Human Reproduction vol.8 no.11 pp.1942–1947, 1993

Reduced circulating placental protein concentrations during the first trimester are associated with preterm labour and low birth weight

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Serum concentrations of human chorionic gonadotrophin (HCG), Schwangerschaftsprotein 1 (SP-1), pregnancy-associated plasma protein A (PAPP-A), progesterone and oestradiol were measured at weekly intervals between the fifth (embryo transfer plus 3 weeks) and 13th week of gestation during the first trimester of pregnancies achieved following in-vitro fertilization (IVF) and embryo transfer in a group of women who delivered before (n = 8) or at term (n = 52). Those women who had a preterm delivery had significantly lower concentrations of PAPP-A (weeks 7–13; P = 0.0001–0.028) and SP-1 (weeks 6–8 and 10–12; P = 0.004–0.04). After correction of birth weight for sex and gestational age at delivery, preterm delivery was found not to be associated with growth retardation. However, comparison of the circulating concentrations of the substances analysed in mothers who delivered babies of <85% of the 50th centile of the normal range of birth weight for a given gestational age and sex, with those who delivered babies of >85% revealed that the concentrations of HCG (P = 0.012–0.04 on weeks 6–9) and SP-1 (P = 0.003–0.03 on weeks 7, 9–13) were significantly lower in the former group. Weak, inconsistent associations were found between the circulating concentrations of HCG, SP-1 and PAPP-A and both corrected birth weight and gestational age at delivery. Thus, both the gestational age at delivery and low birth weight may be related to impaired placental development/function during the first trimester.

Key words: birth weight/placental proteins/preterm labour

Introduction

The factors which determine the time of onset of labour, whether at term or before, are uncertain. Pivotal roles have been ascribed to prostaglandin production, oxytocin receptor expression and altered ratios of the circulating concentrations of oestradiol to progesterone. Each has been suggested to be regulated by maternal, fetal or placental factors. Circulating concentrations of placental hormones have been shown to have no relationship (Stabile *et al.*, 1988), or only a weak relationship with gestational age at delivery (Westergaard *et al.*, 1984a; Hercz *et al.*, 1987). However, as these studies have been carried out during the third trimester of pregnancy, they may have failed to detect any abnormality in placental development or function which later determines the time of the onset of labour. Indeed, elevated concentrations of corticotrophin releasing factor (CRF), which are derived predominantly from the placenta during pregnancy, have been reported to precede the onset of premature labour by up to 11 weeks (Kurki *et al.*, 1991). In addition, further support for a placental role in the determination of the time of onset of labour is given by the reported association between growth retardation and preterm labour (White *et al.*, 1986).

Although weak relationships between birth weight and Schwangerschaftsprotein 1 (SP-1) and human placental lactogen (HPL) concentrations in the last trimester of pregnancy have been reported (Gordon *et al.*, 1977; Biswas *et al.*, 1980), endocrine parameters have not proved to be reliable predictors of birth weight (Obiekwe *et al.*, 1980; Westergaard *et al.*, 1984b; Stabile *et al.*, 1988). Most of these studies were carried out in the third trimester of pregnancy (Gordon *et al.*, 1977; Biswas *et al.*, 1980; Obiekwe *et al.*, 1980; Westergaard *et al.*, 1984b), and would not have detected any abnormality in placental development which influences birth weight later.

Trophoblast volume has been related to human chorionic gonadotrophin (HCG) concentration in early pregnancy (Merchiers *et al.*, 1991). Thus, measurement of the circulating concentration of HCG, and of the other trophoblast-derived proteins, SP-1 and pregnancy-associated plasma protein A (PAPP-A), may give an indication of placental development. This study investigates the hypothesis that impaired placental development during the first trimester is associated with preterm labour and reduced birth weight.

Materials and methods

Maternal serum concentrations of HCG, SP-1, PAPP-A, progesterone and oestradiol were measured at weekly intervals between the fifth (embryo transfer plus 3 weeks) and 13th week of gestation in 62 women with singleton pregnancies achieved following in-vitro fertilization (IVF) and embryo transfer. Comparisons were made between the serum concentrations of these substances in those women who delivered before term (range 33–36, median 35 weeks, n = 8) and those who delivered at term (n = 54). The protocol was approved by the Research Ethics Committee of King's College Hospital.

Ovulation induction was achieved with clomiphene citrate

(Clomid; Merrel Dow Pharmaceuticals Ltd, Uxbridge, Middlesex, UK; 100 mg orally) on days 2–6 of their menstrual cycle, with either human menopausal gonadotrophin (HMG; Pergonal, 75 IU follicle stimulating hormone (FSH) and 75 IU luteinizing hormone (LH) per ampoule; Serono Laboratories, Welwyn Garden City, Herts, UK), or purified FSH (Metrodin, 75 IU FSH and 1 IU of LH; Serono) as described by Sharma *et al.* (1988). Two days after oocyte retrieval, one to four cleavage stage embryos were transferred to the uterus. Subjects received either no luteal phase support (n = 18) or HCG (2000 IU i.m. on the day of embryo transfer and 3 days later) (n = 36), or progesterone (Cyclogest vaginal pessaries, progesterone 200 mg; Hoechst UK Ltd, Hounslow, Middlesex, UK; 400 mg/twice daily, for 8 weeks) (n = 8).

Immunoassays

Blood was collected into plain tubes and the serum separated and stored at −20°C within 2 h. Serum progesterone and oestradiol were extracted with diethyl ether and measured by radioimmunoassay using tritiated antigens and monoclonal antibodies to progesterone: 11 α-succinyl-bovine serum albumin (BSA) and oestradiol: 6-carboxymethyl oxime-BSA, respectively. The samples were diluted to check for parallelism against the dose-response curve and analysed in batches with appropriate quality control. The precision (intra- and inter-assay) for both methods over the period of the study was <10%.

HCG was measured by a non-competitive fluoroimmunoassay (Pharmacia Wallac, Milton Keynes, UK); SP-1 and PAPP-A were analysed by radioimmunoassay (Grudzinskas *et al.*, 1977; Sinosich *et al.*, 1982).

Statistical analysis

The data for each substance analysed and stage of gestation were log-normally distributed. Consequently, the concentrations were expressed as geometric means. Differences between the serum concentrations in the groups of women delivering before and at term were assessed using an ANOVA of the log transformed data. Further comparisons were made after the exclusion of those women in whom ovulation induction had been achieved with clomiphene citrate and FSH.

Birth weights were corrected for gestational age and sex by calculating the ratio of the 50th centile of the normal range of birth weight for a given gestational age and sex (Altman and Coles, 1980) to actual birth weight (birth weight ratio). Comparisons were made between the circulating concentrations of the substances analysed in women with babies with a ratio of more and less than 0.85. The presence of associations between the circulating concentrations and gestational age at delivery and corrected birth weight were investigated using a simple regression analysis.

Results

Eight patients delivered before 37 weeks, in seven of whom ovulation induction had been achieved with clomiphene citrate and HMG, and in one of whom ovulation induction had been achieved with clomiphene citrate and FSH. The serum concentrations of PAPP-A (weeks 7–13; P = 0.0001–0.028) and SP-1 (weeks 6–8 and 10–12; P = 0.004–0.04) were significantly lower in the group of women who delivered before term (Figure 1 and Table I). After the exclusion of women in whom ovulation and induction had been achieved with clomiphene citrate and FSH (n = 18), the concentrations of PAPP-A (weeks 7–13, P = 0.0001–0.019) and SP-1 (weeks 6 and 7; P = 0.022 and 0.029 respectively) were significantly lower in the group of women who delivered before term. Weak inconsistent associations were found between gestational age at delivery and the circulating concentrations of SP-1 (weeks 5, 6, 10–13, r = 0.3–0.5, P = 0.003–0.04), HCG (weeks 5–7, r = 0.25–0.3, P = 0.02–0.049) and PAPP-A (weeks 5–7, r = 0.3–0.4, P = 0.001–0.02).

Data regarding placental mass were not available. None of the women who delivered before term had pregnancy-induced hypertension, uterine malformation or premature rupture of membranes. The range of the birth weight ratio of the babies that were delivered before term was between 0.59 and 1.15, median 0.94.

Using the 10th centile (i.e. a rate of 10% false positive) of the normal range for HCG, SP-1 and PAPP-A to identify those pregnancies at risk from preterm delivery, the following percentages of cases would be detected during the first trimester: HCG between 13 (week 5) and 50% (week 13), mean 32%; SP-1 between 0 (week 5) and 63% (week 7), mean 48%; and PAPP-A between 13 (week 5) and 60% (week 12), mean 42%.

The circulating concentrations of HCG (P = 0.012–0.04 on weeks 6–9) (Figure 2a) and of SP-1 (P = 0.003–0.03 on weeks 7, 9–13) (Figure 2b) were significantly greater in women with babies with a birth weight ratio >0.85 than in those with a ratio of <85. There were no consistent differences in the circulating concentrations of PAPP-A, progesterone or oestradiol (data not shown). The mean gestational ages of the two groups were 38.8 weeks (SD = 1.88) for those >0.85 and 38.6 weeks (SD = 2.4) for those <0.85. Weak inconsistent associations were found between corrected birth weight and the circulating concentrations of SP-1 (weeks 7–11, r = 0.31–0.39, P = 0.007–0.03), HCG (week 8, r = 0.29, P = 0.04) and PAPP-A (weeks 8 and 9, r = 0.28 and 0.37, and P = 0.04 and 0.012 respectively).

Discussion

The findings of this study suggest that impaired placental development or function in the first trimester of pregnancy after IVF and embryo transfer may be associated with preterm delivery and low birth weight. However, in the case of preterm labour, only the concentrations of PAPP-A and SP-1 were reduced, and those of the former more than the latter, while those of HCG were not significantly lower. In contrast, in the case of low birth weight, the concentrations of SP-1 and HCG only were reduced. In normal pregnancies, the concentrations of all three placental proteins, PAPP-A, SP-1 and HCG, are associated (Johnson *et al.*, 1993a), but in pathological pregnancies, such as those destined to miscarry or with Down syndrome, PAPP-A concentrations are differentially reduced (Brambati *et al.*, 1993;

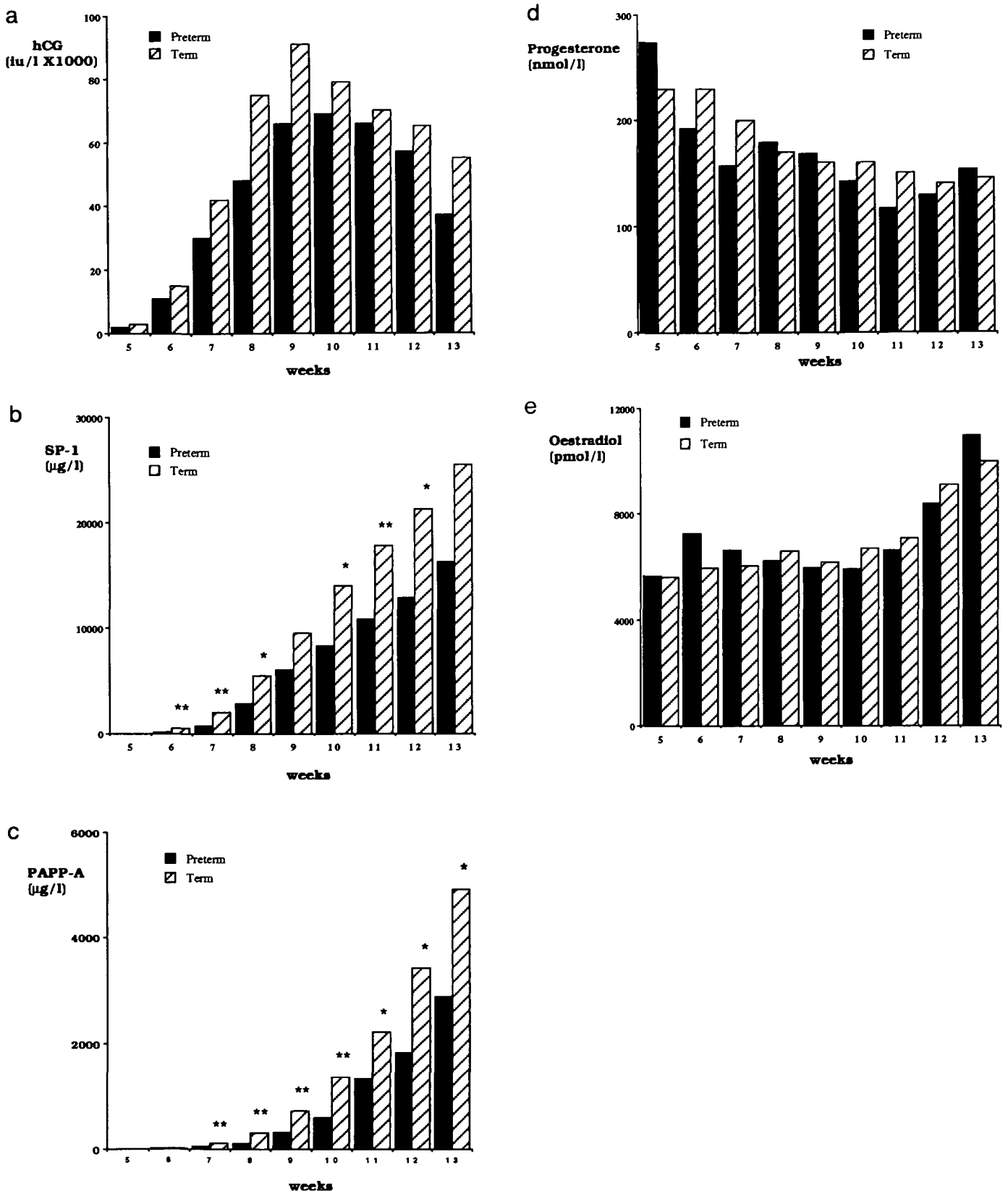

Fig. 1. The geometric mean of the circulating concentrations of human chorionic gonadotrophin (HCG; **a**), Schwangerschaftsprotein 1 (SP-1; **b**), pregnancy-associated placental protein A (PAPP-A; **c**), progesterone (**d**) and oestradiol (**e**) in patients with singleton pregnancies who became pregnant following ovulation induction and subsequent in-vitro fertilization and embryo transfer, and who delivered either before (<37 weeks, $n = 8$) or at term (>37 weeks, $n = 54$). *Denotes a difference of $P < 0.05$ and ** a difference of $P < 0.01$ in the circulating concentrations.

Table I. Geometric means (range, *n*) of serum levels of HCG, SP-1, PAPP-A, progesterone and oestradiol between weeks 5 and 13 in pregnancies destined to deliver at term or before term

Gestational weeks	HCG (iu × 1000/l)		SP-1 (μg/l)		PAPP-A (μg/l)		Progesterone (nmol/l)		Oestradiol (pmol/l)	
	Term	Preterm	Term	Preterm	Term	Preterm	Term	Preterm	Term	Preterm
5	2.8 (0.1–29) (54)	1.8 (0.3–8) (6)	39 (1–1120) (48)	17 (4–55) (6)	6.5 (1–71) (50)	6.4 (2–17) (6)	230 (42–598) (49)	274 (132–373) (6)	5602 (1.1–24 × 10^3) (49)	5648 (3.2–13 × 10^3) (6)
6	15 (2–76) (54)	11 (4.1–44) (8)	484 (42–4800) (54)	171 (26–600) (8)	23 (2–280) (54)	12.6 (8–35) (8)	226 (38–815) (54)	192 (146–289) (8)	5939 (0.9–24 × 10^3) (54)	7255 (3.5–15 × 10^3) (8)
7	43 (9–132) (53)	30 (12–81) (8)	2057 (240–8900) (51)	746 (190–4800) (8)	110 (19–620) (52)	41 (8–144) (8)	204 (32–820) (50)	157 (106–272) (8)	6054 (0.9–22 × 10^3) (51)	6631 (3.2–13 × 10^3) (7)
8	75 (17–217) (47)	48 (30–119) (7)	5354 (1230–18 000) (49)	2843 (0.6–15 × 10^3) (7)	323 (91–1380) (48)	111 (36–344) (7)	171 (60–621) (48)	179 (84–410) (7)	6607 (1.6–21 × 10^3) (549)	6233 (3 3–9.4 × 10^3)
9	91 (33–281) (41)	66 (37–105) (8)	9349 (3–32 × 10^3) (42)	6100 (1.8–22 × 10^3) (8)	740 (220–2884) (42)	331 (127–692) (8)	160 (31–525) (41)	168 (81–402) (8)	6180 (1.7–22 × 10^3) (41)	5980 (3.1–9.9 × 10^3) (8)
10	79 (25–253) (37)	69 (38–125) (7)	13 834 (4 7–32 × 10^3) (36)	8253 (2–29 × 10^3) (7)	1402 (316–4260) (36)	608 (268–1240) (7)	158 (44–487) (36)	142 (65–251) (7)	6712 (1.9–22 × 10^3) (37)	5934 (2.8–9.4 × 10^3) (7)
11	70 (21–220) (44)	67 (27–211) (8)	17 850 (7.4–53 × 10^3) (44)	10 856 (2.2–30 × 10^3) (8)	2259 (640–6920) (43)	1334 (0.8–2.2 × 10^3) (8)	151 (48–361) (44)	117 (50–301) (8)	7114 (1.3–29 × 10^3) (44)	6633 (3.8–10 × 10^3) (8)
12	65 (24–202) (38)	58 (22–161) (5)	21 396 (8.7–54 × 10^3) (39)	12 873 (2.4–34 × 10^3) (5)	3520 (1060–8760) (38)	1833 (1.0–3.0 × 10^3) (5)	145 (39–320) (38)	129 (89–172) (5)	9116 (3.8–20 × 10^3) (38)	8394 (4.7–16 × 10^3) (5)
13	55 (17–170) (38)	37 (17–124) (6)	25 142 (10–75 × 10^3) (31)	16 285 (3.1–44 × 10^3) (6)	5092 (2060–143 000) (32)	2886 (1.6–5.2 × 10^3) (6)	144 (50–329) (34)	153 (102–329) (6)	9990 (5.4–25 × 10^3) (31)	10 984 (7.2–16 × 10^3) (6)

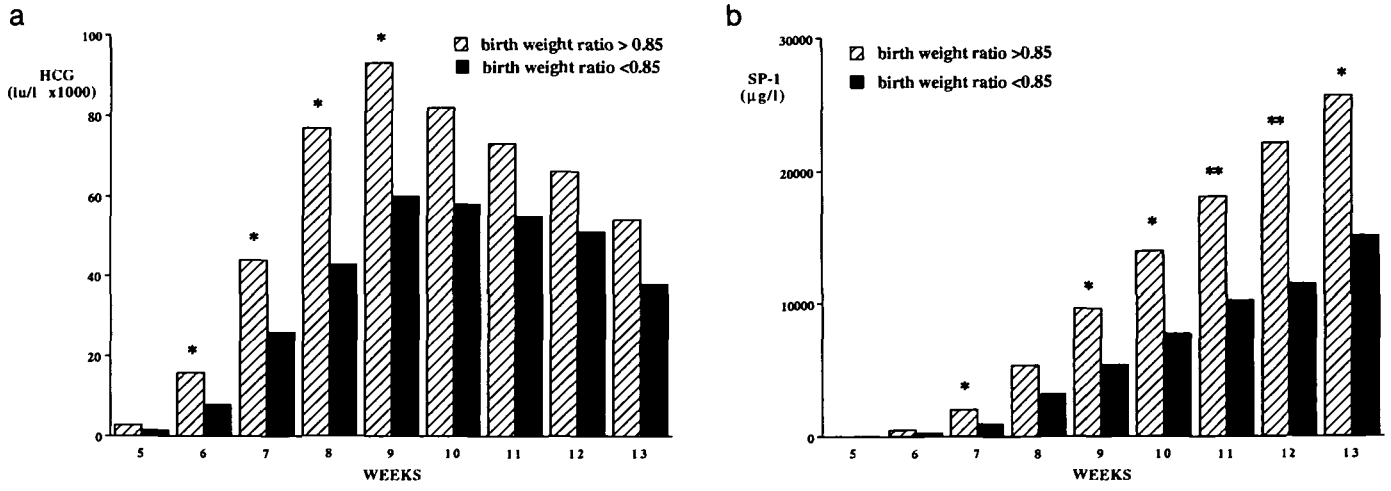

Fig. 2. The geometric mean of the circulating concentrations of human chorionic gonadotrophin (HCG; **a**), and Schwangerschaftsprotein 1 (SP-1; **b**) in patients in whom singleton pregnancies were achieved following ovulation induction and subsequent in-vitro fertilization and embryo transfer and who delivered babies with a ratio of actual birth weight to the 50th centile of the range for a given sex and gestational age either <0.85 (n = 9) or >0.85 (n = 53). *Denotes a difference of $P < 0.05$ and ** a difference of $P < 0.01$.

Johnson *et al.*, 1993b). Clearly, placental protein synthesis is regulated in a more subtle manner than previously thought, and factors other than blood flow and trophoblast mass must be involved. If the production of each placental protein were to be limited to sub-groups of syncytiotrophoblasts, then the differential reduction in the concentrations of placental proteins in various pathological states suggest the existence of a functional specialization hitherto unsuspected.

High oestradiol concentrations, following ovulation induction, have been suggested to lead to impaired implantation (Forman *et al.*, 1988). If such a phenomenon does exist, then it does not seem to impair placental development, as elevated oestradiol concentrations during the first trimester were not associated with reduced concentrations of placental proteins (Johnson *et al.*, 1993a), and in the present study, oestradiol concentrations were not elevated during the first trimester in pregnancies which either delivered before term or resulted in babies of low birth weight. However, a detrimental effect of elevated oestradiol concentrations on endometrial function during implantation cannot be excluded.

Other recent studies have related the concentrations of CRF (Kurki *et al.*, 1991) and relaxin to preterm labour (Petersen *et al.*, 1992). Despite the fact that CRF and relaxin concentrations are higher several weeks before the onset of preterm labour, both may be elevated in response to a second factor, which itself controls the time of onset of labour. In contrast, the lower concentrations of PAPP-A and SP-1 precede the onset of labour by several months, and the longer interval supports the existence of a causal relationship. Thus, the presence of the association between reduced concentrations of placental proteins during the first trimester and preterm labour suggests that the placenta may determine the time of onset of labour, and that this may be programmed early during the first trimester. However, the large overlap between the circulating concentrations of the PAPP-A, SP-1 and HCG in those pregnancies which deliver before term and those that deliver at term suggests that the measurement of these factors is unlikely to be of any clinical value.

The median birth weight ratio of 0.94 in the group which delivered before term suggests that growth retardation was not associated with preterm labour in this group. This is confirmed by the lack of any difference in the mean gestational age of delivery of babies born with a birth weight ratio of <0.85 and >0.85. However, the circulating concentrations of SP-1, and to a lesser extent HCG, were reduced in the lower birth weight group. These findings contrast with attempts later in gestation to relate placental function, in terms of the circulating concentrations of placental proteins, to birth weight (Gordon *et al.*, 1977; Obiekwe *et al.*, 1980; Westergaard *et al.*, 1984b; Biswas *et al.*, 1990), and suggest that birth weight, as well as gestational age at delivery, may be related to placental development or function during the first trimester.

Acknowledgements

The authors are grateful to Dr V.N.Bolton and Dr I.Stabile for advice and other members of the Assisted Conception Units, Kings College School of Medicine and Dentistry and the Hallam Medical Centre for technical assistance. M.R.Johnson is supported by a William's Fellowship from the University of London.

References

Altman,D.G. and Coles,E.C. (1980) Normograms for precise determination of birth weight for dates. *Br. J. Obstet. Gynaecol.*, **87**, 81–86.

Biswas,S., Murrey,M., Buffoe,G., Graves,L., Jelowitz,J. and Dewhurst,J. (1980) Placental lactogen as a reliable index of fetal outcome in threatened abortion during early pregnancy. *J. Obstet. Gynaecol.*, **1**, 75–77.

Brambati,B., McKintosh,M.C.M., Teisner,B., Maguiness,S., Shrimanker,K., Lanzani,A., Bonacchi,I., Tului,L., Chard,T. and Grudzinskas,J.G. (1993) Low maternal serum levels of pregnancy associated plasma protein A (PAPP-A) in the first trimester in association with abnormal fetal karyotype. *Br. J. Obstet. Gynaecol.*, **100**, 324–326.

Forman,R., Fries,N., Testart,J., Belaisch-Allart,J., Hazout,A. and Frydman,R. (1988) Evidence for an adverse effect of elevated serum estradiol concentrations on embryo implantation. *Fertil. Steril.*, **49**, 118–122.

Gordon,Y.B., Grudzinskas,J.G., Lewis,J.D., Jeffrey,D. and Letchworth,A.T. (1977) Circulating levels of pregnancy-specific β1-glycoprotein and human placental lactogen in the third trimester of pregnancy: their relationship to parity, birth weight and placental weight. *Br. J. Obstet. Gynaecol.*, **84**, 642–647.

Grudzinskas,J.G., Gordon,Y.B., Jeffrey,D. and Chard,T. (1977) Specific and sensitive determination of pregnancy specific β1 glycoprotein by radioimmunoassay. *Lancet*, **i**, 333–335.

Hercz,P., Siklos,P., Ungar,L., Farquharson,R.G., Mohari,K. and Kocsar,L. (1987) Change in serum HPL level in maternal vein, umbilical cord vein and artery in mature and premature labour. *Eur. J. Obstet. Gynecol.*, **24**, 189–193.

Johnson,M.R., Riddle,A.F., Grudzinskas,J.G., Sharma,V., Campbell,S., Collins,W.P., Lightman,S.L., Mason,B. and Nicolaides,K.H. (1993a) Endocrinology of IVF pregnancies during the first trimester. *Hum. Reprod.*, **8**, 316–322.

Johnson,M.R., Riddle,A.F., Grudzinskas,J.G., Sharma,V., Collins,W.P. and Nicolaides,K.H. (1993b) The role of trophoblast dysfunction in the aetiology of miscarriage. *Br. J. Obstet. Gynaecol.*, **100**, 353–359.

Kurki,T., Laatikainen,T., Salminen-Lappalainen,K. and Ylikorkala,O. (1991) Maternal plasma corticotrophin releasing hormone: elevated in preterm labour, but unaffected by indomethacin or nylidrin. *Br. J. Obstet. Gynaecol.*, **98**, 685–691.

Merchiers,E., Dhont,M., De Sutter,P. and Vandekerckhove,D. (1991) Correlations between serum HCG and trophoblast volume, determined by transvaginal ultrasound, in early pregnancy. *Hum. Reprod.*, **6** (Suppl. 1), Abstract P673, p. 411.

Obiekwe,B.C., Grudzinskas,J.G. and Chard,T. (1980) Circulating levels of placental protein 5 in the mother: relation to birthweight. *Br. J. Obstet. Gynaecol.*, **87**, 302–304.

Petersen,L.K., Skajaa,K. and Uldbjerg,N. (1992) Serum relaxin as a potential marker for preterm labour. *Br. J. Obstet. Gynaecol.*, **99**, 292–295.

Sharma,V., Riddle,A., Mason,B., Pampiglione,J. and Campbell,S. (1988) An analysis of factors influencing the establishment of a chemical pregnancy in an ultrasound based ambulatory in vitro fertilization program. *Fertil. Steril.*, **49**, 458–468.

Sinosich,M.J., Teisner,B., Folkersen,J., Saunders,D.M. and Grudzinskas,J.G. (1982) Radioimmunoassay for pregnancy associated plasma protein A. *Clin. Chem.*, **28**, 50–53.

Stabile,I., Grudzinskas,J.G. and Chard,T. (1988) Clinical applications of pregnancy protein estimations with particular reference to pregnancy-associated plasma protein A (PAPP-A). *Obstet. Gynecol. Surv.*, **43**, 73–82.

Westergaard,J.G., Teisner,B., Hau,J. and Grudzinskas,J.G. (1984a) Placental protein measurements in complicated pregnancies III. Premature labour. *Br. J. Obstet. Gynaecol.*, **91**, 1230–1233.

Westergaard,J.G., Teisner,B., Hau,J. and Grudzinskas,J.G. (1984b) Placental protein measurements in complicated pregnancies. I. Intrauterine growth retardation. *Br. J. Obstet. Gynaecol.*, **91**, 1216–1223.

White,D.R., Hall,M.H. and Campbell,D.M. (1986) The aetiology of preterm labour. *Br. J. Obstet. Gynaecol.*, **93**, 733–738.

Received on March 31, 1993; accepted on June 13, 1993