REVIEW

IGFs and IGF-binding proteins in the regulation of human ovarian and endometrial function

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Introduction

The key function of reproduction is to propagate inheritable characteristics to new generations. The human female reproductive system consists of the hypothalamic–pituitary–ovarian axis and end organs such as the uterus and fallopian tubes. The endocrine axis involves both peptide hormones (gonadotropin-releasing hormone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH)) and steroid hormones (estradiol and progesterone). In addition, numerous growth factors, including the insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBPs), play an important role at the tissue and cellular level through autocrine and/or paracrine mechanisms. There is substantial evidence that IGFs and IGFBPs are involved in follicular growth/development and steroidogenesis in the ovary, in proliferation and differentiation of the uterine endometrium, and in the implantation of the embryo.

IGFs and IGFBPs

IGF-I and IGF-II are single-chain polypeptides with molecular masses of 7649 Da and 7471 Da respectively (Rinderknecht & Humbel 1978a,b). Both IGFs are structurally homologous to proinsulin and have insulin-like metabolic effects (induced hypoglycemia) (Guler *et al.* 1987). The hypoglycemic effects induced by the IGFs are mediated via the insulin receptor since IGFs at high concentrations bind insulin receptors (Froesch & Zapf 1985). IGFs are synthesized *de novo* in a variety of tissues and exert mitogenic effects within the local tissue environment through autocrine and/or paracrine mechanisms (Holly & Wass 1989). In addition to their roles as mitogens, IGF-I and IGF-II induce cellular differentiation and promote the expression of differentiated functions in a variety of cells (Florini *et al.* 1991, Quinn *et al.* 1993, Jones & Clemmons 1995). IGF-I is growth hormone (GH) dependent and mainly produced by the liver (Schwander

et al. 1983). Two major classes of IGF-I mRNA transcripts have been identified in the liver and in non-hepatic tissues; both transcripts code for the same mature peptide (LeRoith & Roberts 1991). ‘Exon 2 transcripts’ of IGF-I (mRNAs containing a 5′-untranslated region starting in exon 2) are believed to encode the ‘endocrine form of IGF-I’ under the control of GH in the liver. ‘Exon 1 transcripts’ of IGF-I (mRNAs containing a 5′-untranslated region starting in exon 1), which are found in all tissue, are regulated by factors other than GH and may represent the ‘autocrine/paracrine form of IGF-I’ (LeRoith & Roberts 1991). In contrast to IGF-I, IGF-II is not strongly GH dependent (Zapf *et al.* 1981). IGF-II has no growth-promoting effect in the hypophysectomized rat (Brown *et al.* 1986). In the rodent and a number of other species, IGF-II is thought to function as a fetal growth factor since IGF-II gene deletion results in retardation of intrauterine and perinatal growth (Schoenle *et al.* 1985, DeChiara *et al.* 1990). The role of IGF-II in man, however, remains controversial.

The biological effects of IGFs are mediated through two types of cell membrane receptor (Rosenfeld 1989, Nissley & Lopaczynski 1991). The type I receptor is composed of two extracellular α -subunits and two transmembrane β -subunits (Jones & Clemmons 1995). Type I receptors preferentially bind IGF-I and also bind insulin at high concentrations (Nissley & Lopaczynski 1991). The binding affinity of type I receptors is: IGF-I $\geq$ IGF-II $>$ insulin. In contrast, type II receptors bind only IGFs and preferentially bind IGF-II (binding affinity of type II receptors: IGF-II $>$ IGF-I) (Table 1). Structurally, the type II receptor is a monomeric receptor identical to the mannose-6-phosphate receptor (Morgan *et al.* 1987). Functionally, most of the actions of both IGF-I and IGF-II are mediated by the type I receptor. In non-mammalian species, IGF-II does not bind to the mannose-6-phosphate receptor (Clairmont & Czech 1989). In the human, both type I and type II receptors have been identified in a wide range of tissues including ovarian follicles (Hernandez *et al.* 1992), the endometrium (Ghahary & Murphy 1989) and the

Table 1 The binding affinity between insulin, IGFs and their receptors (Rosenfeld 1989, Nissley & Lopaczynski 1991)

	Type I receptor	Type II receptor	Insulin receptor
IGF-I	High	Vey low	Very low
IGF-II	High or intermediate	High	Low
Insulin	Very low	None	High

placenta (Han *et al.* 1996, Liu *et al.* 1996, Sakai *et al.* 1997). However, fetal tissues have greater type I and type II IGF binding activities than adult tissues (Ocrant *et al.* 1988).

The most specific characteristic of IGFs, distinguishing them from other growth factors, is that they are regulated and modulated by a heterogeneous group of binding proteins (IGFBPs) with molecular masses ranging from 17 to 43 kDa (Binoux *et al.* 1991, Cataldo 1997). These high-affinity IGFBPs can compete with the IGF receptor for binding. The physiological roles of IGFBPs include prevention of the hypoglycemic response of IGFs (Yoeh & Baxter 1988), prolongation of clearance time of IGFs (Guler *et al.* 1989), transportation of IGFs to appropriate sites of action and modulation (e.g. enhancement or inhibition) of the action of IGFs (Busby *et al.* 1988, Bar *et al.* 1989). To date, six binding proteins (IGFBP-1 to IGFBP-6) have been identified and characterized. In the circulation, IGFBP-3 is the major carrier protein for the IGFs. This is supported by the fact that IGFBP-3 (as large molecular mass ‘150 kDa’ complexes containing IGFBP-3, IGF and the acid labile subunit) is unable to cross the capillary barrier and is retained in the circulation (Binoux *et al.* 1991). In contrast, IGFBP-1 is believed to regulate acute changes of serum IGFs (Wang & Chard 1992, Lee *et al.* 1997). Additionally, IGFBP-1 can cross the capillary barrier (Binoux *et al.* 1991) and modulate (e.g. enhance or inhibit) the actions of IGFs at the cellular level (Straus *et al.* 1991). The phosphorylation of IGFBP-1 may affect IGF actions; non-phosphorylated IGFBP-1 has 4- to 6-fold lower affinity for IGF-I as compared with phosphorylated IGFBP-1 (Jones *et al.* 1991). Thus, more complete release of IGF-I to receptors would be achieved by non-phosphorylated IGFBP-1. In contrast, the phosphorylated form of IGFBP-1 is more likely to sequester IGF-I and in turn attenuate the actions of IGF-I. IGFBP-2 has been much less extensively studied than IGFBP-1. In general, IGFBP-2 is thought to inhibit IGF actions, particularly those of IGF-II (Reeve *et al.* 1993), although in certain specific cell types it can be a modest stimulator of IGF-I action (Bar *et al.* 1989). In addition, IGFBP-2, as with IGFBP-1, appears to be the major binding protein for IGF-I in the human fetus (Wang & Chard 1992). IGFBP-4 and IGFBP-5 are involved in the control of growth in bone and cartilage (Mohan *et al.* 1989, Kiefer *et al.* 1992), as well as folliculogenesis and steroidogenesis in the rat ovary (Ling *et al.* 1993, Erickson *et al.* 1994).

IGFBP-5 has the unique property of adhering tightly to fibroblast extracellular matrix (ECM). The affinity of IGFBP-5 for IGF-I is lowered by approximately 7-fold when it associates with ECM, suggesting that ECM-bound IGFBP-5 may potentiate IGF actions (Jones *et al.* 1993a). As with IGFBP-4 and IGFBP-5 in the rat ovary, IGFBP-6, stimulated by combined FSH and LH, is also associated with decreased steroidogenesis in response to IGF-I (Rohan *et al.* 1993). However, these effects are substantially weaker than those of IGFBP-4 and IGFBP-5.

Proteolysis of IGFBP-2, IGFBP-3 and IGFBP-4 is a posttranslational processing mechanism that might affect the actions of these binding proteins. In a wide variety of tissues, protease activity may specifically cleave IGFBPs into smaller molecular weight forms that have a markedly reduced affinity for IGFs and in turn potentiate IGF actions (Schmid *et al.* 1991, Conover *et al.* 1993, Mason *et al.* 1996). Thus, proteolytic activity may be an important mechanism by which bioactive IGFs are made available to specific tissues. IGFBP-5 is also cleaved by proteases to a smaller fragment. However, unlike IGFBP-2, IGFBP-3 and IGFBP-4, the smaller fragment of IGFBP-5 does not potentiate the cell growth response to IGF-I (Jones *et al.* 1993a).

IGFs and IGFBPs in the ovary

Follicular growth and development

There is evidence that a tissue-specific and temporal pattern of expression of the IGFs is involved in the development of ovarian follicles (Erickson *et al.* 1994). Studies of the cellular sites of IGF gene expression have shown that, in the rat, IGF-I and IGF-II mRNA are localized to the granulosa cells (Carlsson *et al.* 1989, Hernandez *et al.* 1989, Oliver *et al.* 1989, Zhou *et al.* 1991) and theca-interstitial cells (Hernandez *et al.* 1990) respectively. The granulosa cells begin to express IGF-I during the early follicular phase and a high level of IGF-I activity is detected in the granulosa cells of healthy follicles. In contrast, expression of IGF-I mRNA is not observed in atretic follicles (Carlsson *et al.* 1989, Hernandez *et al.* 1989, Oliver *et al.* 1989, Zhou *et al.* 1991). These findings suggest that, in the rat, selection of the dominant follicle requires continual expression of IGF-I in the granulosa cells.

In the human ovary, the granulosa cell is a site of IGF-II rather than IGF-I gene expression (Hernandez *et al.* 1992, El Roeiy *et al.* 1993, Zhou & Bondy 1993, Mason *et al.* 1994). The concentrations of IGF-II (but not IGF-I) are significantly higher in ovarian venous effluents as compared with the peripheral circulation, implying an ovarian source for this peptide (Jesionowska *et al.* 1990). In contrast, IGF-I mRNA is not detected in granulosa cells of the mature human ovary, while IGF-I receptor mRNA is abundant in granulosa cells of both Graafian and atretic follicles (El Roeiy *et al.* 1993, Zhou & Bondy 1993).

In women undergoing controlled ovarian hyperstimulation (COH) and *in vitro* fertilization (IVF), serum IGF-I levels at the preovulatory stage are substantially higher than those in follicular fluid (Chang *et al.* 1994, Pellegrini *et al.* 1995). These observations imply that IGF-I in follicular fluid is probably derived by diffusion from the peripheral circulation and that local production of IGF-I in granulosa cells of the dominant follicle is unlikely. In preovulatory follicular fluid of patients undergoing COH and IVF, IGF-II levels are approximately 8-fold higher than those of IGF-I (Kubota *et al.* 1993). Additionally, IGF-II increases the release of estradiol from cultured human granulosa cells in a dose-dependent manner (Kubota *et al.* 1993). Taken together, development of human granulosa cells may be regulated by IGFs (IGF-II synthesized *de novo* and IGF-I from the circulation) via IGF receptors, though IGF-I may not be synthesized *de novo* in the follicle.

Immunohistochemical studies with human ovary have localized IGF-I peptides to the theca-interstitial compartment (Hernandez *et al.* 1992). IGF-I cannot stimulate *de novo* steroidogenesis alone because it does not stimulate cholesterol transport into the mitochondria (Magoffin & Weitsman 1996). In theca-interstitial cells, IGF-I, in combination with LH, increases LH receptors, augments LH-stimulation of cAMP and enhances androgen biosynthesis (Cara & Rosenfield 1988, Magoffin & Erickson 1988, Magoffin *et al.* 1990, Bergh *et al.* 1993, Huang *et al.* 1994, Nahum *et al.* 1995). In addition, the synergistic action of LH and IGF-I on the substantial production of androgen has also been observed in cultured human thecal monolayers (Hillier *et al.* 1991). Furthermore, using *in situ* hybridization, IGF-I gene expression is localized to theca cells of atretic and small developing follicles (3–5 mm) in human ovary (El Roeiy *et al.* 1994). This implies that IGF-I of thecal origin may facilitate LH action on androgen production. In contrast, IGF-I in the granulosa cells is capable of augmenting FSH-mediated estrogen synthesis (Adashi *et al.* 1985a, Christman *et al.* 1991); and after luteinization, in synergy with LH, stimulating progesterone synthesis (Adashi *et al.* 1985b, Davoren *et al.* 1985).

At the preovulatory stage, IGFBP-1 levels in follicular fluid are substantially higher than those in serum (Chang *et al.* 1994). As with the preovulatory peak of serum

estradiol, which reflects the production of estradiol by granulosa cells of dominant follicles, serum levels of IGFBP-1 are significantly increased at the preovulatory stage under both non-fasting and fasting conditions (Wang *et al.* 1995, Juul *et al.* 1997). This is consistent with previous results that IGFBP-1 levels in both serum (Martikainen *et al.* 1991) and follicular fluid (Thierry van dessel *et al.* 1996) are substantially elevated at the preovulatory stage in controlled hyperstimulation cycles. In addition, these findings are further supported by results from studies using *in situ* hybridization that abundant expression of IGFBP-1 mRNA is only present in the granulosa cells of dominant follicles (El Roeiy *et al.* 1994). However, there is still a controversy since other investigators fail to find any alterations in serum IGFBP-1 during the normal menstrual cycle (Thierry van dessel *et al.* 1996, Helle *et al.* 1998). It is possible that production of IGFBP-1 by ovarian granulosa cells may or may not be reflected by the circulating IGFBP-1 levels. IGFBP-1 in the ovary during the follicular phase is believed to inhibit IGF-I-induced androgen production by theca cells and in turn inhibit/prevent follicular atresia and anovulation by acting through enhancement of estradiol formation in granulosa cells (a shift from androgen-dominant to estrogen-dominant follicles) (Nobels & Dewailly 1992). These observations indicate that local production of IGFBP-1 (of granulosa cell origin) in dominant follicles at the preovulatory stage is substantial and that it may be important in the regulation of follicular growth and maturation by modulating the actions of IGF-I (from systemic circulation) on the production of androgen in theca cells.

In normal human ovaries, IGFBP-4 can be immunolocalized to the granulosa and theca cells of atretic follicles, but is negligible in primordial, preantral and antral follicles (Peng *et al.* 1996). The follicular fluid of atretic/androgen-dominant follicles contains high levels of IGFBP-2 and IGFBP-4, which are known to inhibit the action of IGF-II (Cataldo & Giudice 1992a, San Roman & Magoffin 1993, Giudice *et al.* 1995). In contrast, an increase in the amount of lower molecular mass forms of IGFBP-2, -3 and -4 (23, 29 and 16.5 kDa respectively) in dominant follicles suggests an increased activity of proteases for IGFBPs (Cwyfan Hughes *et al.* 1997). Moreover, IGFBP-4 protease activity is also observed in the follicular fluid of developing but not atretic follicles (Chandrasekher *et al.* 1995, Iwashita *et al.* 1996). The protease activity may specifically cleave IGFBPs into smaller fragments that have a markedly reduced affinity for IGFs and in turn potentiate IGF actions (Conover *et al.* 1993, Mason *et al.* 1996). *In vitro* studies have shown that IGFBP-4 proteolytic activity is increased when granulosa cells are incubated with IGFs, estradiol or FSH, but not with testosterone. In addition, IGFBP-4 inhibits IGF-I-induced estradiol release by granulosa cells while proteolyzed IGFBP-4 does not (Iwashita *et al.* 1996). Thus, proteolysis of IGFBPs in

Figure 1 Androgen production in 'human' theca cells is controlled by IGF-I in synergy with LH; IGFBP-1 of granulosa cell origin prevents overproduction of androgens by theca cells through inhibition of IGF actions.

follicles may be an important mechanism by which bioactive IGFs are made available to granulosa cells.

Thus, there appear to be two mechanisms by which IGFs and IGFBPs are involved in the control of follicular growth and development. Androgen production in theca cells is controlled by IGF-I in synergy with LH; IGFBP-1 of granulosa cell origin may play an important role in modulating IGF-I bioactivity in theca cells and thereby prevent overproduction of androgens (Fig. 1). On the other hand, FSH-induced estradiol production in granulosa cells is mediated by IGF-II synthesized *in situ*. Concomitantly, less production of certain IGFBPs (e.g. IGFBP-2, -4 and -5) and more production of their specific proteases accentuate the actions of IGF-II. Based on currently available data, the following hypothesis is proposed. In the granulosa cells of the dominant follicle, FSH might stimulate expression of the IGF-II gene and the IGFBP protease genes, and at the same time, expression of the IGFBP genes (e.g. IGFBP-2, -4 and -5) might be inhibited. In addition, the elevated IGFBP proteases cleave IGFBP-2, -4 and -5 into smaller inactive fragments. The resultant activation of IGF might stimulate FSH-induced estradiol production and in turn potentiate proliferation of granulosa cells (Fig. 2). In growing and

developing follicles, events in both theca and granulosa cells progress simultaneously and interact with each other. Failure of progression in either the theca or the granulosa may result in follicular atresia.

Luteal regression

Recent studies have shown that IGFBP-3 is abundant in the follicular fluid of growing follicles (Sarvas *et al.* 1994) and progressively decreased in dominant follicles as they grow to preovulatory size (San Roman & Magoffin 1993). In cultured human luteinizing granulosa cells, synthesis of IGFBP-3 is stimulated by GH and inhibited by FSH. Conversely, IGFBP-3 is also able to inhibit the stimulatory effect of FSH on granulosa cells. As the action of IGFBP-3 is exerted by binding of the IGF peptides, the inhibitory effect on FSH is believed to occur through blocking of the binding between IGF-I and membrane receptors, rather than attenuation of IGF-I potency (Barreca *et al.* 1996). In the luteal phase of the ovarian cycle, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) also stimulates release of IGFBP-3 from human granulosa-luteal cells and inhibits IGF-I-induced progesterone production. This $PGF_{2\alpha}$ -induced stimulation of IGFBP-3 production may be one of the mechanisms

Figure 2 Hypothetically, in the granulosa cells of the 'human' dominant follicle, FSH might activate gene expression of IGF-II and IGFBP proteases and inhibit expression of IGFBP-2, -4 and -5. In addition, the elevated IGFBP proteases cleave IGFBP-2, -4 and -5 into smaller inactive fragments. The net result is increased IGF-II activity in granulosa cells. In synergy with FSH, both IGF-I (from the circulation) and IGF-II (synthesized *de novo*) stimulate production of estradiol (E_2) through IGF receptors and in turn induce proliferation of granulosa cells and follicular growth. White R, FSH receptor; black R, IGF-I and IGF-II receptor.

whereby $PGF_{2\alpha}$ exerts its luteolytic effect via the IGF system (Sarvas *et al.* 1994). Thus, IGFs and IGFBPs are involved in progesterone synthesis and spontaneous regression of the corpus luteum.

Polycystic ovarian syndrome (PCOS)

IGFBPs are thought to play an important role in regulating ovarian function by modifying the effects of IGFs or by directly affecting ovarian steroidogenesis. This is likely to be important in hyperinsulinemic insulin-resistant conditions, such as PCOS.

In both normal human ovaries and polycystic ovaries, granulosa cells may or may not secrete IGFBP-1 in response to FSH (Mason *et al.* 1993). In contrast, the addition of IGF-I to granulosa cells incubated with testosterone or testosterone plus FSH causes complete inhibition of IGFBP-1 production (Mason *et al.* 1993). In women with both polycystic-appearing ovaries and PCOS, serum levels of IGFBP-1 are significantly lower than in those with normal-appearing ovaries (Carmina *et al.* 1997). In

addition, IGFBP-1, analyzed by Western ligand blotting, is not detected in follicular fluid in PCOS (Cataldo & Giudice 1992b). Normally, the ovarian stroma has specific binding sites both for insulin and for IGF-I (Nagamani & Stuart 1990). Additionally, both insulin and IGF-I potentiate LH-induced testosterone and androstenedione secretion in cultured human theca cells (Bergh *et al.* 1993). In women with PCOS, hyperinsulinemia with insulin resistance and stimulation of ovarian androgen synthesis by insulin mediated through the type I IGF receptors are commonly observed (Nagamani & Stuart 1990). *In situ* hybridization studies have shown that abundant expression of IGFBP-1 mRNA is only found in the granulosa cells of dominant follicles and not in those of atretic follicles (El Roeiy *et al.* 1994). Additionally, *in vitro* studies have shown that both insulin and IGF-I inhibit IGFBP-1 production by cultured human luteinizing granulosa cells. Insulin exerts its inhibitory effect on IGFBP-1 production via insulin receptors, while IGF-I appears to exert its effect via IGF receptors (Poretsky *et al.* 1996). These findings indicate that a decrease in IGFBP-1 produced by

Figure 3 The involvement of IGFBP-1 in the pathogenesis of PCOS. Deficient production of IGFBP-1 in granulosa cells results from hyperinsulinemia (insulin resistance) and/or inadequate FSH and therefore intensifies IGF-I activity in theca cells. The high circulating insulin (hyperinsulinemia) may enhance LH-mediated androgen production in theca cells by acting through type I IGF receptors. The resultant overproduction of androgens (as a consequence of elevated insulin, LH and IGF-I activity at the level of theca cells) in turn inhibits aromatase activity in granulosa cells. Reduced estradiol leads to decreased proliferation of granulosa cells and thus defective follicular maturation.

granulosa cells may insufficiently antagonize IGF-I bioactivity in theca-stromal cells, resulting in overproduction of androgens and in turn defective follicular maturation and anovulation (Fig. 3). This is further supported by the clinical observation that the decrease in serum IGF-I concentrations and increase in serum IGFBP-1 induced by combined oral contraceptives appears to decrease ovarian androgen production in women with polycystic ovarian disease (Suikkari *et al.* 1991), resulting in an increased follicular response to ovarian stimulation regimens.

PCOS often develops during puberty. At the onset of puberty, increased pulsatile GH secretion results in insulin resistance and compensatory hyperinsulinemia (Bloch *et al.* 1987). The increased serum insulin levels further decrease hepatic synthesis of IGFBP-1 (Weaver *et al.* 1990) and in turn enhance the bioactivity of IGF-I. Based on the similar changes of IGF-I and IGFBP-1 in PCOS and puberty, it is plausible to speculate that there is an association between PCOS and puberty (Nobels & Dewailly 1992).

In human granulosa cells, IGFBP-1 and estradiol are differentially regulated by IGF-I. In the presence of FSH,

IGF-I stimulates granulosa cells to produce estradiol in a dose-dependent manner (Mason *et al.* 1993). The IGF-I-induced estradiol production is also modulated by other species of IGFBPs. The abundant expression of IGFBP-4 in granulosa and theca cells of preantral and antral follicles of PCOS ovaries may lead to a decrease in the bioavailability of IGF-I in those follicles (Peng *et al.* 1996). The decrease in IGF-I-mediated stimulation of gonadotropin actions on granulosa and theca cells in preantral and antral follicles may in turn impair the induction of aromatase activity, causing an androgenic microenvironment which is characteristic of atretic follicles and PCOS follicles (Peng *et al.* 1996).

IGF-I and IGF-II significantly increase androgen production from the adrenal gland via activation of type I IGF receptors on adrenal cortical cells (Mesiano *et al.* 1997). During steroidogenesis, the cytochrome P450c17 α is expressed as both 17 α -hydroxylase and 17,20-desmolase, and catalyzes androgen production. In addition, IGF-I can stimulate both LH-dependent P450c17 α activity in ovaries and adrenocorticotropin-dependent activity in

adrenals (Penhoat *et al.* 1989). Consequently, both the accentuated IGF-I bioactivity and the activation of type I IGF receptors in the adrenal gland may play a pivotal role in androgen production in PCOS.

IGFs and IGFBPs in the endometrium

Regulation of proliferation and differentiation in the endometrium

In the human endometrium, mRNAs for IGF-I, IGF-II and IGFBPs 1–6 are expressed during the menstrual cycle. Specifically, IGF-I mRNA is substantially higher during the proliferative than the secretory phase, whereas the converse is true for IGF-II (Zhou *et al.* 1994, Gao *et al.* 1995). Furthermore, IGFBP-2, -4, -5 and -6 mRNAs demonstrate a diffuse stromal pattern of expression, whereas IGFBP-1 and -3 are more concentrated in selected subpopulations of endometrial cells (Rutanen *et al.* 1991, 1993, Zhou *et al.* 1994). IGFBP-2 and IGFBP-3 mRNAs are significantly increased in the late secretory phase of the endometrium (Giudice *et al.* 1991). Moreover, IGFBP-3 mRNA is primarily concentrated in endometrial capillaries, largely due to the intensive vascularization of endometrial glands during the secretory phase (Zhou *et al.* 1994). IGFBP-4 and IGFBP-5 mRNAs remain unchanged during the menstrual cycle (Rutanen *et al.* 1993). IGFBP-1 mRNA is not detected in proliferative endometrium but is present in the stromal cells of the secretory endometrium (Julkunen *et al.* 1990, Rutanen *et al.* 1991, Zhou *et al.* 1994).

IGF-I mRNA is present in early proliferative phase endometrium and is most abundant in the late proliferative phase (Boehm *et al.* 1990, Zhou *et al.* 1994). The cyclic changes of IGF-I mRNA in the proliferative phase of the endometrium are coincident with serum estradiol concentrations. Giudice and her coworkers have also reported that endometrial growth in the proliferative phase is accelerated by ovarian estradiol via local production of IGF-I, which in turn stimulates cell proliferation (Giudice *et al.* 1993). IGFs bind to both cell membrane receptors and soluble IGFBP-1 with similar affinity. Thus, IGFBP-1 inhibits binding of IGF-I to its endometrial IGF receptors and reduces the availability of IGF-I at the cellular level (Rutanen *et al.* 1988). After ovulation, progesterone is prominent and stimulates the endometrial stromal cells to secrete IGFBP-1, which in turn modulates the bioactivity of IGF-I (Rutanen & Pekonen 1991). In addition, IGFBP-1 mRNA is localized to predecidualized stromal cells in late secretory endometrium and to decidual cells in pregnancy endometrium (Julkunen *et al.* 1990, Rutanen *et al.* 1991). The timing and cellular localization of IGFBP-1 in human endometrium suggest that this protein is associated with decidual differentiation of the stromal cells. Furthermore, the IGF receptors on human endometrial stromal cells are downregulated by progesterone and in turn inhibit the growth-promoting

actions of IGF-I (Strowitzki *et al.* 1996). Both effects result in inhibition of endometrial stromal growth and acceleration of cell differentiation during the secretory phase.

In women with a progestin (levonorgestrel)-releasing intrauterine contraceptive device (IUCD), IGFBP-1 is present in abundance in decidualized stromal cells of the endometrium (Pekonen *et al.* 1992, Suhonen *et al.* 1996). Furthermore, the decidual reaction and epithelial atrophy induced by intrauterine progestin (levonorgestrel) are associated with expression of IGFBP-1 in decidualized stromal cells (Suhonen *et al.* 1996). In contrast, there is no difference in circulating IGFBP-1 levels between IUCD (without progestin) users and non-IUCD users (Seleem *et al.* 1996). Studies using cell cultures have also shown that progesterone (medroxyprogesterone acetate) stimulates IGFBP-1 secretion in cultured endometrial stromal cells (Bell *et al.* 1991). These findings further confirm that synthesis of endometrial IGFBP-1 is progesterone dependent (Julkunen *et al.* 1988, Rutanen & Pekonen 1991). An immunohistochemical study has shown that administration of micronized progesterone (100 mg/day orally, or 100–200 mg/day vaginally) cannot prevent the proliferative effect of estrogen (Suvanto-Luukkonen *et al.* 1995). In endometrial samples from women treated with micronized progesterone, no signs of progestin effect are detected by microscopic examination; in addition, immunohistochemical staining of IGFBP-1 is completely negative in endometrial stromal cells (Suvanto-Luukkonen *et al.* 1995). Thus, it is tempting to speculate that the immunohistochemical detection of IGFBP-1 in endometrial stromal cells may be used to assess the degree of progestin effect in the endometrium during hormone replacement therapy.

In postmenopausal women, abnormal uterine bleeding is frequently observed as a deficient progesterone effect. Microscopically, there is often simple hyperplasia and hypertrophy of the endometrium. In our own studies, using reverse transcription-polymerase chain reaction (RT-PCR), expression of both IGF-II and IGFBP-1 mRNA was not detected in endometrium from postmenopausal women with abnormal uterine bleeding. In contrast, expression of IGF-II mRNA was detected and that of IGFBP-1 mRNA was relatively abundant in the endometrium from postmenopausal women 3–6 months after progesterone (medroxyprogesterone acetate 5 mg/day) treatment in association with estrogen supplementation (Fig. 4). Expression of IGF-I mRNA was observed in the endometrium with or without progesterone treatment. Histologically, simple hyperplasia and hypertrophy of the endometrium was no longer observed after progesterone treatment. These data provide evidence that the protective effects of progesterone on the endometrium might be mediated through the expression of IGFBP-1, which in turn could inhibit the growth-promoting effects of IGF-I. They also suggest that the differentiation of

Figure 4 Effects of progesterone on expression of mRNAs for IGF-II and IGFBP-1 in the endometrium. Using RT-PCR, expression of both IGF-II and IGFBP-1 mRNA (corresponding to 538 and 417 bp respectively) was not detected in the endometrium from postmenopausal women with unopposed estrogen stimulation and abnormal uterine bleeding (MEM). After progesterone (medroxyprogesterone acetate 5 mg/day) treatment in association with estrogen supplementation for 3–6 months (MEM-P Tx), expression of IGF-II mRNA was detected and that of IGFBP-1 mRNA was relatively abundant in the endometrium from those postmenopausal women. Reverse transcription was performed using 10 µg total RNA from endometrium followed by a 30-cycle PCR. Primers for IGFBP-1 RT-PCR were 5' TACAT CTGGC AGTTG GGGTC TCC 3' (antisense) and 5' TGCAG AGGCA GGGAG CCCTG AAA 3' (sense), for IGF-I RT-PCR 5' ACATC TCCCA TCTCT CTGGA TTCC TTTTC 3' (antisense) and 5' CCCTC TACTT GCGTT CTTC AATGT ACTTCC 3' (sense), and for IGF-II RT-PCR 5' AGTCG ATGCT GGTGC TTCTC ACCTT CTGGC 3' (antisense) and 5' TGCGG CAGTT TTGCT CACTT CCGAT TGCTGG 3' (sense).

the endometrium might be mediated by IGF-II under the regulation of progesterone.

Involvement of IGFs/IGFBPs in implantation – interaction between the endometrium and the embryo

The expression of IGF genes in the uterus can be regulated by estrogen secreted by implanting mammalian embryos of several species, suggesting that IGFs may play a role in implantation (Ko *et al.* 1994). In the presence of progesterone and epidermal growth factor, both IGF-I and IGF-II *in vitro* induce production of IGFBP-1 and prolactin by the human endometrium in a dose-dependent biphasic manner; stimulation of production by low doses of IGFs and inhibition by higher doses (Irwin *et al.* 1994). At the peri-implantation stage, local production of IGFBP-1 by the endometrium in the presence of progesterone appears to stimulate the differentiation and secretory function of the endometrium (Rutanen & Pekonen 1991). After implantation, IGFBP-1 can be detected immuno-

histochemically in both the decidualized endometrium in intrauterine pregnancy and decidualized stromal cells at the implantation site in ovarian pregnancy (Rutanen & Pekonen 1991). IGFBP-1 of decidual origin is believed to inhibit the bioactivity of IGF-I (Pekonen *et al.* 1988, Ritvos *et al.* 1988) and may protect the endometrium from invasion by trophoblast. This is further supported by the fact that IGF-induced mitogenesis is inversely correlated with endogenous IGFBP-1 levels in decidualized stromal cultures (Irwin *et al.* 1994). Furthermore, supplementation with vaginal micronized progesterone during the luteal phase appears to directly stimulate endometrial production of IGFBP-1 (Wang & Soong 1996). The accentuated local production of endometrial IGFBP-1 may in turn inhibit embryo implantation and decrease the pregnancy rate (Wang & Soong 1996).

There is clear evidence that IGF-I and IGF-II stimulate both cell proliferation and differentiation of preimplantation embryos (Smith *et al.* 1993, Shi *et al.* 1994). Embryos co-cultured with various epithelial monolayers show improved development (Lai *et al.* 1996).

In such systems, IGFBP-1, -2, -3, -4 and -5 are produced by the monolayer helper cells (human endometrial and oviductal cells). Of these binding proteins, IGFBP-3 is the most prominent in co-cultured human endometrial monolayers and may play a role in embryo development either by regulating IGF actions or by directly enhancing embryo development (Lai *et al.* 1996).

Using immunocytochemical techniques with a monoclonal antibody to proliferating cell nuclear antigen, an effect of IGF-I on the proliferative activity of trophoblasts is observed during early pregnancy (4–12 weeks of gestation). *In vitro* culture of trophoblasts of early pregnancy, secretion of human chorionic gonadotropin and human placental lactogen by trophoblasts is increased by addition of IGF-I, indicating the effect of IGF-I on the differentiated function of trophoblasts (Maruo *et al.* 1995). These findings suggest that IGF-I acts as an autocrine/paracrine factor in regulating early placental growth and function.

In a normal pregnancy, maternal serum IGFBP-1 of decidual origin increases rapidly during the first trimester and reaches a peak at 12 to 14 weeks of gestation; thereafter it remains high until term (Wang *et al.* 1991). Elevated serum IGFBP-1 levels in the third trimester of gestation have been reported in pregnant women complicated by pre-eclampsia (Than *et al.* 1984, Iino *et al.* 1986, Howell *et al.* 1989). Additionally, in pre-eclampsia concomitant with idiopathic intrauterine growth retardation (IUGR), significantly elevated serum levels of IGFBP-1 (Wang *et al.* 1996, Giudice *et al.* 1997) and decreased circulating IGF-I concentrations (Giudice *et al.* 1997) are found in both the mother and the fetus. Similar observations have also been made in both the maternal and fetal circulation in pregnancies complicated by growth retardation (Crystal & Giudice 1991, Holmes *et al.* 1997, Ostlund *et al.* 1997). Pathologically, inadequacy or failure of intravascular trophoblastic infiltration into intramyometrial segments of the spiral arteries between 12 and 14 weeks of gestation is a specific histological feature of both IUGR and pre-eclampsia (Robertson 1976, Meekins *et al.* 1994, De Groot *et al.* 1996). There is evidence that IGFBP-1 inhibits cytotrophoblast invasion into decidualized stromal multilayers through binding to cytotrophoblast $\alpha 5\beta 1$ integrin (Irwin *et al.* 1996) since IGFBP-1 contains an Arg-Gly-Asp integrin recognition sequence (Jones *et al.* 1993b). In addition, an abundance of IGFBP-1 is found at the maternal-fetal interface in severely pre-eclamptic pregnancies, suggesting that IGFBP-1 may participate in the pathogenesis of the shallow or inadequate placental invasion observed in this disorder (Giudice *et al.* 1997). Functionally, the failure of invasion leads to inadequate circulation and oxygenation of trophoblastic tissue (Khong *et al.* 1986, De Groot & Taylor 1993). Thus, low circulating IGF-I and elevated IGFBP-1 levels may contribute to restricted placental and therefore fetal growth. These findings reflect the association between

elevated IGFBP-1 and IUGR with or without pre-eclampsia, and further indicate that endometrial/decidual IGFBP-1 can play an important role in implantation and placentation.

Conclusions

There is now considerable evidence that IGFs and IGFBPs play a major autocrine and paracrine role in regulation of follicular development and endometrial function. Various molecular forms of IGFBPs act as modulators of the actions of IGFs on reproductive function acting in synergy with pituitary gonadotropins and ovarian steroid hormones. In addition, local production of specific proteases for IGFBPs further mediates the bioactivity of IGFBPs and in turn modulates IGF actions. At various sites in the female reproductive system, small changes (overproduction or deficiency) of IGFBPs may result in pathological conditions such as anovulation and hyperandrogenism, inadequate differentiation of the endometrium, failure of implantation and inadequate placentation.

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