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Circulating placental protein 14: in the first trimester of spontaneous and IVF pregnancies

M.R.Johnson[1], A.Abbas[4], J.Q.Norman-Taylor[2], A.F.Riddle[4], J.G.Grudzinskas[2], T.Chard[3] and K.H.Nicolaides[4]

Neuroendocrinology Unit, Charing Cross and Westminster Medical School, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, [2]The Department of Obstetrics and Gynaecology, The Royal London Hospital, Whitechapel, London E1 1BB, [3]Department of Reproductive Physiology, St Bartholomew's Hospital Medical School, London EC1A 7BE and [4]Harris Birthright Centre for Fetal Medicine, King's College School of Medicine and Dentistry, Denmark Hill, London SE5 8RX, UK

[1]To whom correspondence should be addressed

Circulating placental protein 14 (PP14) levels were measured during the first trimester in three groups of pregnant women: (i) natural conception (n = 15); (ii) pituitary desensitization with buserelin and ovarian stimulation with human menopausal gonadotrophin (HMG) followed by in-vitro fertilization and embryo transfer (IVF–ET) (n = 15); and (iii) ovarian stimulation with clomiphene citrate and HMG, followed by IVF–ET (n = 16). A 7- to 8-fold increase in serum PP14 levels was observed in normal pregnancies between weeks 4 and 10. This increase was earlier and less marked in group (ii) and absent in group (iii). These findings support the concept that endometrial function is altered in pregnancies achieved following ovarian stimulation. Alternatively, if the ovary is an important source of PP14, then these data suggest that in contrast to ovarian synthesis of steroids and the peptide relaxin, ovarian stimulation results in an impairment of PP14 synthesis, and that this is most marked when clomiphene citrate has been used.

Key words: buserelin/clomiphene citrate/Nf-ET/placental protein 14/pregnancy

Introduction

Ovarian stimulation may impair endometrial growth and function (Paulson *et al.*, 1990; Bonoff *et al.*, 1990). Some ultrasound and histological studies report reduced thickness of the endometrium (Bonoff *et al.*, 1990; Rogers *et al.*, 1991) or delayed maturation (Khan *et al.*, 1988; Bonoff *et al.*, 1990); others find no difference from controls (Imoedemhe *et al.*, 1987; Thatcher *et al.*, 1988; Randall *et al.*, 1989). Some of the positive findings may be due to the selection of patients whose oocytes fail to fertilize (Khan *et al.*, 1988; Bonoff *et al.*, 1990). Following ovarian stimulation with clomiphene citrate, compared with human menopausal gonadotrophin (HMG) alone or in combination with buserelin, endometrial thickness is reduced and maturation impaired (Rogers *et al.*, 1991; Paulson *et al.*, 1990; Randall and Templeton, 1991; Eden *et al.*, 1989; Martel *et al.*, 1987; Birkenfeld *et al.*, 1986). These effects may be a result of a direct anti-oestrogenic effect of the drug on endometrial growth (Fritz *et al.*, 1987; Molina *et al.*, 1989) or on the expression of oestrogen and progesterone receptors (Aksel *et al.*, 1986). Alternatively, they could be due to prematurely elevated levels of progesterone (Silverberg *et al.*, 1991) or high levels of oestradiol (Forman *et al.*, 1988); both of these features might impair endometrial function.

During the first trimester, endometrium–conceptus interactions determine the outcome of implantation and placentation. In order to assess endometrial function in pregnancies occurring after different methods of ovarian stimulation, we have compared the circulating levels of placental protein 14 (PP14) in pregnancies that occurred spontaneously or after ovarian stimulation, with human menopausal gonadotrophin (HMG) in combination with clomiphene citrate (Clomid, Merrell Dow) or following pituitary desensitization with buserelin (Suprefact, Hoechst), and in-vitro fertilization and embryo transfer (IVF–ET).

Although initially isolated from the placenta, circulating PP14 levels are thought to be derived solely from the glandular endometrium (Olajide and Chard, 1992). PP14 is a glycoprotein with a molecular weight of 42 000 (Bohn *et al.*, 1982) and showing structural homology with β-lactoglobulin (Huhtala *et al.*, 1987). It may have a role in the transport of small hydrophobic molecules or in the modulation of the maternal immune response to the trophoblast (Bolton *et al.*, 1988). It has been suggested that serum levels reflect endometrial function (Fay *et al.*, 1990).

Materials and methods

Blood samples were obtained at weekly intervals during the first trimester in three groups of pregnant women: (i) natural conception, age range 26–37 years, median 31 years (n = 15, serum, collected in 1991); (ii) pituitary desensitization with buserelin and ovarian stimulation with HMG followed by IVF–ET (age range 26–41 years, median 34 years, n = 15, plasma, collected in 1991); and (iii) ovarian stimulation with clomiphene citrate and HMG, followed by IVF–ET (age range 28–38 years, median 33 years, n = 16, serum, collected in 1988). The methods used for ovarian stimulation with clomiphene citrate and buserelin have been described elsewhere (Sharma *et al.*, 1988).

Blood samples were separated within 2 h of collection. Serum and plasma were stored at −20°C until assayed for PP14 as previously reported (Howell *et al.*, 1989). Levels in serum and plasma were identical (Chapman, 1990). The data were not

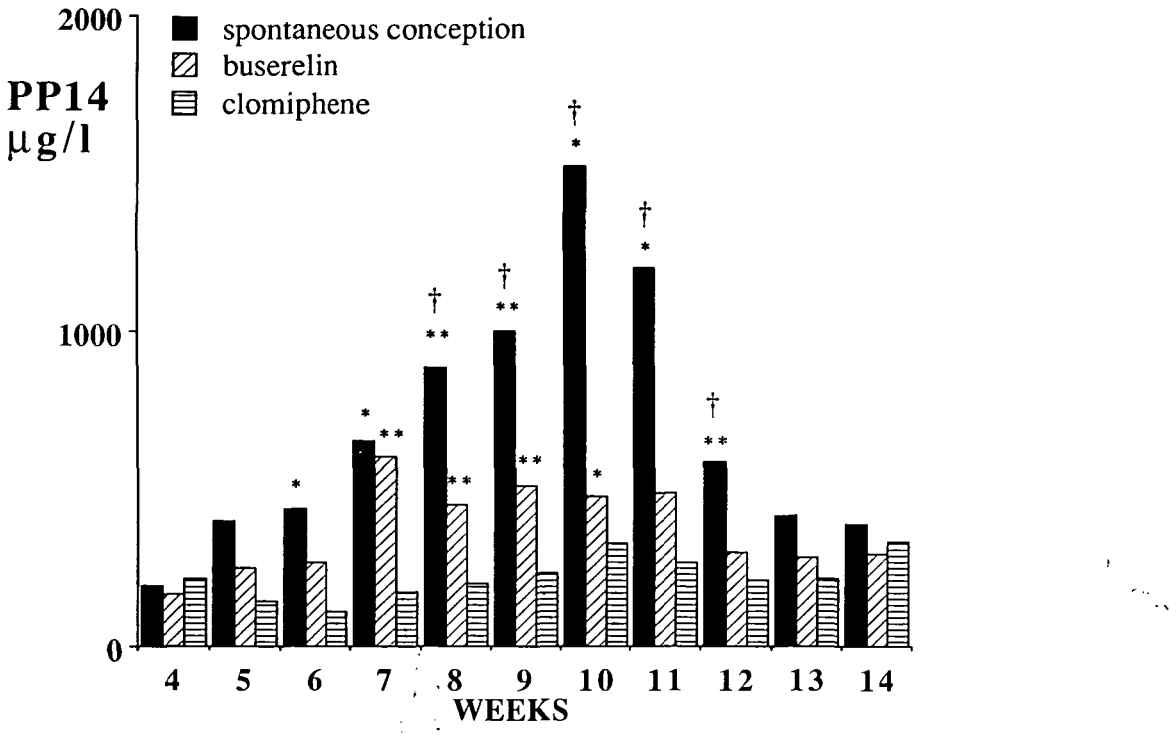

Fig. 1. Circulating levels of placental protein 14 (PP14) (geometric mean) during the first trimester of natural pregnancies, and pregnancies which occurred after superovulation, with human menopausal gonadotrophin and clomiphene citrate or following pituitary desensitization with buserelin acetate. Statistically significant differences between PP14 levels in clomiphene citrate pregnancies and those in spontaneous and buserelin pregnancies: $*P < 0.05$, $**P < 0.01$. Statistically significant differences between PP14 levels in spontaneous and buserelin pregnancies: $\dagger P < 0.05$.

normally distributed and were described by the geometric mean. Comparisons were made using the Mann–Whitney *U* test.

Results

As expected serum PP14 levels in normal pregnancies rose from 4 weeks to a peak at 10 weeks and then fell (Figure 1, Table I). In buserelin pregnancies (group ii), PP14 levels rose until 7 weeks and then showed no change until a slight fall at week 11. In clomiphene pregnancies (group iii), there was no increase in PP14 (Figure 1). In normal pregnancies, the increments of PP14 were statistically significant ($P < 0.05$) between weeks 6 and 7, 8, 9, and 10, while in the buserelin pregnancies, the increments were statistically significant between weeks 4 and 5, 6, 7, and 9. None of the increments in the clomiphene pregnancies were statistically significant.

PP14 levels were significantly lower than normal in buserelin pregnancies (8–12 weeks, $P < 0.05$), and in the clomiphene pregnancies (6–12 weeks); PP14 levels were significantly higher in the buserelin pregnancies than in the clomiphene pregnancies (7–11 weeks, $P < 0.002$–0.05) (Figure 1).

Table I. Geometric mean, range and numbers of subjects at each time point of serum placental protein 14 (PP14) levels in pregnancies following spontaneous ovulation or ovarian stimulation with either buserelin or clomiphene citrate

Weeks	Normal (µg/ml)			Clomiphene (µg/l)			Buserelin (µg/l)		
	Mean	*n*	Range	Mean	*n*	Range	Mean	*n*	Range
4	193	3	32–700	216	4	104–440	163	11	80–520
5	399	5	132–2000	144	5	46–330	246	10	170–520
6	435	11	115–2000	113	8	18–460	267	7	170–700
7	652	10	220–2200	173	7	36–540	604	10	350–1050
8	886	10	286–3100	197	8	115–360	448	9	280–900
9	998	8	407–2800	233	8	115–580	511	8	270–1050
10	1517	8	341–3100	326	8	90–4700	473	7	300–825
11	1200	6	480–3100	265	6	90–4400	486	7	350–1050
12	588	7	280–1000	211	6	90–470	300	6	170–675
13	414	4	290–700	217	4	80–1000	280	7	115–520
14	383	5	260–760	331	2	290–380	292	5	160–540

Discussion

Abnormalities in the processes of implantation or placentation may lead to miscarriage, intra-uterine growth retardation, pre-eclampsia or premature delivery. Structural assessment of the endometrium, its maturation, thickness and echogenicity have been related to the success of implantation (Glissant *et al.*, 1985; Gonen *et al.*, 1989; Gonen and Casper 1990a). Some but not all histological and ultrasound studies show that the process of ovarian stimulation can impair endometrial growth (Paulson *et al.*, 1990; Bonoff *et al.*, 1990); the resulting endometrial dysfunction may contribute to the low rates of pregnancy following ovarian stimulation and IVF–ET. As all of the pregnancies studied here proceeded uneventfully to term, the major differences in the circulating levels of PP14 between the different groups raise the question of whether circulating PP14

reflects endometrial function. Either it does and the defect suggested by this study is irrelevant, or PP14 is derived from another source, such as the ovary (Seppälä *et al.*, 1985; Critchley *et al.*, 1990).

All current literature suggests that PP14 is derived from the endometrium (Olajide and Chard, 1992). Thus, the present study may explain the finding of lower rates of implantation in clomiphene-HMG treated cycles than in gonadotrophin releasing hormone agonist (GnRHa)–HMG treated cycles, despite no difference in embryo quality (Lejeune *et al.*, 1990). This would also explain the impaired endometrial growth in clomiphene–HMG stimulated cycles when compared with HMG stimulated cycles (Gonen and Casper, 1990b). Impaired endometrial growth in women undergoing ovarian stimulation with buserelin and HMG may simply be the result of elevated oestradiol levels (Forman *et al.*, 1988). However, the greater impairment in women treated with clomiphene (Lejeune *et al.*, 1990), together with the lower levels of PP14 found in those women in the present study, suggest that another mechanism is involved. For example, the anti-oestrogen effect of clomiphene inhibits endometrial proliferation in the follicular phase (Lenz and Lindenberg, 1990) and leads to reduced expression of endometrial oestradiol and progesterone receptors in the luteal phase (Aksel *et al.*, 1986). Another possibility is an indirect effect mediated by the elevation in the circulating levels of luteinizing hormone (Archer *et al.*, 1989). Clomiphene might also impair embryonic development (Seibel and Smith, 1989). If endometrial function is important in placentation as well as implantation, then these results may reflect part of the process which leads to an increased incidence of low birth weight and pre-term delivery in pregnancies achieved following ovarian stimulation and IVF–ET (MRC report, 1990).

It was originally proposed that PP14 synthesis was induced by progesterone. However, in-vitro studies show that neither progesterone nor human chorionic gonadotrophin induce PP14 synthesis (Ren and Braunstein, 1990). Furthermore, patients who become pregnant following ovum donation have reduced levels of serum PP14, despite receiving adequate progesterone replacement (Johnson *et al.*, 1993), and no fall occurs in PP14 levels after the progesterone antagonist mifepristone (Howell *et al.*, 1989). In cases reported earlier (Critchley *et al.*, 1990, 1992), serum PP14 levels were reduced in pregnancies achieved following replacement with exogenous oestradiol and progesterone and transfer of cryopreserved embryos. The present findings together with the above suggest that a functional corpus luteum is essential for the increase in circulating PP14 levels (Anthony *et al.*, 1991). Indeed it is possible that the corpus luteum produces PP14, as it has been isolated from follicular fluid (Seppälä *et al.*, 1985). Ovarian stimulation leads to multiple functioning corpora lutea and elevated PP14 in the late luteal phase (Seppälä *et al.*, 1989), but levels of PP14 are reduced in the first trimester. Thus, it is unlikely that the corpus luteum is the source of PP14 during pregnancy, or indeed that PP14 synthesis is regulated directly by other products of the corpus luteum such as relaxin. Instead, the synthesis and release of PP14 by the endometrium is the result of a complex interaction between the endometrium and the corpus luteum. The association between low follicular phase oestradiol levels and low luteal phase PP14 levels (Seppälä *et al.*, 1989), which suggests that endometrial PP14 synthesis in the luteal phase may be regulated by oestrogen in the follicular phase, might also explain why clomiphene pregnancies have depressed levels of PP14 throughout the first trimester.

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