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Human endometrial proteins (1)

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Summary. Human endometrium synthesizes a number of proteins. Two of the major proteins are the low molecular weight insulin-like growth factor-binding protein (IGF-bp25) and endometrial protein PP14 (placental protein 14). Both proteins have been cloned from human decidual cDNA library and their complete amino acid sequences have been deduced. IGF-bp25 is found in various tissues and body fluids including secretory and decidualized endometrium, amniotic fluid, liver, follicular fluid and luteinized granulosa cells of preovulatory ovarian follicles. Clinical studies have shown that there is no systematic variation in the circulating levels during normal menstrual cycle, whereas in hyperstimulated cycles the levels are higher when there are many preovulatory follicles and immediately after follicle aspiration for in vitro fertilization (IVF). Some women with polycystic ovarian disease have a subnormal level of IGF-bp25 in serum. The other protein, PP14, is synthesized by secretory and decidualized endometrium, and it is also abundant in amniotic fluid. PP14 is mainly released by secretory endometrial glands during the last week of ovulatory cycles. There is a consistent variation in serum PP14 levels during normal menstrual cycle. The levels are lowest at the time of ovulation and rise steeply during the last week of luteal phase and peak at the onset of menstruation. Administration of micronized oral progesterone to normally ovulating infertile women brings about elevation in their serum PP14 levels during late luteal phase. In postmenopausal women cyclical estrogen-progestogen replacement causes elevation of serum PP14 level, but this does not take place in hysterectomized postmenopausal women. During ovarian hyperstimulation for IVF the circulating PP14 levels show a similar type of variation as in normal ovulatory cycles. Nadir is seen at the time of follicle aspiration. The higher the estrogen level is in the follicular phase, the higher is the subsequent serum PP14 level in late luteal phase indicating that estrogen priming is important for the subsequent protein secretion by the endometrium. It may become possible in future to assess endometrial function by a blood test based on the circulating PP14 concentration.

Proteins of the human endometrium.

A variety of proteins have been isolated from the human endometrium (Table 1). This paper will review our studies on two of them, namely the small molecular weight insulin-like growth factor-binding protein (IGF-bp25), previously

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known as PP12 (placental protein 12), and endometrial protein PP14 (placental protein 14). Both are abundant in secretory/decidulized endometrium in which their synthesis has been demonstrated (Rutanen *et al.*, 1986 ; Julkunen *et al.*, 1986a).

TABLE 1

Human endometrial proteins (after Seppälä *et al.*, 1987a).

Name	Reference
17β-hydroxysteroid dehydrogenase	Tseng and Gurpide, 1979
Placental protein 12*	Rutanen *et al.*, 1986
Endometrial protein 14*	Bell, 1986
Alpha-1 pregnancy-associated endometrial globulin*	Bell, 1986
Insulin-like growth factor-binding protein*	Koistinen *et al.*, 1986
Placental protein 14**	Julkunen *et al.*, 1986
Progestogen-dependent endometrial protein**	Joshi *et al.*, 1980
Endometrial protein 15**	Bell, 1986
Alpha-2 pregnancy-associated endometrial globulin**	Bell, 1986
Pregnancy-associated plasma protein-A	Sjöberg *et al.*, 1984
Placental protein 5	Bützow *et al.*, 1986
Endometrial proteins 1-17	Bell, 1986

* the names are likely to be synonyms ;
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Insulin-like growth factor-binding protein.

Insulin-like growth factors IGF-I and IGF-II are bound to specific binding proteins in serum. The major IGF-bp in blood has a molecular weight of about 150 kD (Baxter *et al.*, 1986) and it is growth hormone-dependent. The smaller IGF-bp has a molecular weight of 25.3 kD as deduced from its complete cDNA sequence (Julkunen *et al.*, 1988a). IGF-bp25 is not growth hormone dependent, and its levels in serum are inversely correlated to those of serum insulin (Suikkari *et al.*, 1988a). Tissues in which synthesis of IGF-bp25 takes place include secretory endometrium (Rutanen *et al.*, 1986) and the liver (Julkunen *et al.*, 1988a). Preovulatory follicles contain high concentration of IGF-bp25 and, within the follicle, the protein is localized to luteinized granulosa cells (Seppälä *et al.*, 1984), which may actually synthesize this protein.

Clinical conditions in which the circulating IGF-bp25 levels are elevated include pregnancy and trophoblastic disease (Rutanen *et al.*, 1982), primary liver cancer (Rutanen *et al.*, 1984), ovarian cancer (Iino *et al.*, 1986a) and diabetes (Suikkari *et al.*, 1988a). Subnormal levels have been observed in patients with insulinoma (Suikkari *et al.*, 1988a) and polycystic ovarian disease (Suikkari *et al.*, 1988b). During normal menstrual cycle the circulating IGF-bp25 levels show no systematic variation (Suikkari *et al.*, 1987), whereas during ovarian hyperstimulation for IVF the levels rise as the follicles grow, and decline after follicle aspiration (Seppälä *et al.*, 1988a). It is obvious that this elevation cannot derive from secretory endometrium, whereas multiple ovarian follicles are a more likely source. On the other hand, during pregnancy the high IGF-bp25 levels in amniotic fluid and serum probably derive from decidualized endometrium (Rutanen *et al.*, 1985). In preeclampsia the levels are higher than in normal pregnancy indicating increased decidual secretion (Iino *et al.*, 1986b).

Endometrial protein PP14.

PP14 in synthesized by secretory and decidualized endometrium as evidenced by experiments on incorporation of labeled methionine to immunoreactive PP14 by endometrial explants in tissue culture (Julkunen *et al.*, 1986b), and by the detection of PP14 mRNA in the endometrium (Julkunen *et al.*, 1988b). In SDS gel electrophoresis the molecular weight of PP14 is 28 kD, and the protein contains 17 % carbohydrate (Bohn *et al.*, 1982). There is amino acid sequence homology between PP14 and β-lactoglobulins of various species (Huhtala *et al.*, 1987 ; Julkunen *et al.*, 1988b) and between PP14 and a family of retinol-binding proteins (Seppälä *et al.*, 1988b).

PP14 may be used as a marker for endometrial differentiation, as it is synthesized by well differentiated secretory glands, not so much by the stroma. The serum PP14 concentration has the potential to become a marker of endometrial function. PP14 appears to be progesterone-regulated (Julkunen *et al.*, 1986b,c). Evidence that a considerable proportion of circulating PP14 is uterus-derived comes from our studies in which PP14 mRNA was found in the secretory endometrium but not in other tissues (Julkunen *et al.*, 1988b), and the presence or absence of the uterus was found to be critical for the serum PP14 levels to become elevated in response to estrogen-progestogen replacement in postmenopausal women (Seppälä *et al.*, 1988b). We have observed that, after a similar priming with estradiol valerate, the levels during estrogen plus a conventional dose (0.25 mg) of levonorgestrel are higher than those during the same estrogen plus 10 mg medroxyprogesterone acetate (Seppälä *et al.*, 1988b). Also the mode of drug delivery appears to be important. Sustained administration of danazol on patients with endometriosis suppresses rather than elevates the serum PP14 levels (Than *et al.*, 1987). These results indicate that the measurement of endometrial secretory products in blood may tell about endometrial responses to exogenous hormones. Cyclically elevated progestogen levels appear to be critical for subsequent elevation of serum PP14 levels in late luteal phase. During the last week of luteal phase the serum PP14 concentration doubles in

every three days (Julkunen *et al.*, 1986a ; Seppälä *et al.*, 1988a). No similar elevation is seen in anovulatory cycles. Infertile ovulatory women given micronized oral progesterone have higher luteal phase PP14 levels than the same women taking placebo indicating that it is possible to increase the endometrial secretory activity by exogenous progesterone (Seppälä *et al.*, 1987b). Whether this has bearance to subsequent implantation is an obvious question that remains to be answered. Endometrial secretory activity is also influenced by the magnitude of estrogen priming in the follicular phase, irrespective of the subsequent luteal phase progesterone levels. This was evidenced by our finding (to be published) of a positive correlation between follicular phase estradiol and luteal phase PP14 levels.

During pregnancy the circulating PP14 levels show a similar pattern as those of chorionic gonadotropin (hCG). Very high PP14 levels have been found in amniotic fluid, whereas the hCG level is low (Julkunen *et al.*, 1985). It has been suggested that PP14 has an immunosuppressive effect in human reproduction (Bolton *et al.*, 1987).

These and the various other ongoing clinical studies indicate that we are learning about new biochemical ways to assess endometrial responses to endocrine stimuli.

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Résumé. *Les protéines de l'endomètre humain.*

L'endomètre humain sécrète de nombreuses protéines. Deux des protéines majeures sont la protéine légère de liaison des facteurs de croissance, « insulin-like » (IGF-bp25) et la protéine endométriale PP14. Ces deux protéines ont été clonées à partir d'une librairie de cDNA décidual et leur séquence complète a été déduite. L'IGF-bp25 se trouve dans des tissus divers, comprenant l'endomètre sécrétoire ou décidualisé, le foie, les cellules lutéinisées de granulosa provenant de follicules préovulatoires. On la trouve également dans des liquides biologiques tels le liquide amniotique ou le liquide folliculaire.

Des études cliniques il ressort qu'il n'y a pas de variations systématiques dans les niveaux circulants pendant le cycle menstruel normal, tandis qu'après stimulation folliculaire les niveaux sont plus élevés quand il y a beaucoup de follicules préovulatoires et même après aspiration des follicules en vue de pratiquer la FIV. Quelques femmes souffrant d'ovaires polykystiques ont des niveaux sériques subnormaux de IGF-bp25. L'autre protéine PP14 est synthétisée par l'endomètre sécrétoire ou décidualisé et est également présente en abondance dans le liquide amniotique. La PP14 est principalement libérée par les glandes endométriales en phase sécrétoire pendant la dernière semaine des cycles ovulatoires. Il existe une variation nette des niveaux sériques de PP14 pendant le cycle menstruel ; les niveaux sont les plus bas au moment de l'ovulation et s'élèvent fortement pendant la dernière semaine du cycle, le pic se situant au moment de la menstruation. L'administration orale de progestérone micronisée à des femmes stériles bien qu'ovulant, augmente leur taux sérique en fin de phase lutéale. Chez la femme postménopausée une thérapie œstro-progestative de substitution provoque une élévation de la PP14 sérique, mais cette élévation n'a pas lieu si la femme est hystérectomisée. Pendant l'hyperstimulation ovarienne en cas de FIV, la PP14 sérique varie comme au cours d'un cycle normal. Le minimum est observé au moment de l'aspiration des follicules. Plus est élevé le niveau d'estradiol pendant la phase folliculaire, plus le niveau de PP14 est élevé en fin de phase lutéale ; ceci montre l'importance de l'action protéique ultérieure de l'endomètre. Il semble possible dans le futur d'estimer le fonctionnement de l'endomètre par la connaissance du niveau sérique de la PP14.

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