IGFBPs < 40 kDa than does serum, whereas they cannot be detected in highly vascularized preovulatory follicles. IGFBP-3 increases slightly during follicular growth but decreases in small atretic follicles. IGFBP-1 does not seem to be a major IGFBP in the ovary.

preovulatory follicles, whereas synthesis is very low. It is therefore possible that IGF-I in large normal follicles is derived, at least in part, from the circulatory pool *in vivo*.

Changes in intrafollicular IGFBP concentrations during growth and atresia of ovarian follicles

In sheep, analysis of follicular fluid by western-ligand blotting showed that follicular growth is accompanied by a slight increase in the intensity of the 42–44 kDa IGFBP doublet, and clear decreases in those of the 35 kDa and 24 kDa bands; follicular atresia, by contrast, is accompanied by marked increases in the intensity of the 35, 28.5–32 and 24 kDa bands (Fig. 3, Monget *et al.*, 1993). The 35 kDa and the 30 kDa bands have been further identified by immunoblotting as ovine IGFBP-2 and -5, respectively. The 42–44 kDa and 24 kDa bands probably represent the ovine IGFBP-3 and -4, respectively. In pigs, Mondschein *et al.* (1991) and Howard and Ford (1992) also showed an inverse relationship between the concentration of IGFBPs < 40 kDa and the maturity of the follicle. In humans, intrafollicular IGFBP-2 and -4 concentrations are higher in atretic androgenic than in healthy oestrogenic follicles (Cataldo and Giudice, 1992). Hence, in these species, the increase in intrafollicular IGFBP-3:IGFBPs < 40 kDa ratio appears to be strikingly associated with the growth and development of the follicle. The mechanisms that lead to such changes in intrafollicular IGFBP concentration are likely to be different for IGFBPs < 40 kDa and IGFBP-3.

IGFBPs < 40 kDa. Several reports suggest that local paracrine processes are responsible for changes in small molecular weight IGFBPs during growth and atresia. First, in poorly vascularized, atretic follicles, the concentration of small molecular weight IGFBPs is higher in follicular fluid than in serum, suggesting some local synthesis. Moreover, from preliminary *in situ* hybridization experiments, atresia is characterized by a slight increase in IGFBP-4 expression in ovine thecal cells and a marked increase of IGFBP-5 expression in granulosa cells (authors' unpublished data). Similarly in the rat ovary, expression of IGFBP-4 and IGFBP-5 increases in granulosa cells of atretic follicles (Nakatani *et al.*, 1991; Erickson *et al.*, 1992).

Second, in pigs (Mondschein *et al.*, 1991; Howard and Ford, 1992) and sheep (Monget *et al.*, 1993), the concentration of IGFbps < 40 kDa progressively decreases in healthy growing follicles, and cannot be detected by western-ligand blotting in large highly vascularized preovulatory follicles, whereas significant concentrations are present in the corresponding serum. However, as serum proteins with molecular masses below 10^3 kDa can cross the basal membrane of the follicle (Shalgi *et al.*, 1973), significant amounts of these IGFbps should be found in follicular fluid of preovulatory follicles. Moreover, in sheep, *in situ* hybridization experiments have shown that IGFBP-2 is expressed in granulosa cells and IGFBP-4 and -5 are expressed in thecal cells from healthy ovine (particularly large) follicles (authors' unpublished data). El-Roeiy *et al.* (1994) found that, in women, IGFBP-2, -4 and -5 mRNAs are expressed in dominant follicles. What are the mechanisms that explain the absence of small molecular weight IGFbps in follicular fluid from preovulatory follicles?

Inhibition of IGFBP expression by gonadotrophins in follicular cells would be one such mechanism. In sheep, preliminary *in situ* hybridization experiments show that expression of IGFBP-2 in granulosa cells of healthy follicles decreases during follicular growth (authors' unpublished data). Similarly in pigs, Samaras *et al.* (1993) showed a negative relationship between the concentrations of mRNAs encoding IGFBP-2 isolated from individual follicles and the diameter of these follicles or their concentration of progesterone. In rats, Liu *et al.* (1993) showed that expression of mRNAs encoding IGFBP-4 and -5 decreases in FSH-stimulated granulosa cells *in vitro*. Finally, in humans, Cataldo *et al.* (1993) showed that the secretion of IGFBP-2 by luteinizing granulosa cells *in vitro* is decreased by hCG and IGF-II.

Degradation of small molecular weight IGFbps by specific intrafollicular proteases would be another mechanism. Liu *et al.* (1993) showed that FSH induces the production of a protease that degrades IGFBP-4 and -5 in rat granulosa cells *in vitro*.

Hence, in healthy growing follicles, gonadotrophins would not only reduce the expression of small molecular weight IGFbps, but also induce the secretion of a protease(s) which would degrade locally synthesized as well as peripheral IGFbps < 40 kDa, leading to an increase in the intrafollicular IGFBP-3:IGFbps < 40 kDa ratio. In atretic follicles, by contrast, an increase in the expression of IGFbps < 40 kDa and a putative decrease in the expression or activity of proteases would lead to a marked decrease in this ratio.

IGFBP-3. There is evidence for an endocrine origin (at least partly) of intrafollicular IGFBP-3. First, its concentration in preovulatory highly vascularized follicles is slightly lower (sheep: Monget *et al.*, 1993) or quite similar (human: Cataldo and Giudice, 1992) to that in the corresponding serum as assessed by western-ligand blotting. Moreover, the systemic 150 kDa IGFBP-IGF complex has been identified in ovine (Hodgkinson *et al.*, 1989) and human (Kubota *et al.*, 1993) follicular fluid. Furthermore, in rat (Nakatani *et al.*, 1991) and pig (Samaras *et al.*, 1992) ovaries, very low or no expression of IGFBP-3 was shown either in granulosa or thecal cells, unlike in the corpus luteum. Nevertheless, further experiments are necessary to elucidate the respective endocrine or paracrine roles of this IGFBP in the ovary.

Consequences of the bioavailability of IGFs at follicular cells. Previous studies have shown that IGFBP-4 and -5 inhibit both secretion of oestradiol and progesterone by FSH-stimulated rat granulosa cells *in vitro* (Liu *et al.*, 1993). Such an inhibition could be explained by sequestration of endogenously produced IGFs. In our model, to understand the physiological consequences of the observed changes in IGFBP-3:IGFbps < 40 kDa ratio in ovine follicles further, we studied their relationship with intrafollicular free IGF binding activity. In ovine follicles, free IGF binding activity is positively correlated with concentrations of small molecular weight IGFbps, and is higher in small than in large follicles, and in atretic than in normal follicles (Monget *et al.*, 1993). These changes in free IGF binding activity may have important consequences for the bioavailability of IGFs at the granulosa cells. To assess this question, we studied the action of follicular fluid from healthy and atretic follicles on [3 H]thymidine incorporation in ovine granulosa cells cultured with either IGF-I or its analogue [QAYL]-IGF-I, which has a weak affinity for IGFbps. In the presence of 1% follicular fluid from healthy follicles, 1 ng IGF-I ml^{-1} stimulates [3 H]thymidine incorporation. By contrast, IGF-I action is inhibited in the presence of 1% follicular fluid from atretic follicles, whereas the action of the IGF-I analogue remains unchanged, showing that the type

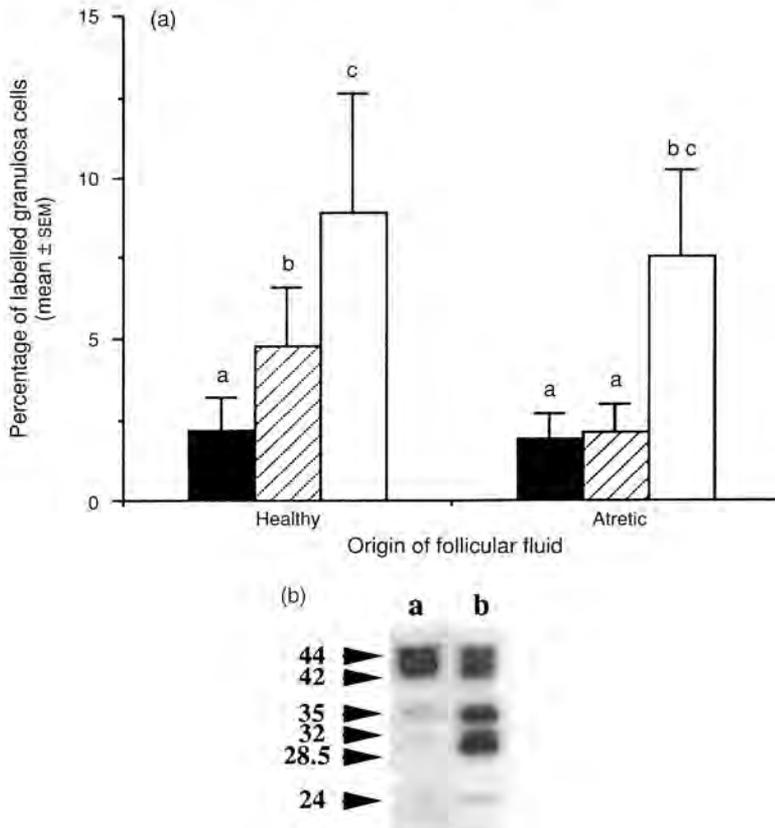


Fig. 4. (a) Effect of insulin-like growth factor I (IGF-I) and an IGF-I analogue on ovine granulosa cells in the presence of follicular fluid from healthy and atretic follicles. Granulosa cells from follicles of 1–3 mm diameter were cultured with 1% follicular fluid from either healthy or atretic follicles in the presence of IGF-I or an IGF-I analogue ([QAYL]IGF-I, which has a weak affinity for insulin-like growth factor binding proteins (IGFBPs)). (■) Control; (▨) with IGF-I (1 ng ml^{-1}); (□) with IGF-I analogue (1 ng ml^{-1}). After culture for 24 h, cells were incubated with $0.25 \mu\text{Ci } [^3\text{H}]\text{thymidine ml}^{-1}$ for 2 h, and then treated for autoradiography. Data represent the percentage of labelled cells, estimated after analysis under a microscope. Columns with different letters are significantly different. (b) Patterns of IGFBPs of follicular fluid from healthy (lane a) and atretic (lane b) follicles used in these experiments were visualized by western-ligand blotting. Redrawn from Monget *et al.* (1993) with permission, © The Endocrine Society.

of follicular fluid in the culture medium affects the action of IGF-I, but not of its analogue (Fig. 4). In similar experiments, Adashi *et al.* (1992b) showed that *in vitro* des(1-3)IGF-I, which has a weak affinity for IGFBPs, is more potent than IGF-I in promoting the FSH-stimulated accumulation of progesterone by rat granulosa cells in circumstances where IGFBPs are present, whereas both peptides are equipotent in circumstances in which IGFBPs are absent.

It should be noted that in the latter experiments, the role of IGFBPs in the action of IGFs was undertaken *in vitro* using soluble IGFBPs. In particular, it seems that soluble IGFBP-5 (present in follicular fluid) is a potent inhibitor of follicular growth. However, Jones *et al.* (1993) showed that IGFBP-5 can bind to laminin, fibronectin and type III and IV collagen. These authors suggested that the lower affinity of IGFBP-5 bound to the ECM could serve as a reservoir of immobilized IGFs, facilitating delivery to type I receptor and then potentiating its action. In healthy ovine follicles, it is possible that IGFBP-5

bound to ECM elements, such as laminin, fibronectin and collagen, enhances rather than inhibits IGF action on granulosa cells. This could also be the case for membrane-bound IGFBPs, particularly IGFBP-2 which binds to cell membranes by an RGD sequence peptide and, in that way, can enhance IGF-I action.

Changes in type II receptor expression during growth and atresia of ovarian follicles

Few data are available concerning the expression of type II receptors in the ovary. In sheep, its expression is closely related to the quality of the follicle, as it is expressed by thecal cells from healthy follicles and by granulosa cells from atretic follicles (Teissier *et al.*, 1994). In humans by contrast, the type II receptor is highly expressed by granulosa cells from dominant follicles (El-Roeiy *et al.*, 1993). The role of this receptor in folliculogenesis is still unknown, and probably depends on the presence of its different ligands, IGF-II, lysosomal enzymes or TGF- β 1. However, a recent study strongly suggests that the IGF-II induced decrease in IGFBP-2 concentrations in luteinized human granulosa cell-conditioned medium is mediated by type II receptors (Cataldo *et al.*, 1993). Hence in human preovulatory follicles, coexpression of high amounts of IGF-II and IGF-II-M6P receptors might contribute to the decrease in intrafollicular concentrations of IGFBP-2. However, further studies are necessary to confirm this hypothesis.

Future Directions

Ovarian folliculogenesis, like many biological functions, is controlled by apparently redundant systems of growth factors, most of them displaying quite similar functions on follicular cells: stimulation of proliferation (EGF, FGF, IGFs), stimulation of differentiation (IGF-I, TGF- β) or inhibition of differentiation (EGF, FGF, TNF- α). Within each system, the many ligands and receptors that have discrete and overlapping functions also lead to functional redundancy: for example, IGF-I and -II, or TGF- α and EGF, seem to display the same functions in the ovary. Natural genetic mutations have provided some information about the role of some of these growth factors. For example, in mice, the *Steel* and the *c-kit* mutations lead to a complete absence of germinal cells in the ovary (as well as in the testis), providing evidence for their crucial role in oogenesis. Moreover, the production of transgenic mice by targeted disruption of genes will provide information on the exact roles of these factors on folliculogenesis. TGF- α , for example, does not seem to be indispensable for ovarian physiology, since transgenic mice lacking these factors are fertile (Lueteteke *et al.*, 1993). The knock-out of the IGF-II gene also does not alter fertility of mice (DeChiara *et al.*, 1991), whereas mice lacking IGF-I are infertile (Baker *et al.*, 1993). However, in this case, it is difficult to conclude definitively on the direct role of IGF-I in the ovary, since such a targeted disruption also affects extraovarian compartments such as pituitary FSH/LH cells or hypothalamic GnRH neurones. Moreover, extrapolation of these results to other species requires some caution. In humans, the Laron syndrome is characterized by a general resistance to GH action and by a great decrease in IGF-I concentrations in serum and follicular fluid, but follicular growth appears quite normal, and women are fertile (Dor *et al.*, 1992).

Moreover, biological effects of growth factors in the ovary depend on many different parameters. First, as suggested above for IGFs, the stage of differentiation of follicular cells (which depends itself on the action of gonadotrophins and other growth factors), and then the set of transcription factors present in the nucleus, may orientate the response to a growth factor. In the same way, TGF- β exerts pleiotropic effects on follicular cells and the cellular response to TGF- β is probably determined by the concentration or activity of intracellular transcription factors. In particular, as suggested by results obtained on Chinese hamster ovary cells (Bissonnette *et al.*, 1992), it is possible that the increase in *c-myc* expression in granulosa cells following stimulation by a mitogenic factor, such as IGF-I or EGF, could respectively increase proliferation rate, or induce apoptosis and follicular atresia, depending on the amount of survival factors such as bcl-2 in the cells. It was reported that *c-myc* is expressed in both highly proliferative and degenerating granulosa cells (Li *et al.*, 1994). Among transcription factors, intracellular steroid receptors may also influence the action of growth factors. In particular, the mitogenic action of IGF-I and EGF on human breast cancer cells is partly mediated by oestradiol receptors (Chalbos *et al.*, 1993). In the same way, we have shown that *in vitro*, in steroid-free culture conditions, the mitogenic action of IGF-I on

ovine granulosa cells is partly inhibited by ICI164-364, a specific inhibitor of the oestradiol receptor (M. Tomanek, C. Pisselet and D. Monniaux, unpublished). The mechanisms of action of growth factor transducing signals on these intracellular transcription factors remain to be investigated in the ovary.

Finally, biological actions of growth factors critically depend on their bioavailability in the vicinity of the cell. Bioavailability is determined by the concentration of ligands, by the concentrations of their binding proteins and by the ability of specific proteases to degrade binding proteins or to cleave latent forms of growth factors. It is the 'fine tuned' combination of these different parameters which determines the window of concentration allowing growth factors to trigger a specific cellular response. For example, overexpression of the proteoglycan syndecan in transfected fibroblasts causes downregulation of bFGF-induced cell proliferation (Mali *et al.*, 1993). In the ovary, there is an abundance of HSPG in degenerating follicles (Bushmeyer *et al.*, 1985 and authors' unpublished observations). It can be hypothesized that, as locally produced IGFBPs in atretic follicles inhibit IGF action, the increased expression of HSPG in degenerating follicles might inhibit FGF action on follicular cells. Moreover, modulation of extracellular bFGF action in the ovary might depend less on its level of expression (which is low) than on the action of proteoglycanases which modulate its bioavailability. It is now crucial to study the involvement of all the elements that can modulate bioavailability of growth factors in the ovary.

It is also important to determine the exact role of growth factors in the mediation of gonadotrophin actions on follicular growth and atresia. *In vitro*, in serum-free culture conditions, IGFBP-4 and -5 (Liu *et al.*, 1993) inhibit FSH-induced steroidogenesis on rat granulosa cells, suggesting a determinant role for endogenous IGF-I in the mechanism of action of FSH. The inhibition of FSH-induced DNA synthesis of hamster follicles *in vitro* by addition of a specific antibody against EGF (Roy and Greenwald, 1991), strongly supports the contention that this factor plays a critical role in the mediation of gonadotrophin-induced follicular growth. Furthermore, growth factors may also be implicated in LH-induced follicular atresia of small antral follicles during the follicular phase. McNeilly *et al.* (1992) suggested that, in sheep, selection of preovulatory follicles during the follicular phase involves a decrease in FSH concentrations and an increase in LH pulse frequency. Such an increase in the LH:FSH ratio would enhance growth of dominant follicles which express LH receptors in thecal and granulosa cells, but induce atresia of smaller antral follicles, devoid of LH receptors in granulosa cells. As TGF- α is expressed mainly in thecal cells from small antral follicles in cows, and putatively in ewes, it is possible that, as suggested by McNeilly *et al.* (1992), theca-derived TGF- α would partly mediate the LH-induced atresia on small antral follicles, by inhibiting terminal differentiation. Further experiments are necessary to investigate whether, during the follicular phase, an increase in the LH:FSH ratio would also play an active role in increasing the expression of IGFBPs < 40 kDa in small non-ovulatory follicles.

Conclusion

To study the role of a growth factor in folliculogenesis, it is necessary to integrate all the elements of the system to which it belongs: the growth factors, their receptors, their binding proteins and proteases that may alter the bioavailability of the ligands. The IGF system is considered as a paradigm for the study of the action of growth factors in the ovary since most of its elements (except proteases) are identified. In sheep, as in most mammals, terminal follicular growth is characterized mainly by a marked decrease in intrafollicular concentrations of IGFBPs < 40 kDa (IGFBP-2, -4 and -5). This decrease is responsible for an increase in bioavailability of IGFs, leading to an amplification of the action of gonadotrophins in preovulatory follicles. By contrast, atresia is characterized by a marked increase in intrafollicular IGFBPs < 40 kDa concentrations and in inhibition of the action of IGFs.

References

- Adashi EY, Resnick CE and Rosenfeld RG (1990) Insulin-like growth factor-I (IGF-I) and IGF-II hormonal action in cultured granulosa cells: mediation via type I but not type II IGF receptors *Endocrinology* **126** 754-760
- Adashi EY, Resnick CE, Hurwitz A, Ricciarelli E, Hernandez ER, Roberts CT, LeRoith D and Rosenfeld RG (1992a) The intra-ovarian IGF system *Growth Regulation* **2** 10-15

- Adashi EY, Resnick CE, Ricciarelli E, Hurwitz A, Kokia E, Tedeschi C, Botero L, Hernandez ER, Rosenfeld RG, Carlsson-Skwrut C and Francis GL (1992b) Granulosa cell-derived insulin-like growth factor (IGF) binding proteins are inhibitory to IGF-I hormonal action *Journal of Clinical Investigation* **90** 1593-1599
- Attisano L, Carcamo J, Ventura F, Weis JMB, Massagué J and Wrana JL (1993) Identification of human activin and TGF- β type I receptors that form heteromeric kinase complexes with type II receptors *Cell* **75** 671-680
- Baker J, Liu J-P, Robertson EJ and Efstratiadis A (1993) Role of insulin-like growth factors in embryonic and postnatal growth *Cell* **75** 73-82
- Bissonnette RP, Echeverri F, Mahboubi A and Green DR (1992) Apoptotic cell death induced by *c-myc* is inhibited by *bcl-2* *Nature* **359** 552-555
- Bushmeyer SM, Bellin ME, Brantmeier SA, Boehm SK, Kubajak CL and Ax RL (1985) Relationships between bovine follicular fluid glycosaminoglycans and steroids *Endocrinology* **117** 879-885
- Cataldo NA and Giudice LC (1992) Insulin-like growth factor binding protein profiles in human ovarian follicular fluid correlate with follicular functional status *Journal of Clinical Endocrinology and Metabolism* **74** 821-829
- Cataldo N, Woodruff TK and Giudice LC (1993) Regulation of insulin-like growth factor binding protein production by human luteinizing granulosa cells cultured in defined medium *Journal of Clinical Endocrinology and Metabolism* **76** 207-215
- Chalbos D, Philips A, Galtier F and Rochefort H (1993) Synthetic antiestrogens modulate induction of p52 and cathepsin-D messenger ribonucleic acid by growth factors and adenosine 3',5'-monophosphate in MCF7 cells *Endocrinology* **133** 571-576
- Conover CA (1992) Potentiation of insulin-like growth factor (IGF) action by IGF-binding protein-3: studies of underlying mechanism *Endocrinology* **130** 3191-3199
- DeChiara TM, Robertson EJ and Efstratiadis A (1991) Parental imprinting of the mouse insulin-like growth factor II gene *Cell* **64** 849-859
- Delidow BC, Lynch JP, White BA and Peluso JJ (1992) Regulation of proto-oncogene expression and deoxyribonucleic acid synthesis in granulosa cells of perfused immature rat ovaries *Biology of Reproduction* **47** 428-435
- Dor J, Ben-Shlomo I, Lunenfeld B, Pariente C, Levran D, Karasik A, Seppälä M and Mashiach S (1992) Insulin-like growth factor-I (IGF-I) may not be essential for ovarian follicular development: evidence from IGF-I deficiency *Journal of Clinical Endocrinology and Metabolism* **74** 539-542
- Echternkamp SE, Spicer LJ, Gregory KE, Canning SF and Hammond JM (1990) Concentrations of insulin-like growth factor-I in blood and ovarian follicular fluid of cattle selected for twins *Biology of Reproduction* **43** 8-14
- El-Roeiy A, Chen X, Roberts VJ, LeRoith D, Roberts CT, Jr and Yen SSC (1993) Expression of insulin-like growth factor-I (IGF-I) and IGF-II and the IGF-I, IGF-II, and insulin receptor genes and localization of the gene products in the human ovary *Journal of Clinical Endocrinology and Metabolism* **77** 1411-1418
- El-Roeiy A, Chen X, Roberts VJ, Shimasaki S, Ling N, LeRoith D, Roberts CT, Jr and Yen SSC (1994) Expression of the genes encoding the insulin-like growth factors (IGF-I and -II), the IGF and insulin receptors, and IGF-binding proteins-1-6 and the localization of their gene products in normal and polycystic ovary syndrome ovaries *Journal of Clinical Endocrinology and Metabolism* **78** 1488-1496
- Erickson GF, Nakatani A, Ling N and Shimasaki S (1992) Localization of insulin-like growth factor-binding protein-5 messenger ribonucleic acid in rat ovaries during the estrous cycle *Endocrinology* **130** 1867-1878
- Frost VJ, Macaulay VM, Wass JAH and Holly JMP (1993) Proteolytic modification of insulin-like growth factor-binding proteins: comparison of conditioned media from human cell lines, circulating proteases and characterized enzymes *Journal of Endocrinology* **138** 545-554
- Geisthovel F, Moretti-Rojas IM, Rojas FJ and Asch RH (1989) Immunoreactive insulin-like growth factor I in human follicular fluid *Human Reproduction* **4** 35-38
- Giudice LC (1992) Insulin-like growth factors and ovarian follicular development *Endocrine Reviews* **13** 641-669
- Hammond JM, Samaras SE, Grimes R, Leighton J, Barber J, Canning SF and Guthrie HD (1993) The role of insulin-like growth factors and epidermal growth factor related peptides in intraovarian regulation in the pig ovary *Journal of Reproduction and Fertility Supplement* **48** 117-125
- Hatey F, Langlois I, Mulsant P, Bonnet A, Benne F and Gasser F (1992) Gonadotropins induce accumulation of insulin-like growth factor I mRNA in pig granulosa cells *in vitro* *Molecular and Cellular Endocrinology* **86** 205-211
- Hernandez ER, Roberts CT, Jr, Hurwitz A, LeRoith D and Adashi EY (1990) Rat ovarian IGF-II gene expression is theca-interstitial cell-specific: hormonal regulation and receptor distribution *Endocrinology* **127** 3219-3226
- Higashiyama S, Abraham JA and Klagsbrun M (1993) Heparin-binding EGF-like growth factor stimulation of smooth muscle cell migration: dependence on interactions with cell surface heparan sulfate *Journal of Cell Biology* **122** 933-940
- Hodgkinson SC, Moore L, Napier JR, Davis SR, Bass JJ and Gluckman PD (1989) Characterization of insulin-like growth factor binding proteins in ovine tissue fluids *Journal of Endocrinology* **120** 429-438
- Holly JMP and Cwyfan Hughes SC (1994) Measuring insulin-like growth factors: why, where and how? *Journal of Endocrinology* **140** 165-169
- Hossenlopp P, Segovia B, Lassarre C, Roghani M, Bredon M and Binoux M (1990) Evidence of enzymatic degradation of insulin-like growth factor binding proteins in the 150K complex during pregnancy *Journal of Clinical Endocrinology and Metabolism* **71** 797-805
- Howard HJ and Ford JJ (1992) Relationship among concentrations of steroids, inhibin, insulin-like growth factor-I (IGF-I), and IGF-binding proteins during follicular development in weaned sows *Biology of Reproduction* **47** 193-201
- Hsu CJ, Holmes SD and Hammond JM (1987) Ovarian epidermal growth factor-like activity concentrations in porcine follicular fluid during follicular enlargement *Biochemical and Biophysical Research Communications* **147** 242-247
- Jones JL, Gockerman A, Busby WH, Jr, Camacho-Hubner C and Clemmons D (1993) Extracellular matrix contains insulin-like growth factor binding protein-5: potentiation of the effects of IGF-I *The Journal of Cell Biology* **121** 679-687
- Koistinen R, Angervo M, Leinonen P, Hakala T and Seppälä M (1993) Phosphorylation of insulin-like growth factor-binding protein-1 increases in human amniotic fluid and decidua from early to late pregnancy *Clinica Chimica Acta* **215** 189-199

- Kubota T, Sakamoto S, Kamada S, Shimizu Y, Ohara M, Aso T and Tagushi M (1993) Insulin-like growth factor II in follicular fluid of the patients with *in vitro* fertilization and embryo transfer *Fertility and Sterility* **59** 844–849
- Lalou C and Binoux M (1993) Evidence that limited proteolysis of insulin-like growth factor binding protein-3 (IGFBP-3) occurs in the normal state outside of the bloodstream *Regulatory Peptides* **48** 179–188
- Li S, Maruo T, Ladines-Llave CA, Kondo H and Mochizuki M (1994) Stage-limited expression of myc oncoprotein in the human ovary during follicular growth, regression and atresia *Endocrine Journal* **41** 83–92
- Liu X-J, Malkowski M, Guo Y, Erickson GF, Shimasaki S and Ling N (1993) Development of specific antibodies to rat insulin-like growth factor-binding proteins (IGFBP-2 to -6): analysis of IGFBP production by rat granulosa cells *Endocrinology* **132** 1176–1183
- Logan A (1990) Commentary: intracrine regulation at the nucleus – a further mechanism of growth factor activity? *Journal of Endocrinology* **125** 339–343
- Luetteke NC, Qiu TH, Peiffer RL, Oliver P, Smithies O and Lee DC (1993) TGF alpha deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice *Cell* **73** 263–268
- McNeilly AS, Crow W, Brooks J and Evans G (1992) Luteinizing hormone pulses, follicle-stimulating hormone and control of follicle selection in sheep *Journal of Reproduction and Fertility Supplement* **45** 5–19
- Mali M, Elenius K, Miettinen HM and Jalkanen M (1993) Inhibition of basic fibroblast growth factor-induced growth promotion by overexpression of syndecan-1 *Journal of Biological Chemistry* **268** 24215–24222
- Mondschein JS, Etherton TD and Hammond JM (1991) Characterization of insulin-like growth factor binding proteins of porcine ovarian follicular fluid *Biology of Reproduction* **44** 315–320
- Monget P, Monniaux D and Durand P (1989) Localization, characterization and quantification of insulin-like growth factor-I-binding sites in the ewe ovary *Endocrinology* **125** 2486–2493
- Monget P, Monniaux D, Pisselet C and Durand P (1993) Changes in insulin-like growth factor-I (IGF-I), IGF-II, and their binding proteins during growth and atresia of ovine ovarian follicles *Endocrinology* **132** 1438–1446
- Monniaux D and Pisselet C (1992) Control of proliferation and differentiation of ovine granulosa cells by insulin-like growth factor-I and follicle-stimulating hormone *in vitro* *Biology of Reproduction* **46** 109–119
- Monniaux D, Pisselet C and Fontaine J (1994) Uncoupling between proliferation and differentiation of ovine granulosa cells *in vitro* *Journal of Endocrinology* **142** 497–510
- Nakatani A, Shimasaki S, Erickson GF and Ling N (1991) Tissue-specific expression of four insulin-like growth factor-binding proteins (1, 2, 3, and 4) in the rat ovary *Endocrinology* **129** 1521–1529
- Nissley SP and Kiess W (1991) Binding of insulin-like growth factor-II (IGF-II) and lysosomal enzymes to the IGF-II/mannose-6-phosphate receptor. In *Modern Concepts of Insulin-like Growth Factors* pp 419–430 Ed. EM Spencer. Elsevier Science Publishers, New York
- Nolan CM, Kyle JW, Watanabe H and Sly WS (1990) Binding of insulin-like growth factor II (IGF-II) by human cation-dependent mannose 6-phosphate receptor/IGF-II receptor expressed in receptor-deficient mouse L cells *Cell Regulation* **1** 197–213
- Rechler MM (1993) Insulin-like growth factor binding proteins *Vitamins and Hormones* **47** 1–114
- Roy SK and Greenwald GS (1991) Mediation of follicle-stimulating hormone action on follicular deoxyribonucleic acid synthesis by epidermal growth factor *Endocrinology* **129** 1903–1908
- Rutter LM and Manns JG (1991) Insulin-like growth factor I in follicular development and function in postpartum beef cows *Journal of Animal Science* **69** 1140–1146
- Samaras SE, Hagen DR, Shimasaki S, Ling N and Hammond JM (1992) Expression of insulin-like growth factor-binding protein-2 and -3 messenger ribonucleic acid in the porcine ovary: localization and physiological changes *Endocrinology* **130** 2739–2744
- Samaras SE, Guthrie HD, Barber JA and Hammond JM (1993) Expression of the mRNAs for the insulin-like growth factors and their binding proteins during development of porcine ovarian follicles *Endocrinology* **133** 2395–2398
- Shalgi R, Kraicer P, Rimon A, Pinto M and Soferman N (1973) Proteins of human follicular fluid: the blood–follicle barrier *Fertility and Sterility* **24** 429–434
- Spicer LJ, Tucker KE, Henderson KA and Doby RT (1991) Concentrations of insulin-like growth factor-I in follicular fluid and blood plasma of mares during early and late oestrus *Animal Reproduction Science* **25** 57–65
- Taipale J, Miyazono K, Heldin CH and Keski-Oja J (1994) Latent transforming growth factor- β 1 associates to fibroblast extracellular matrix via latent TGF- β binding protein *Journal of Cell Biology* **124** 171–181
- Teissier MP, Monget P, Monniaux D and Durand P (1994) Changes in insulin-like growth factor-II/mannose-6-phosphate receptor during growth and atresia of ovine ovarian follicles *Biology of Reproduction* **50** 111–119
- Turnbull JE and Gallagher JT (1993) Heparan sulphate: functional role as a modulator of fibroblast growth factor activity *Biochemical Society Transactions* **21** 477–482
- Veldhuis J, Rodgers RJ and Furlanetto RW (1986) Synergistic actions of estradiol and the insulin-like growth factor, somatomedin-C, on swine ovarian (granulosa) cells *Endocrinology* **119** 530–538
- Voutilainen R and Miller WL (1987) Coordinate tropic hormone regulation of mRNAs for insulin-like growth factor II and the cholesterol side-chain-cleavage enzyme, P450_{ssc}, in human steroidogenic tissues *Proceedings of the National Academy of Sciences of the USA* **84** 1590–1594
- Wandji SA, Pelletier G and Sirard MA (1992) Ontogeny and cellular localization of ¹²⁵I-labeled insulin-like growth factor-I, ¹²⁵I-labeled follicle-stimulating hormone, and ¹²⁵I-labeled human chorionic gonadotropin binding sites in ovaries from bovine fetuses and neonatal calves *Biology of Reproduction* **47** 814–822
- Wong ST, Winchell LF, McCune BK, Earp HS, Teixido J, Massagué J, Herman B and Lee DC (1989) The TGF- α precursor expressed on the cell surface binds to the EGF receptor on adjacent cells leading to signal transduction *Cell* **56** 495–506
- Yayon A, Klagsbrun M, Esko JD, Leder P and Ornitz DM (1991) Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor *Cell* **84** 841–848
- Zhou J, Chin E and Bondy C (1991) Cellular pattern of insulin-like growth factor-I (IGF-I) and IGF-I receptor gene expression in the developing and mature ovarian follicle *Endocrinology* **129** 3281–3288.