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Corpus luteum failure in ectopic pregnancy

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The endocrinology of ectopic pregnancy was studied in order to investigate the origin of the discordance in the circulating amounts of human chorionic gonadotrophin (HCG) and those of oestradiol and progesterone. Serial maternal blood samples were obtained at 4–9 weeks gestation from 93 patients who became pregnant following in-vitro fertilization and embryo transfer including 10 ectopic, 21 anembryonic and 62 normal singleton pregnancies. The samples were analysed for HCG, Schwangerschaft protein-1 (SP-1), pregnancy-associated plasma protein-A (PAPP-A), progesterone and oestradiol. In ectopic pregnancies, concentrations of all substances analysed were significantly reduced compared to singleton pregnancies from 5 weeks gestation ($P < 0.05–0.001$) but they were not significantly different from those of anembryonic pregnancies. In ectopic pregnancies, associations were found between the concentration of both HCG and SP-1 and those of progesterone and oestradiol. No associations were found between PAPP-A and any other substances analysed. This may be due to insensitivity of the PAPP-A assay; alternatively PAPP-A concentrations may be differentially reduced in ectopic pregnancy. These findings suggest that progesterone and oestradiol are derived from the corpus luteum in early ectopic pregnancy but that the corpus luteum fails rapidly and the dominant source of both hormones becomes the trophoblast as early as 5 weeks.

Key words: corpus luteum/ectopic pregnancy/human chorionic gonadotrophin/progesterone/oestradiol

Introduction

It is widely held that in normal pregnancy human chorionic gonadotrophin (HCG) regulates corpus luteum production of progesterone and oestradiol. However, previous studies have shown that a significant relationship between these hormones exists only in anembryonic pregnancy (Johnson *et al.*, 1993a), and not in normal intra-uterine pregnancies (Yoshimi *et al.*, 1969; Tulchinsky and Hobel, 1973; Johnson *et al.*, 1993b). These findings suggest that, in the presence of a viable embryo, an embryonic factor may be the primary determinant of corpus luteum function, and it is only in the absence of this factor that HCG has a major luteotrophic role. Supportive evidence is provided by data from heterotrophic pregnancies where the circulating concentrations of oestradiol and progesterone are similar to those in normal twin pregnancies and yet HCG concentrations are less than those of singletons (Johnson *et al.*, 1993c). On the basis of these findings it was postulated that corpus luteum function is controlled by an endometrial factor, which is synthesized in response to implantation but regulated by an embryonal factor(s) (Johnson *et al.*, 1993c). The aim of the present study was to investigate this hypothesis by examining the relationship between circulating amounts of ovarian (progesterone and oestradiol) and placental [HCG, Schwangerschaft protein-1 (SP-1) and pregnancy-associated plasma protein-A (PAPP-A)] products in ectopic pregnancy.

Materials and methods

Maternal venous blood samples were obtained every week from 4 to 9 weeks gestation from 93 pregnancies achieved following ovulation induction, in-vitro fertilization (IVF) and embryo transfer. In 10 cases the pregnancies were tubal (diagnosed initially by the absence of an intra-uterine gestational sac by ultrasound in the presence of a positive pregnancy test and subsequently at operation), in 21 they were anembryonic (diagnosed by the absence of a fetal pole on ultrasound) and in 62 they were normal singleton. In the uncomplicated pregnancies, variations in patient attendance accounted for the differences in numbers at each time point, while in pathological pregnancies, patient numbers varied for the same reason and because the timing of clinical presentation varied.

The methods used for ovarian stimulation using clomiphene citrate (Clomid; Merrel Dow Pharmaceuticals Ltd, Uxbridge, Middlesex, UK; 100 mg orally), IVF and embryo transfer have been described (Sharma *et al.*, 1988). Ovarian stimulation was achieved with clomiphene citrate on days 2–6 of the 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 Laboratories) (Sharma *et al.*, 1988).

The serum was separated by centrifugation and stored within 2 h at −20°C until assayed. Serum progesterone and oestradiol were extracted with diethyl ether and measured by radioimmunoassay using tritiated antigens and monoclonal antibodies to

11α-succinyl−bovine serum albumin (BSA) or 6-carboxymethyl oxime−BSA for progesterone and oestradiol, respectively. The samples were diluted to check 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 in a non-competitive fluoroimmunoassay (Pharmacia Wallac, Milton Keynes, UK). SP-1 and PAPP-A were analysed by radioimmunoassay as described in detail elsewhere (Grudzinskas *et al.*, 1977; Sinosich *et al.*, 1982). The protocol was approved by the Research Ethics Committee of King's College Hospital.

Statistical analysis

As the data were not normally distributed, they were expressed as geometric means and further analysis was performed by non-parametric methods. The data were compared at the same time points by a Mann−Whitney *U*-test. A simple regression analysis was used to determine correlation between different substances analysed at the same time points.

Results

In ectopic pregnancies, the serum concentrations of all substances analysed were reduced when compared to the concentrations found in intra-uterine singleton pregnancies (Figure 1, Table I). Although the concentrations of the substances analysed were not consistently significantly different between the anembryonic and ectopic pregnancies, there were trends in early pregnancy for the concentration of progesterone and oestradiol to be greater in anembryonic pregnancies (Figure 1, Table I).

In ectopic pregnancy, significant associations were found between the serum concentrations of SP-1 and progesterone (Figure 2e; weeks 5, 6 and 7, $P = 0.02-0.006$), oestradiol (Figure 2e; weeks 5 and 7, $P = 0.02-0.006$) and HCG (Figure 2a; weeks, 6, 7 and 8, $P = 0.004-0.001$); significant associations were also observed between serum concentrations of HCG and those of progesterone (Figure 2b; weeks 6, 7 and 8, $P = 0.05-0.003$), and oestradiol (Figure 2b; week 7, $P = 0.02$); and between serum concentrations of progesterone and those of oestradiol (Figure 2c; weeks 5 and 7, $P = 0.02$ and 0.01, respectively). No associations were found between the serum concentrations of PAPP-A and any of the other substances analysed (Figure 2a and d). When the serum concentrations of HCG and progesterone from each week of gestation were pooled no correlation was found between them ($r = 0.105$).

Discussion

It has been suggested that HCG does not regulate corpus luteum function (Johnson *et al.*, 1993b; Lower *et al.*, 1993). This may explain the failure to detect any relationship between the circulating concentrations of progesterone and either the immunoreactive or bioactive concentrations of HCG (Hubinot *et al.*, 1987; Norman *et al.*, 1988). However, a relationship has been reported between the rate of rise of HCG and the concentration of progesterone (Kratzer and Taylor, 1990). This association may be explained if the trophoblast is the dominant source of progesterone. Thus, rising HCG, previously assumed to be luteotrophic, reflects improving trophoblast function and progesterone production. Indeed, the associations between the serum concentrations of both SP-1 and HCG, and progesterone and oestradiol support the idea that in ectopic pregnancy, progesterone and oestradiol are derived predominantly from the trophoblast. Alternatively, the failure of previous studies to detect a relationship between the circulating concentrations of HCG and progesterone may be because data from different weeks of gestation were pooled, as suggested by the absence of any association between the pooled values for HCG and progesterone in the present study.

The presence of associations between SP-1 and HCG and between progesterone and oestradiol in ectopic pregnancies is in contrast to our earlier results in anembryonic pregnancies,

Table I. Geometric means (range; *n*) of HCG, SP-I, PAPP-A, progesterone and oestradiol in control, ectopic and anembryonic pregnancies between 5 and 9 weeks gestation

Gestation (weeks)	HCG (IU × 1000/l)			SP-1 (μg/l)			PAPP-A (μg/l)			Progesterone (nmol/l)			Oestradiol (pmol/l)		
	Control	Ectopic	Anembryonic	Control	Ectopic	Anembryonic	Control	Ectopic	Anembryonic	Control	Ectopic	Anembryonic	Control	Ectopic	Anembryonic
4	0 5 (0.2−4) (15)	0.3 (0.03−6) (4)	0 2 (0.02−1.2) (9)	4 (1−48) (16)	1.3 (0.5−3 0) (4)	4 (1−48) (9)	5 (1−11) (18)	3.6 (1−10) (4)	3.0 (1−5) (10)	225 (45−618) (15)	113 (38−283) (4)	170 (41−349) (9)	3023 (630−19 280) (15)	2620 (820−8160) (4)	3390 (860−8400) (9)
5	2 6** (0.1−29) (53)	0.9 (0.2−7) (6)	0.5 (0.05−2 8) (20)	34** (1−1120) (53)	4.2 (0.6−50) (6)	8 (1−68) (19)	6 (1−71) (55)	5.1 (1−18) (6)	3.3 (1−20) (20)	237** (42−598) (54)	64.4 (33−217) (6)	161* (47−479) (20)	5602* (1110−24 120) (49)	1836 (300−9670) (6)	3610 (1100−18 960) (20)
6	14** (2−76) (61)	2.95 (1 2−12) (9)	0.7 (0.02−7.2) (21)	420** (42−4800) (61)	25.8 (1.7−410) (9)	20 (1−240) (21)	21** (2−280) (61)	4.6 (1−30) (9)	6.2 (1−32) (21)	224** (38−815) (61)	49 (20−164) (9)	68 (4−450) (21)	6077** (930−23 780) (54)	1866 (680−8180) (9)	2065 (380−20 830) (21)
7	41** (9−132) (60)	3.2 (0.42−25) (7)	1 3 (0.01−22) (19)	1786** (240−8900) (58)	80.9 (1 5−1100) (7)	47 (1−1400) (20)	97** (8−620) (59)	12.3 (4−28) (7)	11 (1−120) (20)	198** (32−820) (57)	29 (14−59) (7)	51 (5−381) (20)	6119* (900−21 910) (51)	1374 (690−8660) (7)	1244 (150−17 870) (20)
8	71** (17−217) (53)	7.6 (0.42−36) (4)	1.3 (0.02−33) (15)	4964* (1.2−17×10^3) (55)	793 (250−9622) (4)	137 (2−2400) (14)	284** (36−130) (54)	45.9 (32−64) (4)	20 (3−360) (15)	173** (60−621) (54)	29 (17−46) (4)	45 (16−241) (54)	6565** (1.6−21×10^3) (49)	1789 (1080−4540) (4)	824 (90−16 510) (14)
9	87 (33−281) (49)	23 (9.4−57) (2)	13 (1.0−38) (8)	8732* (3.0−32×10^2) (50)	900 (325−2500) (2)	1085 (89−4700) (8)	651* (127−2884) (50)	110 (85−142) (2)	85 (24−800) (7)	161* (31−525) (49)	21 (13−35) (2)	86 (18−233) (8)	6147* (1.7−22×10^3) (41)	863 (780−956) (2)	1460 (250−9200) (8)

* and ** indicate a significant difference of $P < 0.5$ and 0.01 respectively between the levels of an analyte in the control or anembryonic pregnancies and those in ectopic pregnancies.
HCG = human chorionic gonadotrophin; SP-1 = Schwangerschaft protein-1; PAPP-A = pregnancy-associated plasma protein-A.

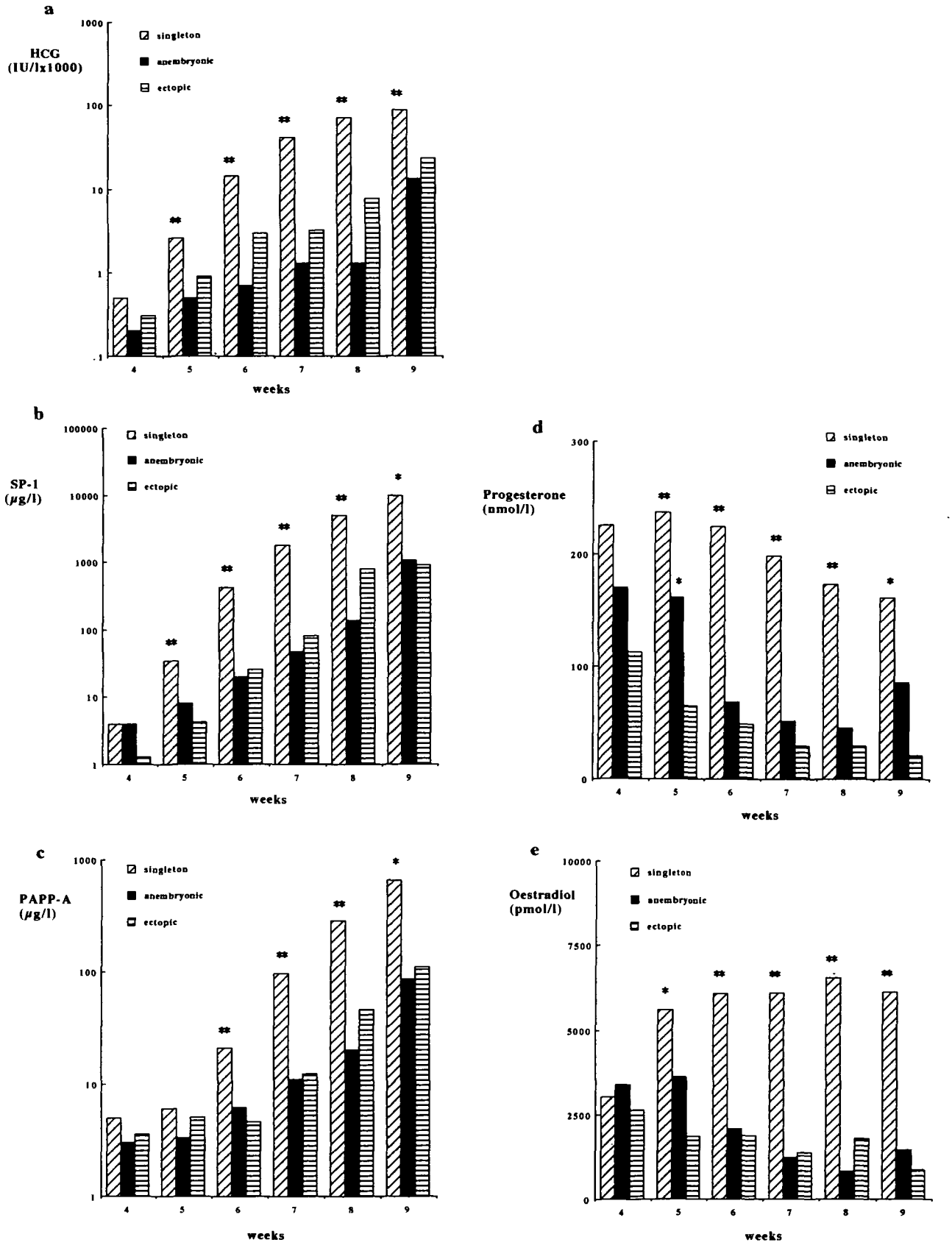

Fig. 1. The circulating concentrations of human chorionic gonadotrophin (HCG) (**a**), Schwangerschaft protein-1 (SP-1) (**b**), pregnancy-associated plasma protein-A (PAPP-A) (**c**), progesterone (**d**) and oestradiol (**e**) measured in normal, anembryonic and ectopic pregnancies plotted against weeks of gestation. Data for HCG, SP-1 and PAPP-A were log-transformed. * indicates a significant difference of $P < 0.05$, and ** indicates a significant difference of $P < 0.01$ between the circulating concentration in normal or anembryonic pregnancies and that in ectopic pregnancies.

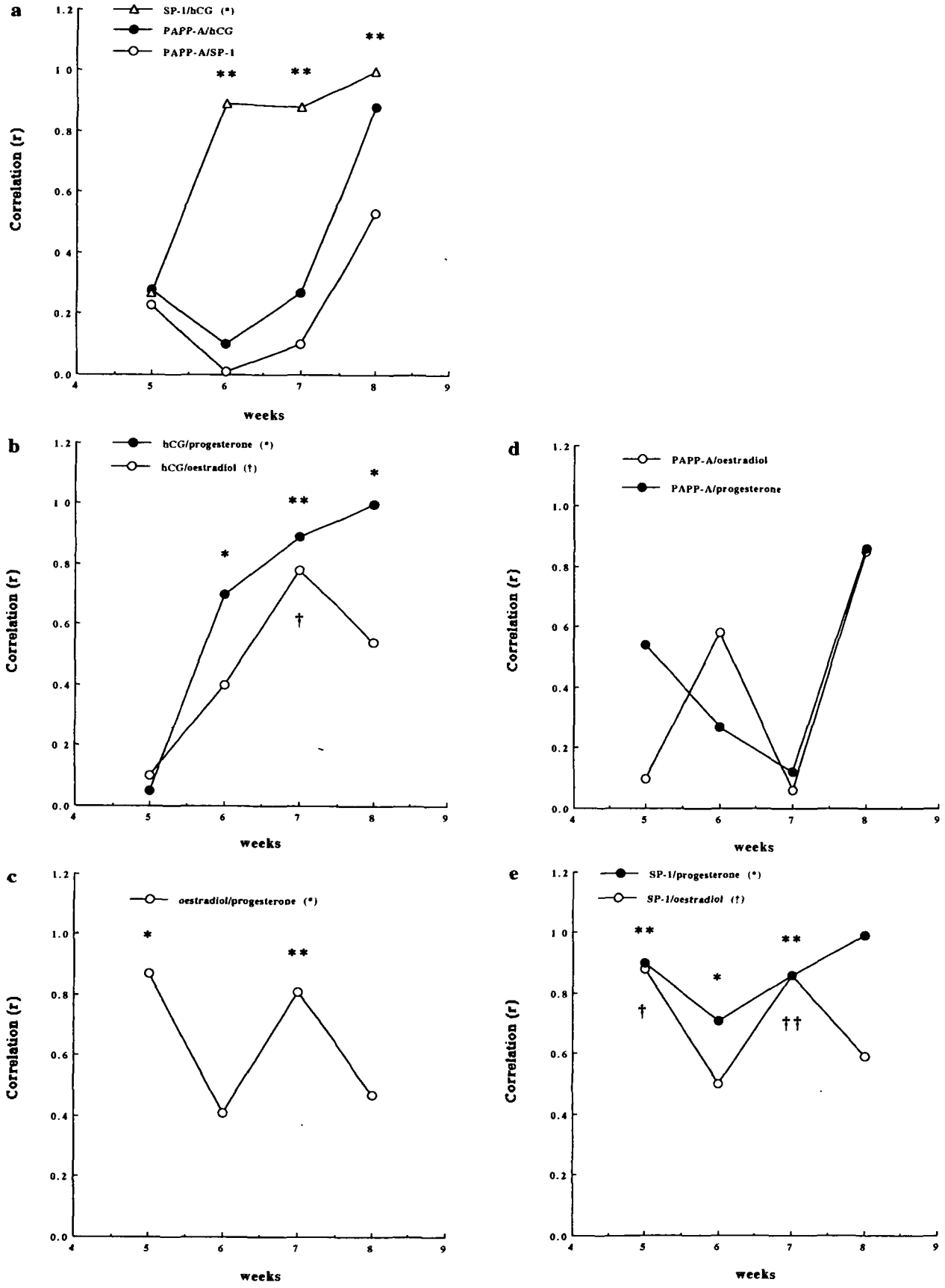

Fig. 2. The correlation coefficient *r* between the circulating concentrations of HCG, SP-1, PAPP-A, progesterone and oestradiol in ectopic pregnancies. */† indicates a significant association of $P < 0.05$, and **/†† indicates a significant association of $P < 0.01$. Particular associations tested are shown in the symbol key to each figure. Abbreviations are the same as for Figure 1.

where significant associations were found between both progesterone and oestradiol and HCG alone, suggesting that the dominant source of progesterone and oestradiol remains the corpus luteum in anembryonic pregnancies (Johnson *et al.*, 1993a), as opposed to the trophoblast in ectopic pregnancies. Ectopic and anembryonic pregnancies differ in two ways: first, the site of implantation, and second the presence of a viable embryo. Previously, we presented data to suggest that both are essential for the maintenance of normal corpus luteum steroidogenesis, and postulated that corpus luteum function is controlled by an endometrial factor, synthesized in response to implantation but regulated by embryonal factor(s) (Johnson *et al.*, 1993c). Hence, in anembryonic pregnancies, implantation in the uterus triggers the synthesis of the putative luteotrophic endometrial factor which is enhanced by the embryo until its demise at between 4 and 5 weeks gestation. In contrast, in ectopic pregnancies, as implantation is not within the uterus, the synthesis of the endometrial factor is not initiated. Thus, after its rescue by HCG, corpus luteum function would be expected to deteriorate earlier in ectopic pregnancies than in anembryonic pregnancies. This is confirmed by the demonstration of a significant reduction in the circulating concentration of progesterone in ectopic pregnancies, but not anembryonic pregnancies, both 16 days after spontaneous conception (Lower *et al.*, 1993) and 3 weeks after fertilization *in vitro* (Johnson *et al.*, 1993a), and the significantly higher concentration of progesterone in anembryonic pregnancies compared to ectopic pregnancies shown 5 weeks after fertilization *in vitro* in the present study. Hence the lower concentration of progesterone in ectopic pregnancies supports the notion that corpus luteum function is controlled by an endometrial factor, synthesized in response to implantation but regulated by embryonal factors. That the pattern of reduction in progesterone concentration was similar in anembryonic and ectopic pregnancies, whether conceived spontaneously (Lower *et al.*, 1993), or following ovulation induction as described in the present study and in an earlier study (Johnson *et al.*, 1993a), suggests that although ovulation induction results in a greater mass of luteal tissue, maintenance of this tissue is dependent on the same factors, and its demise occurs over the same time scale in situations where the production of these factors is impaired.

PAPP-A concentrations have been demonstrated to be differentially reduced in several pathological states, including miscarriage (Johnson *et al.*, 1993d), premature labour (Johnson *et al.*, 1993e) and Down's syndrome (Brambati *et al.*, 1993). The absence of any association between the circulating concentrations of PAPP-A and any of the other substances analysed in the present study may be a further manifestation of this trend for PAPP-A synthesis to be differentially affected in pathological pregnancies. Alternatively, differences in the low serum concentrations of PAPP-A in ectopic pregnancies may not be detected by the relatively insensitive PAPP-A assay.

Thus, these data suggest that the lower concentrations of oestradiol and progesterone in ectopic pregnancies are a result of the involution of the corpus luteum, and that the circulating concentrations of progesterone and oestradiol may be derived from the trophoblast rather than the corpus luteum. In addition, they lend support to the hypothesis that following its rescue the corpus luteum is regulated not by HCG but by an endometrial factor.

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