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Interactions between the embryo and corpus luteum

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A total of 102 patients who had become pregnant following in-vitro fertilization (IVF) and embryo transfer were studied at weekly intervals between 4 and 14 weeks gestation. The pregnancies were classified as follows: (i) normal singleton, $n = 52$; (ii) normal twin, $n = 24$; (iii) heterotopic, $n = 4$ (weeks 4–8 only); and (iv) anembryonic with a viable intra-uterine singleton, $n = 22$. The serum concentrations of human chorionic gonadotrophin (hCG), Schwangerschaft protein-1 (SP-1) and pregnancy-associated plasma protein-A (PAPP-A), oestradiol and progesterone were measured. The mean serum concentrations of HCG, SP-1 and PAPP-A were significantly less in heterotopic than in singleton, singleton/anembryonic or twin pregnancies ($P < 0.01–0.05$), while those of progesterone and oestradiol were not different at any time. There were no significant differences between the serum concentrations of any of the substances analysed in singleton/anembryonic and singleton pregnancies, but the concentrations of all the substances analysed were significantly greater in twin pregnancies from as early as 7 weeks ($P < 0.01–0.05$). These data show that in heterotopic pregnancies trophoblast function is reduced, as suggested by the lower concentrations of the placental proteins. Despite this the concentrations of oestradiol and progesterone, derived predominantly from the corpus luteum between 4 and 8 weeks, are equivalent to those found in twin pregnancies, and greater than those found in singleton and singleton/anembryonic pregnancies. These findings support the notion that although HCG may rescue the corpus luteum it does not subsequently have a direct effect on its function, and suggest that the embryo may influence corpus luteum function.

Key words: corpus luteum/embryo

Introduction

It is accepted that trophoblast-derived human chorionic gonadotrophin (HCG) plays a major role in the rescue of the corpus luteum, preventing the normal progression to atresia, and maintains the synthesis of progesterone and oestradiol. Several studies have failed to find any relationship between the circulating immunoreactive or bioactive concentrations of HCG and those of progesterone or oestradiol, leading to the suggestion that they are not related (Hubinont *et al.*, 1987; Norman *et al.*, 1988; Kratzer and Taylor, 1990). This conclusion was emphasized by the findings of our recent study in pregnancies achieved after in-vitro fertilization (IVF), in which the circulating concentrations of both progesterone and oestradiol were seen to decline despite the presence of increasing concentrations of HCG. It has been suggested that either another factor is involved in the modulation of steroid synthesis by the corpus luteum (Norman *et al.*, 1988; Johnson *et al.*, 1993a), or that the corpus luteum is pre-programmed to decline. Further evidence from anembryonic pregnancies shows that there is a direct relationship between the circulating concentrations of HCG and both progesterone and oestradiol in the absence of embryonic tissue (Johnson *et al.*, 1993b). Thus, the embryo may either synthesize a factor itself, or induce the synthesis of a factor in either the trophoblast or endometrium, which is superimposed upon the luteotrophic effect of HCG.

In order to examine the possible effect of the embryo on corpus luteum function and to elucidate the mechanisms involved in such an interaction, the serum concentrations of HCG, Schwangerschaft protein-1 (SP-1), pregnancy-associated plasma protein-A (PAPP-A), oestradiol and progesterone were measured during the first trimester of the following series of pregnancies achieved following ovarian stimulation and IVF with embryo transfer: (i) normal singleton; (ii) normal twin; (iii) heterotopic (ectopic with a viable intra-uterine singleton); and (iv) anembryonic with a viable intra-uterine singleton.

Materials and methods

A total of 102 patients who had become pregnant following IVF were studied between 4 and 14 weeks gestation. The patients in this group were classified as follows: (i) normal singleton, $n = 52$; (ii) normal twin, $n = 24$; (iii) heterotopic, $n = 4$ (weeks 4–8 only); and (iv) anembryonic with a viable intra-uterine singleton, $n = 22$. The methods used for ovarian stimulation and IVF have been described previously (Sharma *et al.*, 1988).

Venous blood samples were obtained at 1 week intervals from 4 weeks gestation (oocyte retrieval plus 2 weeks) until week 14. Blood was collected into tubes without preservative or anti-coagulant. The serum was further separated by centrifugation, and stored at −20°C within 2 h. Samples were analysed for oestradiol, progesterone, HCG, PAPP-A and SP-1.

Serum progesterone and oestradiol were extracted with diethyl ether and measured by radioimmunoassay using tritiated antigens and monoclonal antibodies to P-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 coefficients of variation) 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 as described previously (Grudzinskas *et al.*, 1977; Sinosich *et al.*, 1982).

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

Statistical analysis

The data appeared to follow a log normal distribution; the level of significance for the differences in the concentrations of the substances analysed between groups at the same time points was assessed by the Mann Whitney *U*-test. The geometric mean values were used to illustrate trends with time in Figures 1 and 2.

Results

Singleton and twin

The serum concentrations of the substances analysed in the singleton and twin pregnancies have been reported previously (Johnson *et al.*, 1993b) and are shown in Table I.

Heterotopic

(i) *Singleton.* In heterotopic pregnancies, the serum concentrations of HCG in weeks 7 and 8 ($P < 0.05$), SP-1 in weeks 6–8 ($P < 0.05$) and PAPP-A in weeks 6–8 ($P < 0.01$–0.05) were significantly less than in singleton pregnancies (Figure 1a–c). Although the serum concentrations of oestradiol and progesterone were greater in heterotopic than in singleton pregnancies, these differences were not statistically significant (Figure 1d and e).

(ii) *Twin.* The serum concentrations of HCG (weeks 6–8,

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

Weeks of gestation	HCG (IU × 1000/l)		SP-1 (μg/l)		PAPP-A (μg/l)		Progesterone (nmol/l)		Oestradiol (pmol/l)	
	Singleton	Twin	Singleton	Twin	Singleton	Twin	Singleton	Twin	Singleton	Twin
4	0.5	0.7	4	5	4	5	225	364	3023	8397
	(0.2–4.0)	(0.5–1.0)	(1–48)	(1–17)	(1–11)	(2–10)	(45–618)	(254–628)	(630–19 280)	(4250–17 920)
	(15)	(4)	(16)	(4)	(18)	(4)	(15)	(4)	(15)	(4)
5	2.6	5*	34	66	6	6	237	336	5602	8519
	(0.1–29)	(1–16.2)	(1–48)	(1–430)	(1–71)	(1–20)	(42–598)	(111–870)	(1.1–24 × 10^3)	(2.2–25 × 10^3)
	(53)	(19)	(55)	(19)	(54)	(18)	(18)	(53)	(54)	(18)
6	14	24*	420	755	21	25**	224	306*	6077	8108*
	(2–76)	(8–50)	(26–4800)	(190–7020)	(2–280)	(2–152)	(38–815)	(111–943)	(0.9–23 × 10^3)	(1.7–27 × 10^3)
	(61)	(24)	(61)	(24)	(61)	(24)	(61)	(24)	(61)	(24)
7	41	74*	1786	3885**	97	136**	198	256	6119	8540*
	(9–132)	(34–170)	(190–8900)	(720–14 000)	(8–620)	(23–790)	(32–820)	(90–742)	(0.9–22 × 10^3)	(2.1–21 × 10^3)
	(60)	(24)	(58)	(23)	(59)	(23)	(57)	(23)	(58)	(23)
8	71	139**	4964	10 082**	284	469**	173	246*	6565	10 148*
	(17–217)	(35–293)	(630–18 000)	3.3–48 × 10^3)	(36–1380)	(98–1356)	(60–621)	(87–736)	(1.6–21 × 10^3)	(3.1–29 × 10^3)
	(53)	(23)	(55)	(23)	(54)	(22)	(54)	(22)	(55)	(23)
9	87	183**	8732	17 373**	651	1127**	161	215	6147	11 047**
	(33–281)	(44–268)	(1.8–32 × 10^3)	(6.1–45 × 10^3)	(127–2884)	(392–3900)	(31–525)	(76–746)	(1.7–22 × 10^3)	(3.8–24 × 10^3)
	(49)	(21)	(50)	(21)	(50)	(21)	(49)	(20)	(49)	(21)
10	78	187**	12 884	24 701**	1241	2364**	158	185	6573	11 304**
	(25–253)	(46–392)	(2.0–37 × 10^3)	(11–53 × 10^3)	(268–4260)	(900–6870)	(44–487)	(35–612)	(1.9–22 × 10^3)	(3.6–25 × 10^3)
	(43)	(18)	(42)	(18)	(42)	(18)	(42)	(18)	(43)	(18)
11	70	161**	16 715	31 702**	2095	4514**	147	230**	7069	14 416**
	(27–220)	(63–273)	(2.2–53 × 10^3)	(18–61 × 10^3)	(640–6920)	(1.4–11 × 10^3)	(48–361)	(100–679)	(1.3–29 × 10^3)	(5.7–29 × 10^3)
	(51)	(21)	(51)	(19)	(50)	(20)	(51)	(20)	(51)	(21)
12	65	124**	19 277	41 090**	3303	7619**	145	223**	9033	16 412*
	(22–202)	(74–255)	(2.0–54 × 10^3)	(19–93 × 10^3)	(1010–8760)	(3.6–17 × 10^3)	(39–320)	(62–780)	(3.8–20 × 10^3)	(6.3–35 × 10^3)
	(42)	(17)	(43)	(18)	(42)	(16)	(42)	(17)	(42)	(17)
13	52	122**	23 712	54 236**	4646	12 471**	147	242**	10 096	21 503**
	(17–170)	(64–233)	(3.1–75 × 10^3)	(20–146 × 10^3)	(1610–14 300)	(4.1–26 × 10^3)	(50–329)	(90–489)	(5.4–25 × 10^3)	(8.1–50 × 10^3)
	(38)	(17)	(36)	(18)	(37)	(18)	(39)	(17)	(36)	(18)
14	48	161*	25 228	71 204*	6403	21 000**	121	232*	9522	16 630
	(24–156)	(123–243)	17–55 × 10^3)	(46–109 × 10^3)	(2.7–20 × 10^3)	(12–70 × 10^3)	(42–289)	(105–597)	(1760–15 830)	(10–23 × 10^3)
	(14)	(3)	(15)	(3)	(14)	(5)	(14)	(6)	(14)	(3)

*Statistically significant difference of $P < 0.05$ and **$P < 0.01$ between serum concentration of a substance in singleton and twin pregnancies at the same time point.

$P < 0.01-0.05$), SP-1 (weeks 6–8, $P < 0.01-0.05$) and PAPP-A (weeks 6–8, $P < 0.05$) were significantly greater in twin pregnancies than in heterotopic pregnancies from 6 weeks (Figure 1a–c), while for progesterone and oestradiol there were no significant differences between twin and heterotopic pregnancies at any time (Figure 1d and e).

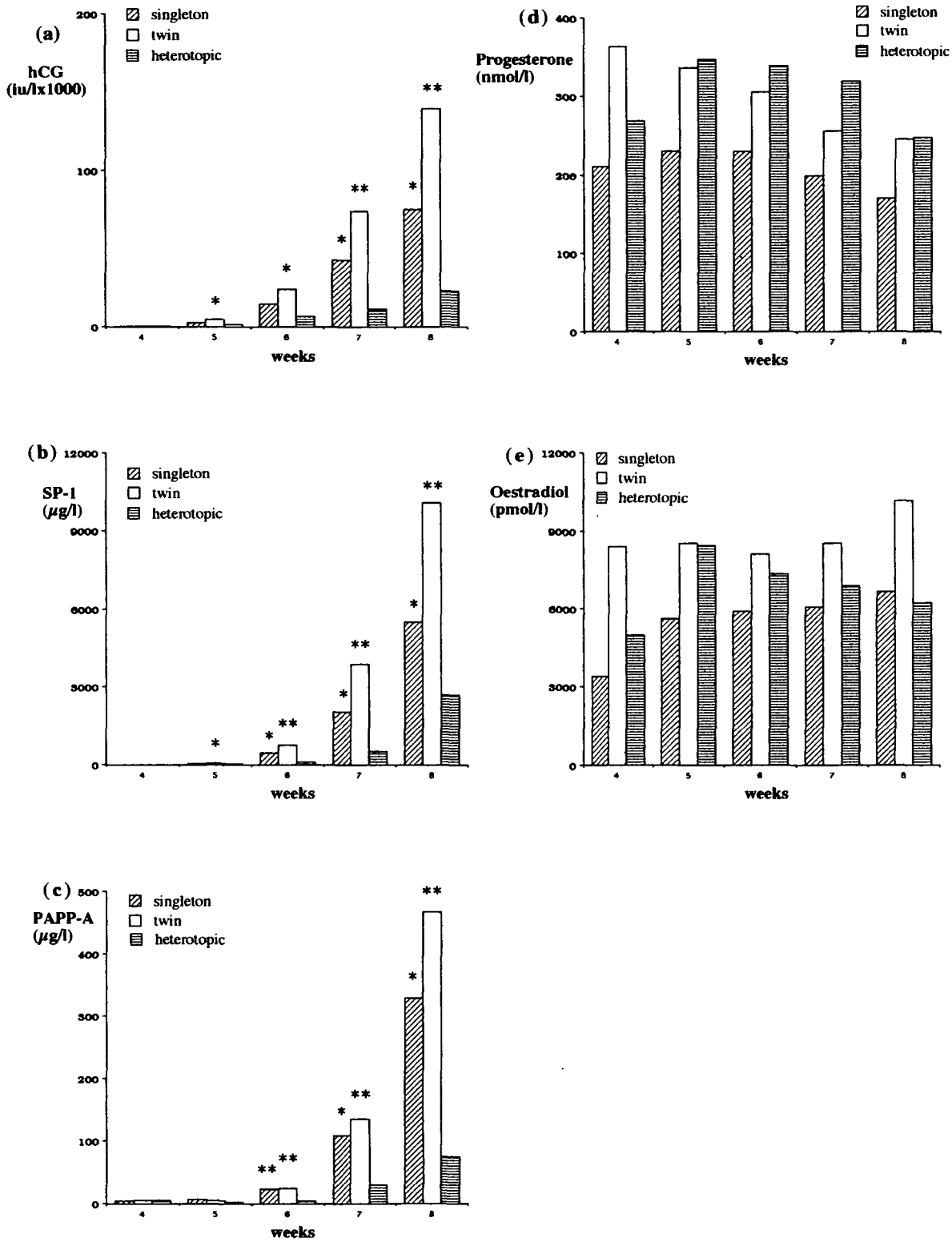

Fig. 1. The circulating concentrations of human chorionic gonadotrophin (HCG; **a**), Schwangerschaft protein-1 (SP-1; **b**), pregnancy-associated plasma protein-A (PAPP-A; **c**), progesterone (**d**) and oestradiol (**e**) in singleton, twin and heterotopic pregnancies between 4 and 8 weeks' gestation. * Denotes a significant difference of $P < 0.05$ and ** of $P < 0.01$ between the serum concentrations of each analyte in heterotopic pregnancies and in either singleton or twin pregnancies.

Singleton/anembryonic

(i) *Singleton*. There were no significant differences in the serum concentrations of any of the substances analysed between singleton/anembryonic and singleton pregnancies (Figure 2a–e).

(ii) *Twin*. The serum concentrations of HCG in weeks 7–13 ($P < 0.01–0.05$), SP-1 in weeks 9–12 ($P < 0.01–0.05$) and

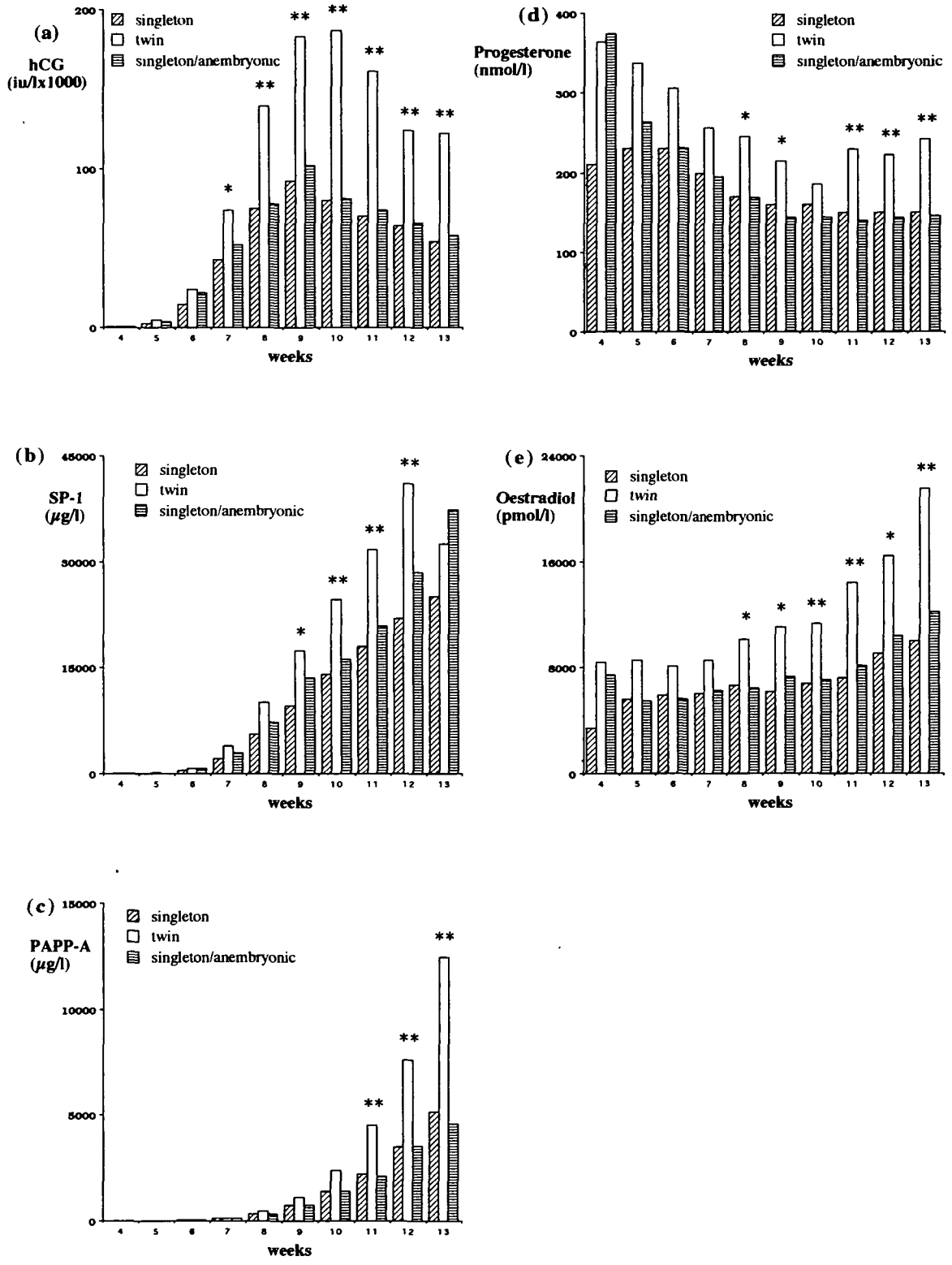

Fig. 2. The circulating concentrations of HCG (**a**), SP-1 (**b**), PAPP-A (**c**), progesterone (**d**) and oestradiol (**e**) in singleton, twin and singleton/anembryonic pregnancies between 4 and 13 weeks' gestation. * Denotes a significant difference of $P < 0.05$ and ** of $P < 0.01$ between the serum concentrations of each analyte in singleton/anembryonic pregnancies and in either singleton or twin pregnancies.

PAPP-A in weeks 11–13 ($P < 0.01$) were significantly greater in twin than in singleton or singleton/anembryonic pregnancies (Figure 2a–c), while for oestradiol and progesterone, the serum concentrations in twin pregnancies were significantly greater than in singleton or singleton/anembryonic pregnancies from 8–13 weeks ($P < 0.01$–0.05) (Figure 2d and e).

Heterotopic and singleton/anembryonic

The serum concentrations of HCG in week 6 ($P < 0.05$), SP-1 in weeks 6–8 ($P < 0.01$–0.05), PAPP-A in weeks 6–8 ($P < 0.01$–0.05) were greater in the singleton/anembryonic than the heterotopic pregnancies; there were no significant differences between the serum progesterone and oestradiol concentrations in the two groups (Table II).

Discussion

A role for HCG in the rescue of the corpus luteum is well established. The hormone is synthesized and secreted by the preimplantation embryo as early as 7 days post-fertilization *in vitro* (Dokras *et al.*, 1991), and there is an abundance of HCG receptors on the corpus luteum at the appropriate time during the luteal phase (Rajaniemi *et al.*, 1981). However, the absence of a direct relationship between the circulating concentrations of HCG and those of progesterone and oestradiol suggests that steroid synthetic activity of the corpus luteum is independent of HCG during pregnancy, and that other factors, possibly embryonic, placental, endometrial or intrinsic to the corpus luteum itself, may be involved (Hubinot *et al.*, 1987; Norman *et al.*, 1988; Kratzer and Taylor, 1990; Johnson *et al.*, 1993a). Indeed, that this putative modulator of corpus luteum function is derived from or regulated by the embryo is suggested by the presence of a direct relationship between the circulating concentrations of HCG and both progesterone and oestradiol in anembryonic pregnancies (Johnson *et al.*, 1993b). Thus the embryo itself, either directly or indirectly, becomes the prime determinant of steroid synthesis by the corpus luteum. This effect may occur by the expression of HCG receptors in the corpus luteum being reduced, as has been reported previously

Table II. Geometric means (range, *n*) of serum concentrations of HCG, SP-1, PAPP-A, progesterone and oestradiol between weeks 4 and 14 of gestation in singleton/anembryonic (S/A) and between 4 and 8 weeks gestation in the heterotopic pregnancies

Weeks of gestation	HCG (IU × 1000/l)		SP-1 (μg/l)		PAPP-A (μg/l)		Progesterone (nmol/l)		Oestradiol (pmol/l)	
	S/A	Heterotopic	S/A	Heterotopic	S/A	Heterotopic	S/A	Heterotopic	S/A	Heterotopic
4	0.7 (0.2–1.75) (7)	0.5 (0.1–0.9) (2)	4 (1–7) (6)	2.3 (1–7) (2)	3.4 (1–6) (6)	6 (5–7) (2)	334 (110–1150) (7)	269 (255–284) (2)	5369 (1440–14 730) (6)	4984 (2940–8450) (2)
5	4.0 (1–35) (22)	1.5 (1–3.0) (4)	60 (6–3000) (22)	10.4 (2–28) (4)	5.4 (1–27) (20)	2.5 (1–8) (4)	265 (36–720) (22)	347 (173–776) (4)	5773 (0.8–29 × 10^3) (22)	8114 (3.2–43 × 10^3) (4)
6	22.1* (2–105) (22)	6.4 (2–13) (4)	733** (33–4800) (22)	92.3 (27–210) (4)	70** (4–590) (20)	3.7 (1–12) (4)	233 (58–511) (22)	340 (165–799) (4)	6016 (1.1–18 × 10^3) (22)	7355 (2.1–34 × 10^3) (4)
7	52.6 (12–171) (22)	11.5 (2–44) (4)	2898** (340–11 000) (22)	415 (140–1430) (4)	105* (17–598) (20)	29.7 (14–64) (4)	199 (61–408) (22)	320 (137–843) (4)	6526 (1.4–16×10^3) (22)	6877 (2.6–27×10^3) (23)
8	77.7 (11–226) (19)	23.2 (3–74) (4)	7331* (3100–15 000) (19)	1569 (310–6000) (4)	332* (108–1308) (17)	76 (34–260) (4)	166 (56–340) (19)	248 (125–682) (4)	6413 (1.6–14 × 10^3) (19)	6236 (2.2–29 × 10^3) (4)
9	97.6 (47–299) (19)		11 581 (5–28 × 10^3) (18)		723 (240–2576) (17)		149 (47–318) (19)		7417 (2.7–13 × 10^3) (19)	
10	78.3 (45–298) (20)		14 391 (8.0–30 × 10^3) (19)		1404 (396–4576) (18)		145 (51–315) (20)		7248 (2.9–14 × 10^3) (20)	
11	70.7 (40–245) (18)		17 320 (6.8–32 × 10^3) (15)		2090 (800–7460) (16)		141 (57–287) (18)		8366 (3.2–13 × 10^3) (18)	
12	64.6 (19–269) (16)		21 513 (13–28 × 10^3) (13)		3520 (1160–9520) (15)		138 (58–302) (17)		10 491 (4.0–26 × 10^3) (16)	
13	58 (28–256) (13)		23 465 (15–31 × 10^3) (7)		4554 (1500–16 150) (13)		146 (71–397) (12)		11 191 (5.9–22 × 10^3) (12)	
14	33.4 (12–73) (3)		22 115 (16–26 × 10^3) (3)		6697 (3.1–49 × 10^3) (4)		125 (73–221) (3)		9604 (6420–18 230) (3)	

*Statistically significant difference of $P < 0.05$ and **$P < 0.01$ between serum concentration of a substance in singleton/anembryonic and heterotopic pregnancies at the same time point.
For abbreviations, see Table I.

(Khan-Dawood and Dawood, 1991), or via direct antagonism of HCG activity.

In the present study, the concentrations of the placental proteins were always lower in the heterotopic pregnancies than in the singleton, twin or singleton/anembryonic pregnancies, while oestradiol and progesterone concentrations in heterotopic and twin pregnancies were equivalent. Thus, the additional embryo in the heterotopic pregnancies appears to increase corpus luteum activity to the level seen in twin pregnancies, while trophoblast function is less than that in singleton pregnancies. If this were simply an effect of the presence of a second, albeit ectopic conceptus, then corpus luteum function, as shown by circulating oestradiol and progesterone, in ectopic pregnancies would be expected to be equivalent to that in normal singleton, intra-uterine pregnancies; however, this is not the case (Norman *et al.*, 1988; Johnson *et al.*, 1993c). Therefore, the interaction between trophoblast and endometrium occurring at implantation must be essential for the induction of the synthesis of a luteotrophic factor, the production of which is then regulated by the developing embryo. That the endometrium, rather than the trophoblast, is likely to be the source of this factor is suggested by the lower concentration of not only HCG, but also PAPP-A and SP-1, in heterotopic compared with singleton pregnancies. Further evidence in support of embryonic, rather than trophoblast regulation of this factor is provided by the similarity in the concentrations of all substances analysed in the singleton and singleton/anembryonic pregnancies, which suggests that the trophoblast of the additional anembryonic pregnancy does not affect its synthesis. These conclusions are based on small numbers of patients. Nevertheless, the trends are consistent in all four patients over 5 weeks of observation, and the marked contrast between the circulating concentrations of placental proteins and ovarian steroids provides additional support.

Thus, while it is established that in early pregnancy HCG synthesized by the developing trophoblast acts on the corpus luteum, preventing atresia and maintaining steroid synthesis, it seems likely that the developing embryo supercedes this effect of HCG to control corpus luteum function itself. The embryo may achieve this by regulating the synthesis of a factor produced by the endometrium in response to implantation.

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