Human Reproduction vol.8 no.2 pp.316–322, 1993

Endocrinology of in-vitro fertilization pregnancies during the first trimester

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The endocrine function of the corpus luteum and placenta and the inter-relationships between ovarian steroids and the placental proteins in pregnancies achieved following ovarian stimulation, in-vitro fertilization and embryo transfer (IVF–ET) have been investigated. The serum concentrations of human chorionic gonadotrophin (HCG), Schwangerschaft protein-1 (SP-1), pregnancy-associated plasma protein A (PAPP-A), progesterone and oestradiol were measured at weekly intervals between the 4th (ET plus 2 weeks) and 14th week of gestation in 86 pregnancies. The mean concentrations of the placental proteins and oestradiol were significantly higher in twin than in singleton pregnancies from as early as 5 weeks gestation, but the mean concentrations of progesterone were significantly higher only at the end of the first trimester. Ranking, as demonstrated by the presence of statistically significant correlations between serum levels of each substance analysed in week 13 with those of preceding weeks, was established for progesterone and SP-1 from the 5th week, for oestradiol and PAPP-A from the 7th week and for HCG from the 8th week of gestation. The presence of statistically significant correlations between each substance analysed suggests that the placenta becomes the dominant source of oestradiol from 8 weeks gestation and of progesterone not until 12 weeks gestation, and that the placental synthesis of HCG, SP-1, PAPP-A, oestradiol and progesterone appear to be linked. There were no statistically significant correlations between the serum concentrations of HCG and either progesterone or oestradiol until the production of each had become predominantly placental.

Key words: birth weight/oestradiol/placental proteins/premature delivery/progesterone

Introduction

The endocrine changes that occur during early pregnancy are induced by the activities of the corpus luteum, endometrium, placenta and embryo acting independently or together. The interactions between the fetus, placenta, endometrium and corpus luteum, and the factors which regulate the synthetic and secretory activity of the feto-placental unit are poorly understood. The corpus luteum produces progesterone, oestradiol and relaxin in response to human chorionic gonadotrophin (HCG). Placental production of progesterone and oestradiol renders the corpus luteum redundant after the 6th week of pregnancy (Csapo and Pulkkinen, 1978), although it remains functional in terms of relaxin secretion throughout gestation (Weiss *et al.*, 1977). In addition, the endometrium produces various hormones and proteins including prolactin, insulin-related growth factor binding protein-1 [IGF-BP-1, synonym, placental protein 12 (PP12) and progesterone-dependent endometrial protein (PEP; synonym, placental protein 14 (PP14)]. However the feto-placental unit accounts for most of the endocrine changes of pregnancy; it produces large quantities of oestrogens, progestagens and a variety of hypothalamic and pituitary hormones together with several proteins physiologically unique to pregnancy such as HCG, Schwangerschaft protein 1 (SP-1), and pregnancy-associated plasma protein A (PAPP-A) (Grudzinskas and Chard, 1990).

The endocrine changes of naturally conceived singleton pregnancies are well documented (Tulchinsky and Hobel, 1973; Aspillaga *et al.*, 1983; Grudzinskas and Chard, 1990), while those in multiple pregnancies and pregnancies achieved through in-vitro fertilization (IVF) and embryo transfer (ET) have not been studied extensively (Westergaard *et al.*, 1985; Yovich *et al.*, 1985). The present study investigates the endocrine function of the corpus luteum and placenta and the inter-relationships between ovarian steroids and the placental proteins in pregnancies achieved following ovarian stimulation and IVF–ET.

Materials and methods

Subjects

A total of 86 patients (aged 22–39 years) who had become pregnant following IVF and ET were studied between March 1986 and November 1987; 82 of these had received clomiphene citrate (Clomid, Merrel Dow Pharmaceuticals Ltd, Uxbridge, Middlesex, UK; 100 mg orally) on days 2–6 of the menstrual

cycle. 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] 2–6 ampoules/day i.m. (n = 58), or purified FSH (Metrodin, 75 IU FSH and 1 IU of LH; Serono Laboratories, Welwyn Garden City, Herts, UK) 2–6 ampoules/day i.m. (n = 24) were started on days 2 or 4, depending on previous ovarian responses to stimulation and the length of the normal cycle, in accordance with a protocol described in detail elsewhere (Sharma *et al.*, 1988). The FSH stimulated cycles had either FSH alone, or FSH on days 1–5 followed by HMG on day 6 until the day of oocyte collection. The gonadotrophin stimulation regimen was chosen randomly. Four subjects who had been treated with buserelin (Hoechst UK Ltd, Hounslow, Middlesex, UK) were excluded from the analysis except in the comparison between singletons and twin pregnancies of the circulating concentrations of the hormones and proteins analysed.

Treatment was continued until the leading follicle reached a maximum diameter of 17 mm and at least three follicles ≥ 14 mm in diameter were present. HCG (Profasi, Serono Laboratories, Welwyn Garden City, Herts, UK) was administered (5000 IU i.m.) and the oocytes were collected transvaginally under ultrasound guidance 34–36 h later. Two days later 1–4 cleavage stage embryos were transferred to the uterus (Sharma *et al.*, 1988). Subjects were allocated to receive luteal phase support

Table I. Geometric means (range, n) of serum concentrations of human chorionic gonadotrophin (HCG), Schwangerschaft protein-1 (SP-1) and pregnancy-associated plasma protein A (PAPP-A) between weeks 4 and 14

Weeks	HCG (IU × 10^3/l)		SP-1 (μg/l)		PAPP-A (μg/l)	
	Singleton	Twin	Singleton	Twin	Singleton	Twin
4	0.5 (0.2–4.0) (15)	0.7 (0.5–1.0) (4)	4 (1–48) (16)	5 (1–17) (4)	4 (1–11) (18)	5 (2–10) (4)
5	2.6 (0.1–29) (53)	5† (1–16.2) (19)	34 (1–48) (55)	66 (1–430) (19)	6 (1–71) (54)	6 (1–20) (18)
6	14** (2–76) (61)	24**/† (8–50) (24)	420** (26–4800) (61)	755** (190–7020) (24)	21** (2–280) (61)	25**/†† (2–152) (24)
7	41** (9–132) (60)	74**/† (34–170) (24)	1786** (190–8900) (58)	3885**/†† (720–14000) (23)	97** (8–620) (59)	136**/†† (23–790) (23)
8	71** (17–217) (53)	139**/†† (35–293) (23)	4964** (630–18000) (55)	10082**/†† (3.3–48 × 10^3) (23)	284** (36–1380) (54)	469**/†† (98–1356) (22)
9	87** (33–281) (49)	183**/†† (44–268) (21)	8732** (1.8–32 × 10^3) (50)	17373**/†† (6.1–45 × 10^3) (21)	651** (127–2884) (50)	1127**/†† (392–3900) (21)
10	78** (25–253) (43)	187**/†† (46–392) (18)	12884** (2.0–37 × 10^3) (42)	24701**/†† (11–53 × 10^3) (18)	1241** (268–4260) (42)	2364**/†† (900–6870) (18)
11	70** (27–220) (51)	161**/†† (63–273) (21)	16715** (2.2–53 × 10^3) (51)	31702**/†† (18–61 × 10^3 (19)	2095** (640–6920) (50)	4514**/†† (1.4–11 × 10^3) (20)
12	65** (22–202) (42)	124**/†† (74–255) (17)	19277** (2.0–54 × 10^3) (43)	41090**/†† (19–93 × 10^3) (18)	3303** (1010–8760) (42)	7619**/†† (3.6–17 × 10^3) (16)
13	52** (17–170) (38)	122**/†† (64–233) (17)	23712** (3.1–75 × 10^3) (36)	54 236**/†† (20–146 × 10^3) (18)	4646** (1610–14300) (37)	12471**/†† (4.1–26 × 10^3) (18)
14	48** (24–156) (14)	161†† (123–243) (3)	25228** (17–55 × 10^3) (15)	71204† (46–109 × 10^3) (3)	6403* (2.7–20 × 10^3) (14)	21000†† (12–70 × 10^3) (5)

*P < 0.05, **P < 0.01 compared to week 5 value, †P < 0.05, ††P < 0.01 between protein concentrations in singleton and twin pregnancies at the same time point.

with either HCG (2000 IU, i.m. on the day of ET and 3 days later) (n = 44) or progesterone (Cyclogest vaginal pessaries, progesterone 200 mg, Hoechst UK Ltd, Hounslow, Middlesex, UK; 400 mg/twice daily, for a minimum of 8 weeks) (n = 8). Thirty women did not receive luteal phase support. Of the 86 patients, 62 conceived singleton and 24 twin pregnancies.

Peripheral venous blood samples were taken at weekly intervals from 4 weeks gestation (oocyte retrieval plus 2 weeks) until week 14. Blood was collected into plain tubes, the serum separated by centrifugation and stored within 2 h at −20°C prior to analysis.

The protocol was approved by the Research Ethics Committee of King's College Hospital.

Immunoassays

Serum progesterone and oestradiol were extracted with diethyl ether and measured by radioimmunoassay using tritiated antigens and monoclonal antibodies to P-11 a-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 coefficient of variation (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

Table II. Geometric means (range, n) of serum concentrations of progesterone and oestradiol between weeks 4 and 14

Weeks	Progesterone (nmol/l)		Oestradiol (pmol/l)	
	Singleton	Twin	Singleton	Twin
4	225 (45−618) (15)	364 (254−628) (4)	3023 (630−19280) (15)	8397 (4250−17920) (4)
5	237 (42−598) (18)	336 (111−870) (53)	5602 (1.1−24 × 10^3) (54)	8519 (2.2−25 × 10^3) (18)
6	224 (38−815) (61)	306† (111−943) (24)	6077 (0.9−23 × 10†) (61)	8108† (1.7−27 × 10^3) (24)
7	198** (32−820) (57)	256** (90−742) (23)	6119 (0.9−22 × 10^3) (58)	8540† (2.1−21 × 10^3) (23)
8	173** (60−621) (54)	246**/† (87−736) (22)	6565 (1.6−21 × 10^3) (55)	10148† (3.1−29 × 10^3) (23)
9	161** (31−525) (49)	215** (76−746) (20)	6147 (1.7−22 × 10^3) (49)	11047†† (3.8−24 × 10^3) (21)
10	158** (44−487) (42)	185** (35−612) (18)	6573 (1.9−22 × 10^3) (43)	11304†† (3.6−25 × 10^3) (18)
11	147** (48−361) (51)	230**/†† (100−679) (20)	7069 (1.3−29 × 10^3) (51)	14416*/†† (5.7−29 × 10^3) (21)
12	145** (39−320) (42)	223**/†† (62−780) (17)	9033** (3.8−20 × 10^3) (42)	16412*/†† (6.3−35 × 10^3) (17)
13	147** (50−329) (39)	242**/†† (90−489) (17)	10096** (5.4−25 × 10^3) (36)	21503*/†† (8.1−50 × 10^3) (18)
14	121* (42−289) (14)	232† (105−597) (6)	9522 (1760−15830) (14)	16630 (10−23 × 10^3) (3)

*P < 0.05, **P < 0.01 compared to week 5 value, †P < 0.05, ††P < 0.01 between hormone concentrations in singleton and twin pregnancies at the same time point.

were analysed by radioimmunoassay as described by Grudzinskas *et al.* (1977) and 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 groups were assessed by non-parametric methods. The data at the same time points were compared by the Mann–Whitney U-test and to week 5 with the Wilcox signed-rank test. A simple regression analysis was used to determine the correlation between the concentration of each substance analysed and the week of gestation (from 5–13), and between different substances at the same time points.

Results

There was no significant difference in the ages of women who conceived singleton and twin pregnancies (31.9 versus 32.7 years, respectively), or between those receiving HMG or FSH (singletons: 31.9 versus 32.1 years respectively; twins: 32.8 versus 32.3 years respectively). The mean number of embryos transferred to those women who conceived singletons was significantly ($P < 0.05$) less than the mean number transferred

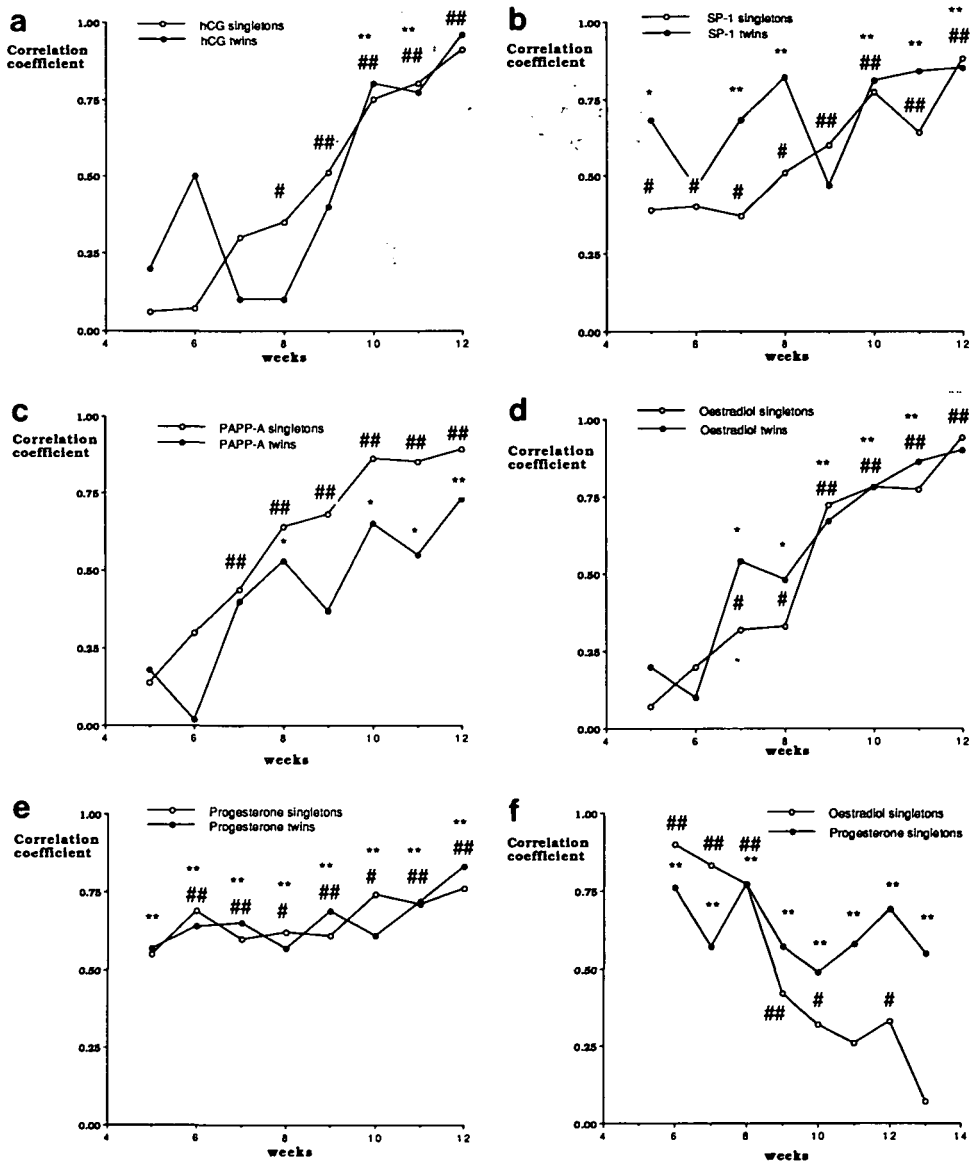

Fig. 1. (a–e) Representations of the correlation coefficient, *r*, plotted against time, between circulating human chorionic gonadotrophin (HCG) (a), Schwangerschaft protein-1 (SP-1) (b), pregnancy-associated plasma protein A (PAPP-A) (c), oestradiol (d) and progesterone (e) of each individual on week 13 and on the preceding weeks in twin and singleton pregnancies. * and ** denote a significant correlation of $P < 0.05$ and $P < 0.01$ respectively in singleton pregnanices; # and # # denote a significant correlation of $P < 0.05$ and $P < 0.01$ respectively in twin pregnancies. (f) Representation of the correlation coefficient *r* plotted against time, between circulating oestradiol and progesterone of each individual on week 5 and on the succeeding weeks in singleton pregnancies. * and ** denote a significant correlation of $P < 0.05$ and $P < 0.01$ respectively in progesterone levels; # and # # denote a significant correlation of $P < 0.05$ and $P < 0.01$ respectively in oestradiol levels.

to those who conceived twins (3.07 ± 0.97 versus 3.6 ± 1.0; mean ± SD).

Singleton and twin pregnancies

Mean serum HCG concentration increased significantly with gestation in both singleton and twin pregnancy to a peak at 9 weeks for singletons and 10 weeks for twins, after which they fell for the remainder of the study period (Table I). Mean serum concentration of SP-1 and PAPP-A rose exponentially throughout the study period (Table I). Mean serum oestradiol concentration increased in singletons between weeks 4 to 5 (Table II) and thereafter remained unchanged until a statistically significant increase on weeks 12 and 13 (compared to week 5). For twins, mean serum oestradiol concentration rose slowly from week 5 and was significantly higher than week 5 from week 11 onwards (Table II). The mean serum progesterone concentration declined progressively in singletons throughout the study period and in twins until week 10, thereafter a small increase was observed (Table II). The concentrations of serum HCG from week 5, PAPP-A from week 6, SP-1 from week 7 (Table I), oestradiol from week 6 and progesterone on weeks 6, 8, 11, 12, 13 and 14 only (Table II) were significantly higher in women with twin, rather than singleton, pregnancies.

Correlations

In singleton and twin pregnancies, the correlations between the concentrations of an individual analyte on week 13 and on each of the preceding weeks were raised for SP-1 (Figure 1b) and progesterone (Figure 1e) throughout the study period, but were raised for HCG only after week 10 (Figure 1a), for PAPP-A after week 8 (Figure 1c) and for oestradiol after week 9 (Figure 1d). For singleton pregnancies, high correlations were observed between week 5 and all subsequent weeks for progesterone, but only until week 8 for oestradiol (Figure 1f).

Correlations between the concentrations of individual substances analysed at the same time points of singleton and twin pregnancies combined were assessed. Correlations between SP-1 and HCG were high throughout. Those between PAPP-A and

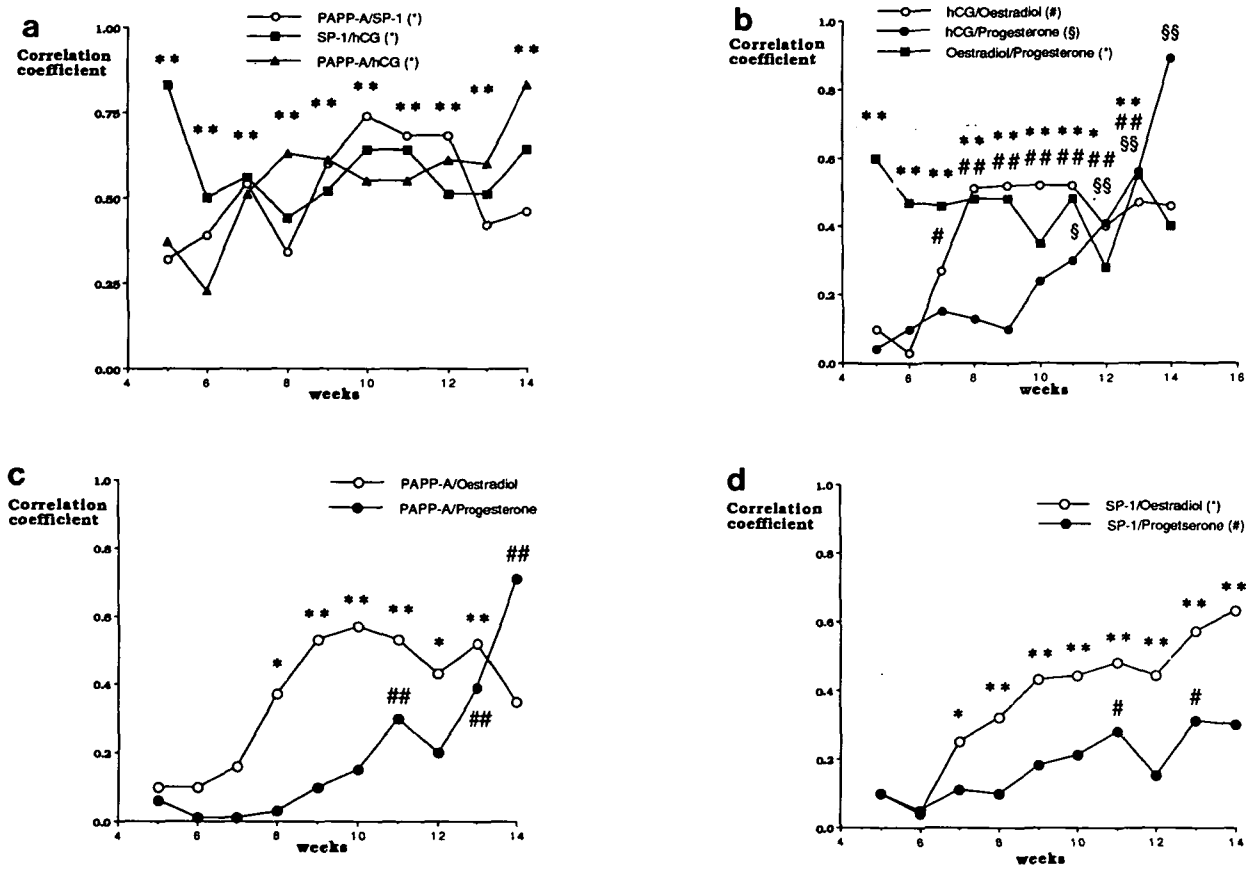

Fig. 2. (a) Representation of the correlation coefficient r plotted against time, between circulating HCG, SP-1 and PAPP-A of each individual. ** denotes a significant correlation of $P < 0.01$ between (i) PAPP-A and HCG; (ii) SP-1 and HCG; and (iii) PAPP-A and SP-1. For abbreviations see Figure 1. (b) Representation of the correlation coefficient, r, plotted against time, between circulating HCG, oestradiol and progesterone, of each individual. * and ** denote a significant correlation of $P < 0.05$ and $P < 0.01$ respectively between oestradiol and progesterone; # and # # denote a significant correlation of $P < 0.05$ and $P < 0.01$ respectively betwen HCG and oestradiol; § and §§ denote a significant correlation of $P < 0.05$ and $P < 0.01$ respectively between HCG and progesterone. (c) Representation of the correlation coefficient r plotted against time, between circulating PAPP-A and both oestradiol and progesterone of each individual. * and ** denote a significant correlation of $P < 0.05$ and $P < 0.01$ respectively between PAPP-A and oestradiol; # and # # denote a significant correlation of $P < 0.05$ and $P < 0.01$ respectively between PAPP-A and progesterone. (d) Representation of the correlation coefficient r plotted against time, between circulating SP-1 and both oestradiol and progesterone of each individual. * and ** denote a significant correlation of $P < 0.05$ and $P < 0.01$ respectively between SP-1 and oestradiol; # and # # denote a significant correlation of $P < 0.05$ and $P < 0.01$ respectively between SP-1 and progesterone.

both HCG and SP-1 were initially weak, but improved with gestation, and by 8 weeks were similar to those between SP-1 and HCG but deteriorated with time. The reverse was true of PAPP-A and HCG (Figure 2a). The initial correlations were poor between HCG and both oestradiol and progesterone, but improved with time, rapidly for oestradiol, and more slowly for progesterone (Figure 2b). The correlation between oestradiol and progesterone remained high throughout (Figure 2b). Correlations between PAPP-A and oestradiol were higher and observed sooner than between PAPP-A and progesterone (Figure 2c); a similar pattern was observed in the correlations between SP-1 and both oestradiol and progesterone (Figure 2d).

Discussion

The maternal serum concentrations of placental proteins and their changes with gestation in singleton IVF pregnancies are similar to those reported for naturally conceived pregnancies (Grudzinskas and Chard, 1990). In twin pregnancies the concentrations were higher from as early as 5 weeks gestation and this finding may be a consequence of the greater trophoblast mass. The concentrations of oestradiol and progesterone were much higher than in naturally conceived pregnancies reflecting the use of ovarian stimulation. The concentration of progesterone was consistently higher in twin than in singleton pregnancies only after week 11. In contrast, the mean concentration of oestradiol was higher in twin than in singleton pregnancies as early as week 7, suggesting that the placenta becomes the major source of oestradiol earlier than progesterone. This is supported by the earlier and stronger association between oestradiol and the placental proteins. Thus, the decrease in progesterone concentration with time must be due to declining corpus luteum secretion despite increasing HCG concentration. The oestradiol concentration did not decline with time, but the increase compared with the concentration at week 5 was significant for twins only from week 11 and for singletons from week 12, suggesting that the corpus luteum secretion of oestradiol was declining throughout the study period also. Thus in terms of steroid secretion, the corpora lutea of pregnancies achieved following IVF are maximally active at 4–5 weeks gestation, as has been suggested for natural pregnancies (Yoshimi *et al.*, 1969), and thereafter their activity declines. The placenta becomes the dominant source of oestradiol around the 8th week of gestation and of progesterone around 12–13 weeks gestation. These results contrast with those reported for ovum donation pregnancies, in which the circulating concentrations of progesterone and oestradiol are significantly greater than those on replacement alone at 6 weeks for oestradiol and 7 weeks for progesterone (Scott *et al.*, 1991). The difference is due to the use of ovarian stimulation in IVF–ET pregnancies.

A previous shorter study compared the endocrine responses to pregnancy in singleton and twin pregnancy achieved following IVF and ET, and found similar results for oestradiol and progesterone, while the increments in HCG were smaller and peaked earlier for singletons; furthermore, the difference between singleton and twin pregnancies was only apparent later (Khazen *et al.*, 1986). SP-1 concentrations have been previously shown to be higher in twin than in singleton pregnancies (Sorensen, 1978), but no such study has been performed previously for PAPP-A in the first trimester.

The absence of any correlation between HCG and either progesterone or oestradiol in early gestation and the contrast in their circulating concentrations implies that although HCG may be directly involved in the rescue of the corpus luteum, subsequently it plays no regulatory role in the control of steroid secretion. Indeed, the improved correlations between HCG and both oestradiol and progesterone with time can be a reflection only of their common placental origin in later gestation. This notion is supported further by the improving correlation with gestation between the other placental proteins, SP-1 and PAPP-A, and both oestradiol and progesterone. The present assessment is based on the circulating concentration of HCG and takes no account of either its bioactivity or the expression of its receptors in the corpus luteum. It is possible that further factors regulate steroid synthesis and secretion by the corpus luteum, or that the corpus luteum is pre-programmed to undergo a gradual decline in steroid synthetic activity. The former is more probable in view of higher levels of oestradiol and progesterone in twin pregnancies.

The suggestion that 'ranking' occurs and is maintained through pregnancy was first made by Aspillaga *et al.* (1983), who found high correlations between a group of individuals' serum concentrations of progesterone, oestradiol, human placental lactogen and HCG from late in the first trimester to the third trimester. Strong correlations between circulating concentrations of a substance analysed at the beginning and end of pregnancy suggest that the rate of synthesis and clearance of the substance have remained constant over the period of investigation. In the present study, the possibility that such ranking is established earlier in pregnancy has been investigated. While for oestradiol, HCG and PAPP-A no evidence for such a process exists, for progesterone and SP-1 high correlations were found between concentrations in individuals at the beginning and the end of the first trimester. The high correlation (between progesterone concentration at the beginning and the end of the first trimester) implies that progesterone production by the corpus luteum is linked to that of the placenta. In addition, the concentrations of progesterone and oestradiol correlated well throughout the study period. While early in the first trimester this could be explained by the source of both being the corpus luteum, later, when the source of oestradiol is predominantly placental, the correlation supports the suggestion that progesterone production by the corpus luteum is linked to steroid production by the placenta. For SP-1, it implies that the rate of synthesis and release is determined in early pregnancy, in contrast to PAPP-A and HCG. The findings for HCG suggest that the regulation of HCG synthesis in early pregnancy, during its rapid rise, differs from that occurring later on. For PAPP-A, however, the explanation may be the lack of sensitivity of the assay prior to 6 weeks gestation.

The behaviour of the circulating levels of placental proteins and ovarian steroids in the first trimester in singleton and twin pregnancies achieved following ovarian stimulation and IVF–ET have been described. Furthermore, ranking has been shown to be established early in the first trimester for SP-1 and progesterone and later for the remaining substances analysed.

The importance of HCG in the regulation of steroid synthesis by the corpus luteum has been questioned and the possible existence of a mechanism linking progesterone synthesis by the corpus luteum to steroid synthesis by the placenta has been suggested. In addition, the inter-relationships between the placental products has been explored and the normal patterns in IVF pregnancies established.

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 Williams Fellowship from London University.

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Received on May 18, 1992; accepted on October 6, 1992