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Insulin-like growth factor binding protein-1 (IGFBP-1): a multifunctional role in the human female reproductive tract

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Insulin-like growth factor-1 (IGFBP-1) is particularly important in human female reproductive physiology, where it is involved with other factors in a complex system which regulates menstrual cycles, puberty, ovulation, decidualization, implantation and fetal growth. This has implications for clinical obstetrics and gynaecology, where there is evidence for a pathophysiological role for IGFBP-1 in pre-eclampsia, intrauterine growth restriction, polycystic ovarian syndrome and trophoblast and endometrial neoplasms.

Key words: female reproductive tract/IGFBP-1/intrauterinegrowth restriction/polycystic ovarian syndrome/pre-eclampsia

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Introduction

Insulin-like growth factor binding protein-1 (IGFBP-1) was the first member of a family of structurally related soluble proteins to be characterized. This family is involved in modulating the effects of the insulin-like growth factors I and II (IGF-I and II) which have important roles in growth and apoptosis, metabolism and development. IGFBP-1 is an endocrine factor which alters serum IGF bioavailability, and also acts in a paracrine/autocrine fashion (Table I).

In the endometrium and during early human pregnancy the IGF family, including IGF peptides, IGFBPs and IGFBP proteases, are thought to have major effects on implantation and trophoblast invasion. IGFBP-1 is the predominant binding protein species in secretory (progesterone-phase) endometrium and decidualized stromal endometrial cells, and its expression is tightly controlled.

Polycystic ovarian syndrome (PCOS) is associated with obesity, insulin resistance and cardiovascular risk later in life, as well as menstrual disturbance and anovulatory infertility. Low serum concentrations of IGFBP-1 occur in this complex syndrome, with high concentrations of circulating androgens and a greater risk of endometrial hyperplasia and neoplasia.

Implantation is similar to tumour invasion. However, the trophoblast invasion seen in implantation is regulated and not dysregulated, as in choriocarcinoma. Invasive trophoblast,

Table I. Summary of proposed roles for IGFBP-1 in human female reproductive physiology and pathology

System	Physiological	Pathological
Ovary	Ovulation	Polycystic ovary disease
	Follicular growth	Anovulation
	Steroidogenesis	Hyperandrogenism
Endometrium	Differentiation	Hyperplasia
	Proliferation	Neoplasia
	Decidualization	
Trophoblast[a]	Implantation	Implantation failure
	Invasion	Impaired placentation Pre-eclampsia/pregnancy-induced hypertension
Feto-placental unit	Fetal growth	Placental insufficiency Fetal growth restriction

[a]Abnormal trophoblast invasion in early pregnancy may contribute to the clinico-pathological entities of impaired placentation which have implications for the feto-placental unit. Impaired placentation may present clinically in later pregnancy as pre-eclampsia, placental insufficiency and intrauterine growth restriction.

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expressing IGF-II, and decidual IGFBP-1 interact in a highly controlled way. IGFBP-1 inhibits IGF-II, providing maternal restraint on invasion. This IGFBP-1 inhibition is important because IGF-II overexpression has been implicated in tumorigenesis and anti-apoptosis.

Conversely, high concentrations of decidual IGFBP-1 which limit invasion may predispose to poor implantation, uterine receptivity and subsequent miscarriage. Abnormal trophoblast invasion is also thought to be important in the aetiology of placental insufficiency and pre-eclampsia. Transgenic animals overexpressing IGFBP-1 exhibit poor fertility and reproductive function, and there is evidence that excessive concentrations of IGFBP-1 in human maternal and fetal serum may contribute to fetal growth restriction.

Genetic regulation of IGFBP-1

The complementary deoxyribonucleic acid (cDNA) sequence of IGFBP-1 was reported in 1988. The complete amino acid sequence of human IGFBP-1 was predicted from the cDNA sequence, and confirmed by direct sequencing of purified IGFBP-1 protein (Lee *et al*., 1988). A family of related proteins (IGFBP-2 to IGFBP-6) have been cloned and partially characterized; these proteins are related by structural and functional homologies, but have distinct physiological characteristics (Jones and Clemmons, 1995) and vary in their tissue expression and regulation by other hormones and growth factors.

The IGFBP-1 primary amino acid sequence contains 12 N-terminal and six C-terminal cysteine residues which are conserved in other mammalian IGFBP-1 sequences and between other IGFBPs. These cysteine-rich areas are essential for optimal IGF binding. IGFBP-1 protein also contains integrin-binding and phosphorylation sites (Lee *et al*., 1993). Recently identified cDNA sequences have been predicted to encode proteins with lower IGF-binding affinities compared with the present described family of IGFBP peptides. The N-terminal region of these predicted proteins contains a cysteine-rich area, and would be structurally homologous to IGFBP-1 to -6 (Rosenfield *et al*., 1999). These may represent new members of the IGFBP family.

IGFBP-1 and -3 genes are contiguous and close to the homeobox A (HOXA) gene cluster on chromosome 7 (Figure 1). HOX genes, which are present in organisms ranging from yeasts to humans, encode transcription factors that are important in early morphogenesis. The first 3.6 kb 5′ to the IGFBP-1 transcription site functions as a IGFBP-1 promotor in several human in-vitro cell systems, for example Hec1B endometrial carcinoma cells and primary endometrial stromal cells. The IGFBP-1 promotor has six DNA boxes shown to be of functional significance *in vitro*. Box 1 contains the TATA element, which is important in transcription initiation. Box 2 has a binding site for hepatic nuclear factor 1 (HNF1), a DNA-binding protein likely to be responsible for the tissue-specific patterns of IGFBP-1 gene expression in ovary and decidua, as well as liver and kidney. Boxes 3 and 5 contain the glucocorticoid response elements, GRE1 and 2, which bind the glucocorticoid receptor. Box 4 contains an element which binds hepatic nuclear factor 3, and is known as the insulin response element (IRE), since it confers the inhibitory effect of insulin on IGFBP-1 promotor activity. Box 6 contains a cAMP response element (CRE) which confers cAMP stimulation on the IGFBP-1 promotor (Lee *et al*., 1997). The TATA element, HNF1 binding region, IRE and GRE2 sequences are highly conserved among the human, rat and mouse promotors, suggesting a crucial evolutionarily conserved role for these gene promotor regions in IGFBP-1 regulation (Upton *et al*., 1993).

The association of the IGFBP genes and HOX clusters show how important these binding proteins are in evolutionary and developmental terms. The various elements of the IGFBP-1 promotor give insight into the various roles of IGFBP-1 protein in metabolism and growth, as well as its tissue-specific expression in the reproductive tract.

IGFBP-1 is unusual among the IGFBPs in that it is rapidly and dynamically regulated. It appears to modulate the bioavailability of free serum IGF-I (Lee *et al*., 1997), and excess concentrations may contribute to growth failure in intrauterine growth restriction (Fant *et al*., 1993; Hills *et al*., 1996). Conversely, low IGFBP-1 concentrations are associated with obesity and cardiovascular risk factors in insulin-resistance syndromes, including the spectrum of disorders seen in PCOS (Gibson *et al*., 1996; Mogul *et al*., 1996; Poretsky *et al*., 1999).

As might be inferred from the presence of IREs in the IGFBP-1 promotor, insulin appears to be the primary determinant of IGFBP-1 expression via its inhibition of IGFBP-1 transcription. IGF-I and II also have inhibitory effects on IGFBP-1 expression. Glucocorticoids and cAMP stimulate IGFBP-1 transcription, but only when insulin concentrations are low or absent. Similarly, cytokines and growth hormone may affect IGFBP-1 expression by altering the regulatory actions of insulin. Studies performed both *in vitro* and *in vivo*, including the use of transgenic animals, have confirmed the IGF-inhibitory actions of IGFBP-1 which has been shown to inhibit linear growth, weight gain, tissue growth and glucose metabolism (Murphy *et al*., 1996).

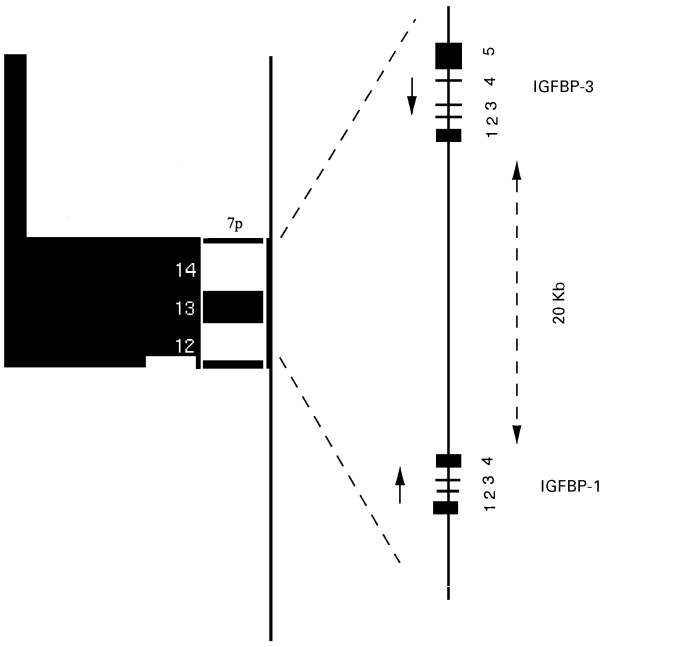

Figure 1. Chromosomal location of IGFBP-1 and IGFBP-3. 7p12–p14 showing location and organization of gene for hIGFBP-1, consisting of four exons separated by three introns, bordering hIGFBP-3 gene in tail-to-tail orientation.

IGFBP-1 in normal and pathological ovaries

Insulin growth factors stimulate ovarian mitosis and steroidogenesis, IGF-II predominating over IGF-I in the corpus luteum (Giudice *et al*., 1993). Non-phosphorylated IGFBP-1 is a potent inhibitor of IGFs *in vitro* (Rutanen *et al*., 1988; Burch *et al*., 1990; Crellier *et al*., 1996) and *in vivo* (Cox *et al*., 1994). Using human granulosa cells *in vitro*, other workers have also shown that insulin and IGFs inhibit IGFBP-1, with IGF-I and -II being the most potent (Poretsky *et al*., 1996a,b). IGFBP-1, primarily non-phosphorylated, is found in follicular fluid from women with normal menstrual cycles and from gonadotrophin-stimulated luteinizing follicles (Giudice *et al*., 1996). IGFBP-1 is expressed in granulosa cells of the dominant follicle following the LH surge, and IGFBP-1 mRNA is expressed in corpora lutea (Seppala *et al*., 1984; El-Roeiy *et al*., 1994). There may be paracrine feedback cycles operating because IGFBP-1 inhibits IGF-II, and in turn IGF-II inhibits IGFBP-1 production by luteinized human granulosa cells *in vitro*. In human ovarian granulosa cells IGF-II is expressed rather than IGF-I, which has not been detected (Hernandez *et al.*, 1992; El-Roeiy *et al.*, 1994; Mason *et al.*, 1994; Zhou *et al.*, 1994).

Other IGFBPs (-2, -3, -4 and -5) are known to play a role in ovarian follicular development and steroid production (Ling *et al.*, 1993; Rohan *et al.*, 1993). Degradation by specific IGFBP proteases after translation (particularly for IGFBP-2, -3 and -4) may decrease the activity of IGFBPs and potentiate IGF actions (Mason *et al.*, 1996). IGFBP-5 is proteolysed, but the lower-molecular weight form has not been found to increase IGF-1 activity (Jones *et al.*, 1993a). The regulatory mechanisms operating in theca and granulosa cells (Figure 2) help to explain the abnormal follicular development and steroid production seen in pathological clinical syndromes such as PCOS.

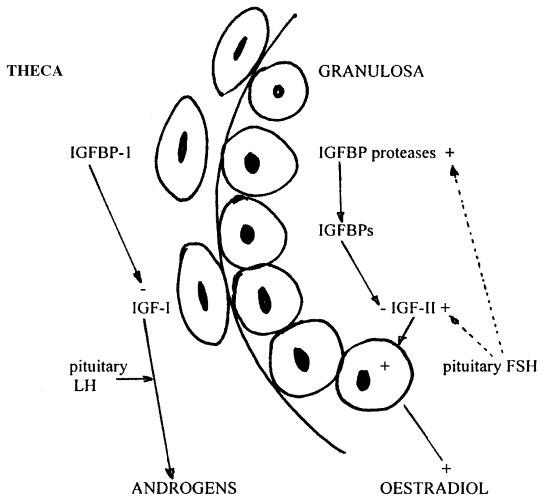

Figure 2. Proposed mechanisms of theca and granulosa cell endocrine and paracrine interactions. Granulosa cell: pituitary FSH-induced oestradiol production is mediated by IGF-II. FSH may stimulate IGF-II expression and IGFBP proteases which degrade IGFBP-2, -4 and -5 into smaller inactive fragments, resulting in a net activation of IGF-II. Increased IGF-II expression may stimulate further FSH-induced oestradiol and proliferation of granulosa cells, leading to normal follicular development. Theca cell: IGF-I and pituitary LH control androgen production. IGFBP-1 acts as an inhibitor on IGF-I action. Physiologically, there are likely to be theca–granulosa interactions; failures in the theca, granulosa or interactive pathways may lead to failure of follicular development and follicular atresia/apoptosis.

PCOS is the most common form of anovulatory infertility, and leads to significant morbidity in affected women of reproductive age. The basic mechanisms underlying PCOS remain unclear. The condition is characterized clinically by menstrual disturbance and sub- or infertility secondary to chronic anovulation, central obesity, virilization, acne and hirsutism secondary to elevated androgens. In addition, there are long-term health risks associated with insulin resistance, such as dyslipidaemia and predisposition to non-insulin-dependent diabetes and cardiovascular disease (Poretsky *et al*., 1999)

Diagnosis is based on the presence of the common clinical features with positive biochemical markers and exclusion of other similar clinical syndromes such as late-onset congenital adrenal hyperplasia, thyroid disease, hyperprolactinaemia and androgen-secreting tumours. In fact, most women with congenital adrenal hyperplasia have PCOS or menstrual problems (Conway, 1998). It is interesting that many of the metabolic and endocrine problems associated with PCOS mimic puberty. Although patients tend to present with clinical problems later in reproductive life, the onset of PCOS may begin in puberty. During puberty, there are increases in insulin resistance and pulse amplitude of LH, increasing circulating androgens and irregular cycles. In normal puberty, multiple small follicles are commonly seen on ultrasound (Nobels and Dewailley, 1992). Women with PCOS may continue to have endocrine profiles resembling puberty, and insulin sensitivity/resistance may not normalize. There is evidence for a single gene or group of genes predisposing the ovaries to insulin stimulation of androgens secondary to increased insulin sensitivity and blocking follicular maturation (Carey *et al*., 1993). Insulin resistance in adipose and skeletal muscle can be demonstrated, but the ovary remains relatively sensitive to insulin: both insulin and IGF-I have stimulatory effects on thecal androgen production. Insulin acts on the liver to inhibit sex hormone-binding globulin (SHBG) and IGFBP-1 production. Reduced SHBG concentrations tend to increase biologically free testosterone. Similarly, IGFBP-1 inhibition tends to increase circulating free IGF-I, which acts synergistically with LH to increase ovarian androgen production (Cara, 1994). Recently, multiple regression analysis has been used (Pakarinen *et al*., 1999) to show that there is no significant association between the concentrations of SHBG, IGFBP-1, glucose, insulin and levonorgestrel, and age and waist–hip ratio in women with normal body mass index (BMI).

The metabolic axis in both obese and lean women with PCOS is characterized by low concentrations of growth hormone, elevated insulin, free IGF-I and LH, and low concentrations of IGFBP-1 (Buyalos *et al*., 1995). Oral contraceptive treatment results in increased serum IGFBP-1, decreased circulating LH concentrations, and decreased IGF-I, associated with decreased androgen production. Interestingly, oral contraceptives do not cause these changes in women with normal ovulatory cycles (Suikkari *et al*., 1993). In PCOS, the progestagen intrauterine device appears to have an endometrial protective effect by increasing endometrial IGFBP-1 expression. Increased IGFBP-1 binding of circulating IGFs would be expected to reduce ovarian androgen production. However, there is no evidence for an increase in circulating IGFBP-1, despite an increase in endometrial IGFBP-1 expression (Rutanen *et al*., 1997)

IGFBP-1 abnormalities have been reported in other clinically important disorders of ovarian function. Adolescent girls with insulin-dependent diabetes mellitus (IDDM) and irregular cycles were reported to have lower IGF-I and higher IGFBP-1 concentrations compared to girls with IDDM, but normal cycles. This finding of low IGF-I and high IGFBP-1 concentrations was associated with higher BMI, higher haemoglobin A1c concentrations, and ultrasound evidence of polycystic ovarian changes (Adcock *et al*., 1993). Elevated BMI is usually highly correlated with elevated insulin, which would correlate with decreased IGFBP-1. However, in a scenario of insulin resistance, for example, syndrome X and non-insulin-dependent diabetes, the normal inverse correlation between insulin and IGFBP-1 is lost. Recent studies on obesity in animals have demonstrated the interaction between IGF-1 and IGFBP-1 in the proliferation of adipocyte precursors, differentiation of preadipocytes and development of obesity. In transgenic mice overexpressing IGFBP-1 and given a sucrose-enriched diet, there was significantly less weight gain compared with wild-type mice.

IGF-1 induction of glycerol-3-phosphate dehydrogenase—a measure of adipocyte differentiation—was reduced in preadipocytes from transgenic mice (Rajkumar *et al*., 1999).

Elevated IGFBP-1 has also been associated with exercise-induced amenorrhoea and irregular cycles in athletes and dancers (Jenkins *et al*., 1993) who have hypothalamic amenorrhoea and are hypo-oestrogenic. It is possible that increased IGFBP-1 concentrations may further inhibit oestradiol production and normal ovarian function in this situation.

In contrast with endometrial neoplasms, IGFBP-1 has not been implicated in the pathogenesis of ovarian malignancy. IGFBP-1 has not been detected in human ovarian carcinoma cell lines or in primary cultures of human epithelial ovarian carcinoma cells (Hofmann *et al.*, 1994).

The role of IGFBP-1 in non-pregnant endometrium

The IGF system is one of several growth factor systems which may play a physiological or a pathological role in the endometrium. The IGF/IGFBP axis is important in endometrial cyclic development and blastocyst implantation, and may have a role in the pathogenesis of neoplasia (Giudice *et al*., 1994).

Uterine endometrium is a dynamic tissue undergoing cyclical changes and responding to circulating ovarian steroids, oestrogen and progesterone, which cause cellular (hypertrophy) and tissue responses (menstruation), possibly via growth factors and related peptides such as the IGF family (Figure 3). However, in neoplasia, these peptides may have a role in pathogenesis; for instance, IGF-I may be an oestrogen mediator in endometrial cancer (Rutanen *et al*., 1994b). IGFs may cause normal or abnormal proliferation and differentiation depending on the presence of modulators, such as binding proteins.

It was found earlier (Koistinen *et al*., 1986) that the major secretory protein of decidualized endometrium was an IGF-binding protein known as placental protein12 (now identified and re-named IGFBP-1), after its primary structure and high-capacity binding characteristics with IGFs were independently reported (Bell *et al*., 1988; Julkunen *et al*., 1988).

There is tissue and developmental time-specific expression of the IGF/IGFBP system and its receptors based on data using immunohistochemical localization and in-situ hybridization (Giudice *et al.*, 1993; Zhou *et al*., 1994; Han *et al.*, 1996). In particular, ovarian stromal IGFBP-1 expression is dependent on menstrual cycle phase, the proliferative phase coinciding with high circulating oestrogen and the secretory phase being progesterone-dominant. This phase-specific pattern, with IGFBP-1 and IGF-II being expressed in secretory (progesterone phase) stroma suggests interaction between these two peptides in endometrial regulation (Giudice *et al.*, 1993). The timing and localization of IGFBP-1 suggests a role in decidual differentiation of stroma (Wang and Chard, 1999). If secretory endometrium differentiates to decidua in the pregnant state, the differential expression of decidual IGFBP-1 and trophoblast IGF-II suggests a paracrine interaction at the decidua–trophoblast interface (Giudice *et al.*, 1993). In contrast to stroma, endometrial epithelium does not appear to express IGF-II or IGFBP-1 in the proliferative or secretory phases. Stromal IGF-I is expressed preferentially in the proliferative (oestrogen-dominant) phase, whereas stromal IGF-II is expressed preferentially in the secretory (progesterone-dominant) phase (Zhou *et al.*, 1994). IGFBP-1 mRNA and IGFBP-1 protein have not been detected in myometrium or fibroid tissue (Tommola *et al*., 1989; Vollenhoven *et al*., 1993). The IGF/IGFBP-1 system is another example of spatial and temporal relationships and differential expression which appear to be important in developing dynamic systems.

In non-human primates, endogenous oestrogen and progesterone act synergistically to increase endometrial IGFBP-1 concentrations, and progesterone is important for maximal IGFBP-1 expression. In-vitro studies show that without progesterone, endometrial stromal cells synthesize and secrete very low concentrations of IGFBP-2, -3 and -4, and no detectable

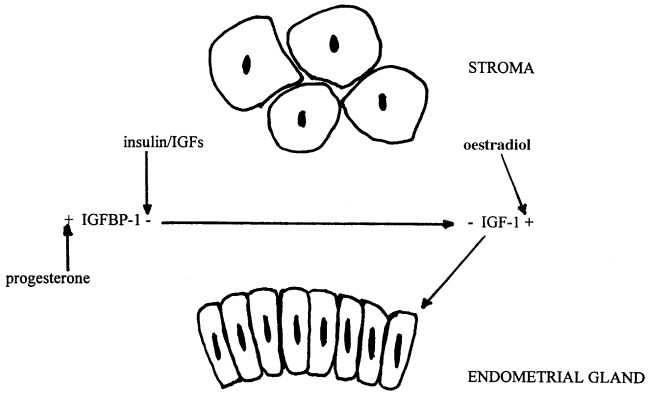

Figure 3. Schematic of non-pregnant endometrium, showing paracrine interactions between stroma and endometrial epithelium. In the secretory phase, stroma expresses IGFBP-1 and IGFs (Zhou *et al.*, 1994) which have mitotic and possibly anti-apoptotic effects on endometrial epithelium. Oestradiol and progesterone have differential effects, depending on the phase of the cycle. In the proliferative (oestrogen-dominant) phase of the menstrual cycle, IGF-I in stroma is differentially expressed, whereas stromal IGF-II is more abundantly expressed in the secretory (progesterone-dominant) phase. Endometrial epithelium appears not to express IGF-I, -II or IGFBP-1 in the proliferative or secretory phases, but may be a target organ for IGFs under the influence of oestrogen. Insulin/IGFs tend to inhibit IGFBP-1, and IGFBP-1 itself inhibits the IGFs, creating a feedback loop. Oestrogen is thought to exert its effects by acting through IGF-I in the proliferative phase. Progesterone tends to stimulate IGFBP-1, which is expressed differentially in stromal cells in the secretory phase. – = inhibition; + = stimulation.

IGFBP-1. Treatment with progesterone causing decidualization leads to very high concentrations of IGFBP-1 secretion compared with another decidual marker, prolactin (Richards *et al*., 1995). As predicted, this action is blocked by the progesterone antagonist RU486. Endometrial cells cultured in the presence of medroxyprogesterone show a dramatic 4000-fold increase in IGFBP-1 concentrations (Lane *et al*., 1994). IGF-II, interleukin-1β (IL-1β) and progesterone appear to be particularly important in endometrial IGFBP-1 regulation.

There are complex relationships between oestradiol, progesterone, IGFBP-1 and IGF-I which may have implications for understanding the pathogenesis of endometrial hyperplasia and neoplasms which are oestrogen-dependent. In order to explore these relationships further, IGFBP-1 transgenic mouse models have been used. In this in-vivo system IGFBP-1 is overexpressed in the uterine glandular epithelium, and this impairs the ability of oestradiol to stimulate DNA synthesis compared with wild-type controls (Rajkumar *et al*., 1996). This suggests that IGFBP-1 inhibits IGF-1 and endometrial oestradiol action. Inhibition of IGF receptor binding by IGFBP-1 in human endometrium was originally demonstrated in 1988 (Rutanen *et al*., 1988). This IGF inhibition raises the possibility of devising treatments for endometrial hyperplasia and neoplasia which will result in oestrogen inhibition, such as progestagens which stimulate IGFBP-1 production, IGFBP-1 agonists as anti-oestrogens, or IGF-I inhibitors. High concentrations of IGFBP-1 would be expected to inhibit oestradiol and therefore IGF-I, causing endometrial glandular atrophy and reduction in hyperplasia and neoplastic change.

Species differences in endometrial regulation between other mammals and humans make extrapolations difficult. Recently, an intrauterine microdialysis device has made possible the dynamic in-vivo measurement of uterine paracrine interactions in humans. Locally applied human chorionic gonadotrophin (HCG) appears to inhibit prolactin, macrophage colony stimulating factor (M-CSF) and IGFBP-1; the authors suggest that HCG used for ovulation induction and luteal support may directly alter endometrial physiology (Licht *et al*., 1998).

The role of IGFBP-1 in implantation and pregnant endometrium

During blastocyst implantation, there may be an important interaction between decidual IGFBP-1 and trophoblast-derived IGF-II (Figure 4). IGFBP-1 mRNA and protein are highly expressed in decidua and at the decidua–trophoblast interface in human early pregnancy samples (Hustin *et al*., 1994). IGFBP-1 immunoreactivity has been localized to the extracellular matrix and stroma of decidua and the periarteriolar regions (Bryant-Greenwood *et al*., 1993). IGF-II mRNA is highly expressed by trophoblast, and there is a gradient of mRNA abundance, with the greatest concentrations expressed at the invading front. At conception, the proximity of invading trophoblast, producing active peptides, and decidua imply that growth factors and cytokines at this interface regulate decidual IGFBP-1 production. It is known that IGF-II and IL-1β are inhibitory for IGFBP-1, with IL-1β inhibiting decidualization, whereas other growth factors such as transforming growth factor-β (TGF-β), stem cell factor (SCF), colony stimulating factor-1 (CSF-1) and leukaemia inhibitory factor (LIF) have not been reported to affect IGFBP-1 (Mark *et al*., 1996).

The role and regulation of HCG in pregnancy remains relatively unclear in reproductive physiology. Experimental data on HCG/IGFBP-1 interactions are few, *in vitro*, and conflicting. Using an in-vitro model, it was found that HCG and progesterone did not stimulate the production of PP14, IGFBP-1 (PP12) or prolactin in decidualized endometrium (Ren and Bruanstein, 1990). However, another in-vitro model yielded contrasting results. Glycoprotein hormone α-subunit, secreted by the anterior pituitary throughout the menstrual cycle and by placenta, tends to act synergistically with progesterone to induce more rapid decidualization with higher output of prolactin and IGFBP-1 compared with progesterone alone (Moy *et al*., 1996). This implies that HCG α-subunit would have the same stimulatory effect. From other data it is known that IGFBP-1 is differentially expressed in secretory stroma in the progesterone-dominant phase of the menstrual cycle (Giudice *et al*., 1993; Zhou *et al*., 1994; Han *et al*., 1996). This supports the previous findings (Moy *et al*., 1996) which suggested that the stimulatory effects of HCG and progesterone lead to decidualization and increased IGFBP-1 production.

Similar processes operate in both normal and abnormal trophoblast invasion: attachment to extracellular matrix, local proteolysis, cell migration and varying degrees of inhibition by the tissue being invaded (Steler-Stevenson *et al*., 1993).

IGFBP-1 itself has been shown to have inhibitory effects on IGF binding and IGF-mediated mitogenesis in choriocarcinoma cells *in vitro* (Ritvos *et al*., 1989); a similar relationship may exist at the trophoblast–decidua interface. However, precise mechan-

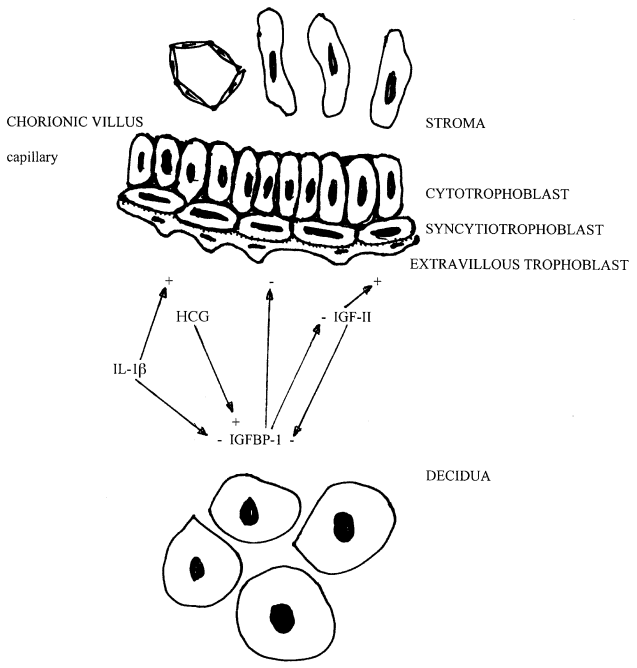

Figure 4. Schematic of pregnant endometrium, illustrating blastocyst-derived trophoblast/endometrial decidua interactions and the roles of human chorionic gonadotrophin (HCG), interleukin-1β (IL-1β), IGF-II and IGFBP-1. Decidually expressed IGFBP-1 and trophoblast-expressed IGF-II (Han *et al*., 1996) interact in a highly controlled way during implantation. −= inhibition; += stimulation.

isms are unclear, and there is much controversy as to whether in-vitro studies involving choriocarcinoma cell lines can be applied to physiological situations *in vivo*.

Additionally, IGFBP-1 may have IGF-independent actions mediated via its internal (Arg-Gly-Asp) integrin-binding sequence. Invading trophoblast has a unique phenotype as it expresses the $\alpha_5\beta_1$ integrin/fibronectin receptor which may be the binding site for the IGFBP-1 integrin-binding domain (Irving and Lala, 1995). IGFBP-1 binds specifically and inhibits fibronectin binding to this integrin in trophoblasts. This action would tend to suppress fibronectin inhibition of trophoblast cell migration, potentiating invasion (Jones *et al*., 1993a). However, in contrast, trophoblast invasion into decidual stromal cell multilayers appears to be suppressed by IGFBP-1. A possible explanation is that IGF-I and -II are potent stimulators of cell migration, and IGFBP-1 inhibits IGF binding to cell surface receptors, thus inhibiting trophoblast migration and invasion. *In vivo*, it is possible that IGFBP-1 inhibition of IGF-stimulated cell migration may predominate over IGFBP-1 blockade of fibronectin action, resulting in net suppression of trophoblast invasion (Lee *et al*., 1997).

A chance and interesting experimental observation is the selective inhibitory effect on IGFBP-1 and prolactin secretion in decidualized stromal cells plated on laminin, which can bind to integrins (Brar *et al*., 1995). Endometrial laminin is important during decidualization, and is thought to be essential for normal cell adhesion.

Elevated serum LH concentrations have been associated with increased risk for spontaneous miscarriage (Li *et al*., 1993). Premature ovulation or elevated concentrations of LH in the follicular phase may lead to premature decidualization. Whether elevated decidual IGFBP-1 production predisposes to poor implantation, implantation failure and miscarriage is not known.

IGFBP-1 and maternal/fetal physiology in normal and abnormal pregnancies

Interestingly, very high concentrations of IGFBP-1 are found in maternal and fetal fluids (Rutanen *et al*., 1982). Maternal serum IGFBP-1 concentrations are elevated 2-fold relative to those in non-pregnant serum (Wathen *et al*., 1993), this being several orders of magnitude lower than amniotic fluid. The patterns of maternal and amniotic fluid IGFBP-1 are parallel, increasing rapidly in the first trimester to a midgestational peak in the second and early third trimesters, with concentrations decreasing after about 33 weeks gestation (Wang *et al*., 1991; Rutanen *et al*., 1994a). Fetal serum concentrations appear to follow a similar pattern, but limited data are available in this respect (Reece *et al*., 1994). Maternal serum IGFBP-1 concentrations are higher in twins compared with singletons, similar between twin and other multiples, and decrease toward singleton pregnancy levels following embryo reduction (Abbas *et al*., 1995).

The origin of maternal serum IGFBP-1 is likely to be decidua. In the first trimester, the extra-embryonic coelomic fluid contains very high concentrations of IGFBP-1, whereas first-trimester amniotic fluid has low concentrations by comparison. The extra-embryonic coelom is lined by chorion and decidual cells. In the second trimester, the amnion and chorion fuse, obliterating the extra-embryonic coelom, and at this time amniotic fluid concentrations of IGFBP-1 increase by several orders of magnitude (Nonoshita *et al*., 1997).

The role of IGFBP-1 phosphorylation in regulating IGFBP-1 activity is controversial. Integrin-binding (Arg-Gly-Asp consensus sequence for cell attachment) and phosphorylation sites within IGFBP-1 have functional significance *in vitro*, but their physiological role *in vivo* has not been adequately defined. In-vitro studies in which serine phosphorylation sites are mutated result in lower affinity of IGFBP-1 for IGF-I (Jones *et al*., 1993b). There is a similar decrease in affinity for non-phosphorylated versus phosphorylated human amniotic fluid IGFBP-1 (Westwood *et al*., 1995). Despite limited knowledge about the physiological role of IGFBP-1 phosphorylation, the distribution of the phospho-isoforms are useful markers of sites of production. Non-pregnant serum IGFBP-1 circulates as a single, highly phosphorylated species of hepatic origin. In maternal serum, IGFBP-1 exists in this highly phosphorylated state and in partially and non-phosphorylated isoforms. The highly phosphorylated form is likely to be hepatically derived, and the other forms—present at lower concentrations—are likely to be of decidual origin. This is supported by the presence of the partially and non-phosphorylated forms in amniotic fluid and decidua, and an inability to detect highly phosphorylated forms in amniotic fluid (Westwood *et al*., 1994). Parallel gestational age-related changes in phosphoisoform patterns in serum, amniotic fluid and decidual explants further implicate decidua as the major contributor to amniotic fluid IGFBP-1 (Martina *et al*., 1997). Clinically, phosphorylated IGFBP-1 isoforms in cervical secretions of women with intact fetal membranes at term have been measured and found to reflect cervical ripeness (Nuutila *et al.*, 1999). These workers suggested that IGFBP-1 isoforms may predict amenability for labour induction.

Regulation of IGFBP-1 appears to be abnormal in diabetic pregnancy (IDDM), and maternal IGFBP-1 concentrations are higher in diabetic compared with normal pregnancies. In particular, diabetics with prolonged duration of diabetes with retinopathy had higher total IGFBP-1 concentrations than those with fewer complications. In addition, highly phosphorylated IGFBP-1 in diabetic pregnancy is positively related to duration of diabetes and inversely to fetal growth, less phoshorylated IGFBP-1 being related to maternal weight and BMI (Gibson *et al*., 1999).

Pre-eclampsia is a multisystem disorder which carries high maternal and fetal morbidity and mortality, both in the developed and the developing world. The secondary maternal clinical effects—hypertension, renal, liver and cerebral dysfunction and coagulopathy—may be controlled symptomatically, but the primary molecular pathology is still unclear. Fetal effects that are often associated with pre-eclampsia, such as intrauterine growth restriction, abnormal arterial flow and redistribution, may result from a primary placental problem, such as abnormal trophoblast invasion in the first trimester. This would result in abnormal remodelling of the endometrial vasculature with consequent flow, molecular transport and gas exchange problems, leading to placental insufficiency. There may be a variety of causes for abnormal trophoblast invasion, including abnormalities in trophoblast adhesion molecules (Zhou *et al*., 1993) and elevated decidual concentrations of IGFBP-1 which are known to inhibit invasion (Giudice *et al*., 1997). In severe pre-eclampsia, maternal serum IGFBP-1 concentrations in the second and early

third trimesters are 6-fold higher, and at term are 2-fold higher, compared with normal pregnancies (Wang *et al*., 1996). There is a correlation between maternal diastolic blood pressure and IGFBP-1, suggesting that IGFBP-1 reflects the severity of pre-eclampsia and liver involvement. The elevated serum IGFBP-1 concentrations probably derive from both decidua and liver. In pre-eclampsia, vascular permeability and vasospasm may account for increased release of IGFBP-1 from the periarteriolar areas of the decidua into the maternal circulation (Giudice *et al*., 1997).

The role of the IGF/IGFBP system in fetal growth

Birth weight, as a measure of fetal growth, is one of the most challenging human traits to study as there are multiple factors to consider. There is good evidence for familial clustering of birth weight, but external influences such as maternal smoking and nutrition may confound birth weight studies. However, genes which modify birth weight and birth size have been identified (Dunger *et al*., 1998; Hattersley *et al*., 1998), adding fuel to the nature–nurture debate.

Fetal and maternal IGF/IGFBP systems are involved in fetal growth, and their effects have been dissected using a combination of rodent gene knockout and human studies. Fetal blood sampling (18–30 weeks) has been used to establish normal values for IGF-1, IGF-II and IGFBP-1 to -3 in normal human fetal serum (Langford *et al*., 1998). Human trisomy 18 presents in the first trimester with reduced crown–rump length, implying early-onset growth restriction, whereas trisomy 21 is associated with postnatal growth problems. Increased first-trimester maternal serum IGFBP-1 occurs in pregnancies complicated by trisomy 18, which may imply IGFBP-1 inhibition of IGFs resulting in early-onset growth restriction (Miell *et al*., 1997).

In IGF-I and -II gene knockout mice, there is marked growth restriction (Baker *et al*., 1993). Human studies concur with this, showing a direct correlation between cord blood or fetal serum IGF-I concentration and fetal size or birth weight (Giudice *et al*., 1995).

Several workers have shown a marked inverse correlation between fetal size and maternal serum or fetal serum IGFBP-1 concentrations (Spencer *et al* 1995; Nonoshita *et al*., 1997). Transgenic rodent studies are useful because it is possible to induce over- or under-expression of human mRNAs and peptides, characterize the genotype, and observe phenotype behaviour *in vivo*. In transgenic mice overexpressing human IGFBP-1 (human IGFBP-1cDNA under control of the α-1 antitrypsin promotor to obtain liver-specific expression), there is growth retardation as studied by serial weight gain. Reproductive function also appeared to be severely affected, especially in homozygous females, and mating failed to result in pregnancy in half of the homozygous females mated with non-transgenic males, suggestive of impaired fertilization or implantation (Gay *et al*., 1997). These studies indicate that permanent and uncontrolled hepatic expression of IGFBP-1, even at low levels, affects fertility and both pre- and postnatal development. This is strong evidence for the role of IGFBP-1 in reproduction, from implantation to postnatal growth, and it may apply to humans as well as rodents.

Experimental differences have been reported in transgenic models, perhaps because of the different expression levels of human versus rat IGFBP-1 transgenes, or the use of different promotors leading to different patterns of tissue-specific IGFBP-1 expression. Generally however, IGFBP-1 overexpression is associated with evidence of impaired IGF action.

In human pregnancy, maternal serum IGFBP-1 concentrations at 20–24 weeks and 30–34 weeks gestation correlate inversely with fetal size as assessed by ultrasound measurements (Baldwin *et al*., 1993). Fetal serum obtained by cordocentesis at 26–27 weeks showed a greater than 2-fold increase in IGFBP-1 and a 4-fold reduction in IGF-I concentrations in fetuses with uteroplacental insufficiency compared with small and appropriate-for-gestational-age fetuses (Langford *et al*., 1993). Chronic hypoxia may be a regulator of fetal IGFBP-1 and therefore fetal growth. This hypothesis has been addressed (Tazuke *et al*., 1998) using HepG2 cells which expressed increased IGFBP-1 mRNA and protein under hypoxic conditions. A 372 bp fragment of IGFBP-1 intron 1, containing three potential consensus sequences for hypoxia response elements (HREs) was placed downstream to a promotor in a plasmid with a reporter gene. Reporter gene activity rose 30-fold with hypoxia. Mutations in the middle HRE abolished reporter activity in hypoxia. Co-transfection of HRE reporter genes with an hypoxia-inducible factor 1 plasmid in HepG2 cells increased reporter activity. These results support a role for hypoxia-inducible factor 1 and HREs in induction of IGFBP-1 gene expression in conditions of chronic hypoxia. These in-vitro experiments might help to explain the clinical finding of increased maternal and fetal serum IGFBP-1 in placental insufficiency and growth restriction, conditions of chronic hypoxia.

In human pregnancies resulting from superovulation, in-vitro fertilization and embryo transfer, third-trimester maternal serum IGFBP-1 was elevated and birth weights were lower compared with spontaneous conceptions (Johnson *et al*., 1995).

Overall data indicate an important role for IGF-I and -II in fetal growth, and for IGFBP-1 as an inhibitor or regulator of this growth. What stimulates elevated IGFBP-1 in the maternal and fetal circulations in pregnancies complicated by intrauterine growth restriction is not known. Elevated glucocorticoid concentrations associated with an element of fetal stress may be contributory in growth restriction due to placental insufficiency. In animal studies, IGFBP-1 tends to be highly expressed in early gestation, to increase during gestation, and then to decline postnatally. Developmental changes in IGFBP-1 transcription may be due to developmental changes in the expression of nuclear proteins which interact with the IGFBP-1 promotor. From immunohistochemical localization studies the primary source of serum IGFBP-1 in the fetal circulation is likely to be the liver, though IGFBP-1 mRNA has also been detected in human fetal kidney (Hill *et al*., 1993; Pannier *et al*., 1994).

Restricted fetal growth and overgrowth have important implications for fetal and neonatal morbidity and mortality. Fetal growth and development are complex, and involve a variety of mechanisms, most fundamentally involving environmental and genetic effects. Low birth weight is strongly associated with type 2 diabetes and related conditions, dyslipidaemia, hypertension and cardiovascular disease later in life.

Whether it is the fetal phenotype or genotype which predisposes to these problems is controversial. In the phenotype model, intrauterine starvation leads to small, thin babies and triggering of permanent remodelling of physiology. In the

genotype hypothesis, genes influencing insulin secretion and action influence both fetal growth and susceptibility to diabetes. In this phenotype–genotype debate, twin studies may not be helpful since monozygotic twins, as well as sharing genes, may also share the placenta and hence the same intrauterine environment. Common mutations in genes influencing insulin action might provide a genetic explanation accounting for the low birth weight data. This has been addressed (Dunger *et al*., 1998) by examining the relationship between variation at the variable number tandem repeat (VNTR) minisatellite to the insulin gene and birth weight. Interestingly, VNTR neighbours the IGF2 locus (which is maternally imprinted) on chromosome 11; overexpression of IGF-II results in Beckwith–Weidemann syndrome, which is characterized by macrosomia and an excess of tumours.

Thus, birth weight may depend on gene prevalence, polymorphisms, imprinting and relative phenotypic expression during pregnancy and fetal life.

Conclusions

IGFBP-1 to -6 are structurally related peptides which have been conserved in mammalian species, substantiating the evolutionarily important developmental roles for these binding proteins. IGFBP-1 was initially described as a carrier protein modulating the bioavailability of IGF-I and -II, but has other roles which are both dependent and independent of the IGFs. IGFBP-1 can act in a paracrine/autocrine manner, binding cell and possibly nuclear receptors. In particular, IGFBP-1 has been shown to play important roles in ovarian, endometrial, trophoblast and feto-placental physiology and pathology.

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