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The role of trophoblast dysfunction in the aetiology of miscarriage

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ABSTRACT

Objective To investigate the endocrine changes associated with spontaneous miscarriage after fetal heart activity has been demonstrated.

Design Prospective study during the first trimester of pregnancy comparing the circulating levels of human chorionic gonadotrophin (hCG), Schwangerschaft protein 1 (SP-1), pregnancy-associated plasma protein A (PAPP-A), oestradiol (E_2), and progesterone (P), and fetal growth (crown-rump length [CRL] and gestational sac volume [GSV]) in women who miscarried after the identification of fetal heart activity with those of normal singleton and twin pregnancies achieved following *in vitro* fertilisation (IVF) and embryo transfer (ET).

Setting The Assisted Conception Unit of King's College Hospital, London.

Subjects Nine women who miscarried after demonstration of fetal heart activity, 52 normal singleton and 22 normal twin pregnancies.

Interventions Weekly blood tests and ultrasound assessments of CRL and GSV.

Results Four fetuses (all singleton) died between 9 and 12 weeks gestation (Group 1), and seven (three singleton and two twin) died between 16 and 20 weeks gestation (Group 2). In Group 1, both fetal growth and placental function, as assessed by serial measurements of CRL and GSV, and of serum levels of PAPP-A, SP-1 and hCG respectively, were reduced before fetal death. In Group 2, while fetal growth was maintained in all but one case, placental function was reduced in 4 of 5 women.

Conclusion These findings suggest that there may be a relationship between trophoblast dysfunction and some forms of miscarriage. Furthermore, the pattern of the reduction in the circulating levels of the placental proteins in later miscarriages suggests that the function of specific cell types may be impaired.

Several studies have investigated the endocrine changes associated with spontaneous miscarriage. Most have not distinguished between pregnancies in which a fetal heart (FH) has been demonstrated, and those in which it has not (Confino *et al.* 1986; Deutinger *et al.* 1986; Bersinger *et al.* 1987; Bischof *et al.* 1989; Coddington *et al.* 1989; Whittaker *et al.* 1989). Some centres have taken serum samples from women only after their presentation with vaginal bleeding, and compared those who went on to miscarry to those who did not (Bersinger *et al.* 1987; Stabile *et al.* 1989). The consensus of these studies is that biochemical tests are unable to distinguish reliably between the two groups. Furthermore, it has been suggested that the reductions in circulating placental and ovarian hormone levels associated with miscarriage probably occur after fetal demise rather than before (Stabile *et al.* 1989). In order to clarify these points we have studied a group of women during the first trimester who had become pregnant following *in vitro* fertilisation (IVF) and embryo transfer (ET), and who then miscarried after the identification of a FH. Circulating levels of human chorionic gonadotrophin (hCG), Schwangerschaft protein 1 (SP-1), pregnancy-associated plasma protein A (PAPP-A), oestradiol (E_2) and progesterone (P), with ultrasound assessments of crown-rump length (CRL) and gestational sac volume (GSV) in this group have been compared with those in uncomplicated singleton and twin pregnancies achieved following IVF and ET.

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Subjects and methods

Nine women who had become pregnant following ovarian hyperstimulation, IVF and ET were studied, having had between 2 and 5 (median 3) embryos replaced. The 10th and 90th centiles of the circulating levels of each analyte, and the 5th and 95th centiles of the crown rump length (CRL) and gestational sac volume (GSV) were derived from control populations of singleton (n = 52) and twin (n = 22) pregnancies achieved following IVF and ET. The pregnancies of the control groups have proceeded uneventfully to at least 28 weeks. Ovarian hyperstimulation was achieved with clomiphene citrate (Clomid, Merrel Dow Pharmaceuticals Ltd, Uxbridge, Middlesex, UK; 100 mg orally on days 2 to 6 of their menstrual cycle) and either human menopausal gonadotrophin (hMG, Pergonal, 75 IU FSH and 75 IU LH per ampoule; Serono Laboratories, Welwyn Garden City, Herts, UK; 2–6 ampoules intramuscularly per day; n = 6) or purified follicle stimulating hormone (FSH; Metrodin, 75 IU FSH and 1 i.u. of LH; Serono Laboratories, Welwyn Garden City, Herts, UK; n = 3), as described previously (Sharma *et al.* 1988). Four women miscarried (all singleton) within the study period and five (three singletons and two twins) afterwards (16 to 20 weeks). FH activity was identified in all cases between 6 and 8 weeks gestation and at weekly intervals thereafter. The time of miscarriage was defined as the time at which FH activity could no longer be identified.

The women were seen at weekly intervals, ultrasound assessments performed and peripheral blood samples taken into a plain tube. The blood was allowed to clot. The serum was separated after centrifugation, and stored at −20°C within 2 h. Each sample was analysed for hCG, SP-1, PAPP-A, E2 and P.

Immunoassays

Serum P and E_2 were extracted with diethyl ether and measured by radioimmunoassay (RIA) 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) for both methods over the period of the study was less than 10%.

HCG was measured by a noncompetitive fluoroimmunoassay (Pharmacia Wallac, Milton Keynes, UK). SP-1 and PAPP-A were analysed by RIA described in detail elsewhere (Grudzinskas *et al.* 1977; Sinosich *et al.* 1982).

Ultrasonography

Ultrasound examinations were carried out at weekly intervals using the following: Diasonics DRF-1 3.5 MHz GPM-11 probe, (Bedford, UK); Phillips SDR 1500 3.5 and 5.0 MHz abdominal probes, (Hammersmith, London, UK); International General Electric RT3000 3.5 and 5.0 MHz abdominal probes (Slough, Berkshire, UK).

Statistical analysis

The data for each analyte at a given stage of gestation were log-normally distributed. Consequently, the concentrations were expressed as geometric means. Differences between the groups were assessed by nonparametric methods. The data at the same time points were compared by the Mann Whitney U test and to week 5 with the Willcox signed-rank test.

Results

The circulating levels of the analytes in each woman were plotted against the 10th and 90th centiles and the CRL and GSV against the 5th and 95th centiles of normal singleton and twin pregnancies following IVF and ET. The women were divided into two groups: Group 1 consisting of those who miscarried during the study period (patients 1 to 4); and Group 2 consisting of those who miscarried after the study period (patients 5 to 9).

Group 1

Serum PAPP-A levels in all four women, and of both SP-1 and hCG in three women, were consistently beneath the 10th centile (see Figs. 2a, 3a, 4a). In the fourth (patient 3), levels of hCG and SP-1 declined prior to the loss of the FH action at week 13 (Figs. 2a and 3a). The levels of E_2 declined in three cases before the loss of the FH activity, those of P declined in only one case (Fig. 5a and 6a). CRL was reduced in all cases prior to miscarriage, whilst GSV was reduced in three (Fig. 1a and b).

Group 2

In the three singleton pregnancies, the serum levels of hCG were beneath the 10th centile in one case only; those of SP-1 fell in two cases (6 and 7) to beneath the 10th centile and in the last (patient 5) fell between week 10 and 11, while those of PAPP-A were consistently around the 10th centile—one above (patient 5) and two below (patients 6 and 7) (see Figs. 2b, 3b, 4b). In the two twin pregnancies, the serum levels of hCG fell from around the 90th centile to the 50th; SP-1 levels in patient 9 fell from above the 90th to below the 10th centile between weeks 9 and 13; and those of PAPP-A in patient 8 were consistently beneath the 10th centile (Figs. 2c, 3c, 4c). The levels of P and E_2 remained unchanged in all cases with the exception of E_2 in patient 8, which declined between weeks 7 and 11 (Figs. 5b and c, and 6b and c). CRL was reduced in one pair of twins only and GSV was normal in all cases (Figs. 1b and d).

The pattern of vaginal bleeding was not related to hormone levels, fetal growth, or loss of fetal heart activity in either group (Table 1). Evacuation of retained products of conception (ERPC) was carried out in seven of nine cases; in each, histological confirmation of pregnancy was obtained, but karyotyping was not performed.

Discussion

Women undergoing a miscarriage typically present with vaginal bleeding and pain, consequent to endometrial

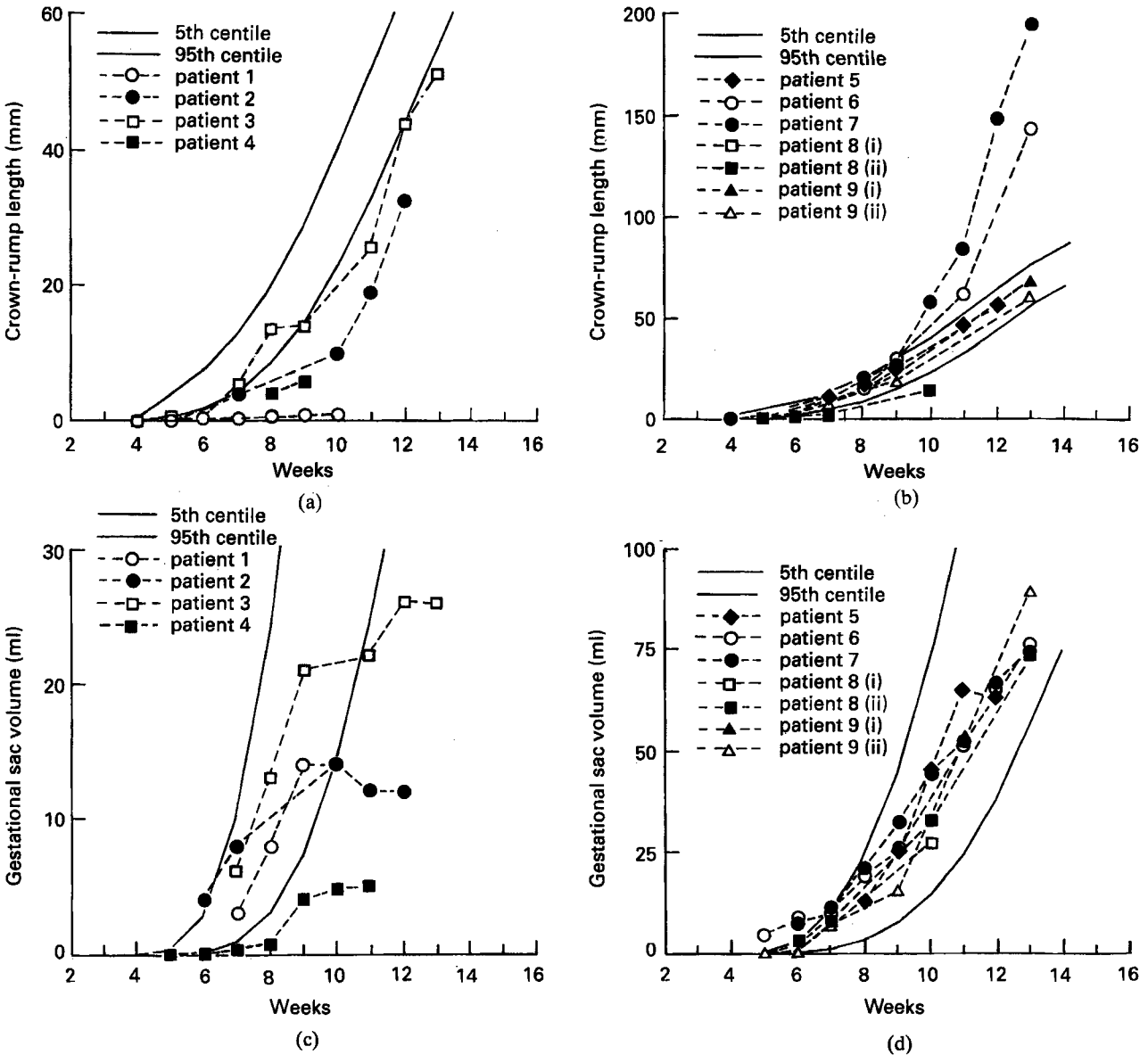


Fig. 1. Crown rump length (CRL) and gestational sac volume (GSV) of pregnancies which spontaneously miscarried following demonstration of fetal heart activity, plotted against the 5th and 95th centiles for CRL and GSV of the control population. Fig. 1a, CRL and Fig. 1b, GSV of pregnancies which miscarried between 10 and 12 weeks; patient 1, at 10 weeks; patient 2, at 12 weeks; patient 3, at 12 weeks; and patient 4, at 10 weeks; and Fig. 1c, CRL and Fig. 1d, GSV of cases 5 to 9 which miscarried between 16 and 20 weeks gestation.

breakdown and uterine contractions, both of which may be induced by falling levels of circulating progesterone (Csapo & Pulkkinen 1978). After the sixth week of gestation the trophoblast is considered to be the principal source of E_2 and P in naturally conceived pregnancies (Csapo & Pulkkinen 1978); hence, miscarriage after this time is due to failing placental function, either secondary to fetal abnormality or demise or as a result of primary trophoblast dysfunction. The former suggestion carries with it the implication that maintenance of the trophoblast is dependent on the presence of a viable fetus, which is supported by the spontaneous abortion of anembryonic pregnancies. This study has demonstrated that trophoblast function, as assessed by the circulating levels of hCG, SP-1 and PAPP-A, decreased prior to fetal death. Thus, it is more likely that miscarriage is precipitated by trophoblast, rather than fetal, dysfunction. This finding is supported further by the reduced circulating levels of placental factors seen when fetal growth was maintained in four of five cases of Group 2. Alternatively, mechanisms which are responsible for fetal demise may also cause trophoblast dysfunction which is detected more easily or earlier.

Fetal growth, judged by CRL, but not GSV, was impaired prior to loss of fetal heart activity in all women in Group 1, while in Group 2 fetal growth was normal in all

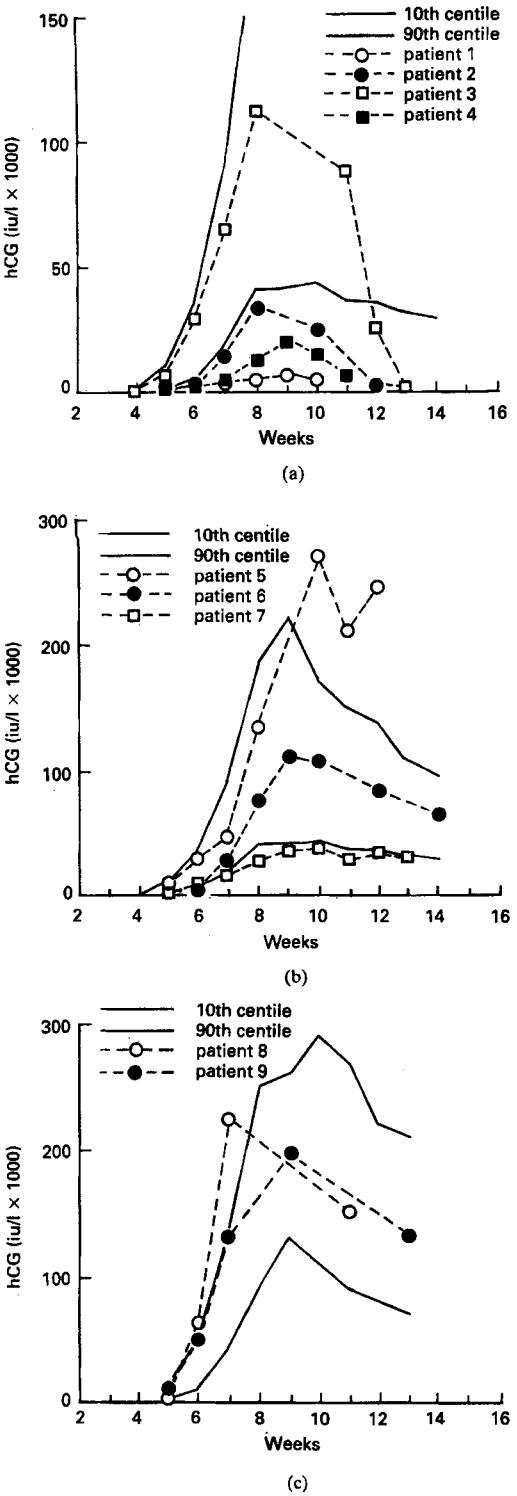


Fig. 2. Serum human chorionic gonadotrophin (hCG) levels of each pregnancy which miscarried after the demonstration of fetal heart activity, plotted against the 10th and 90th centiles of serum hCG levels of the control groups. Fig. 2a, singleton pregnancies which miscarried between 10 and 12 weeks; patient 1, at 10 weeks; patient 2, at 12 weeks; patient 3, at 12 weeks; and patient 4, at 10 weeks; Fig. 2b, singleton pregnancies which miscarried between 16 and 20 weeks gestation; and Fig. 2c, twin pregnancies which miscarried between 16 and 20 weeks gestation.

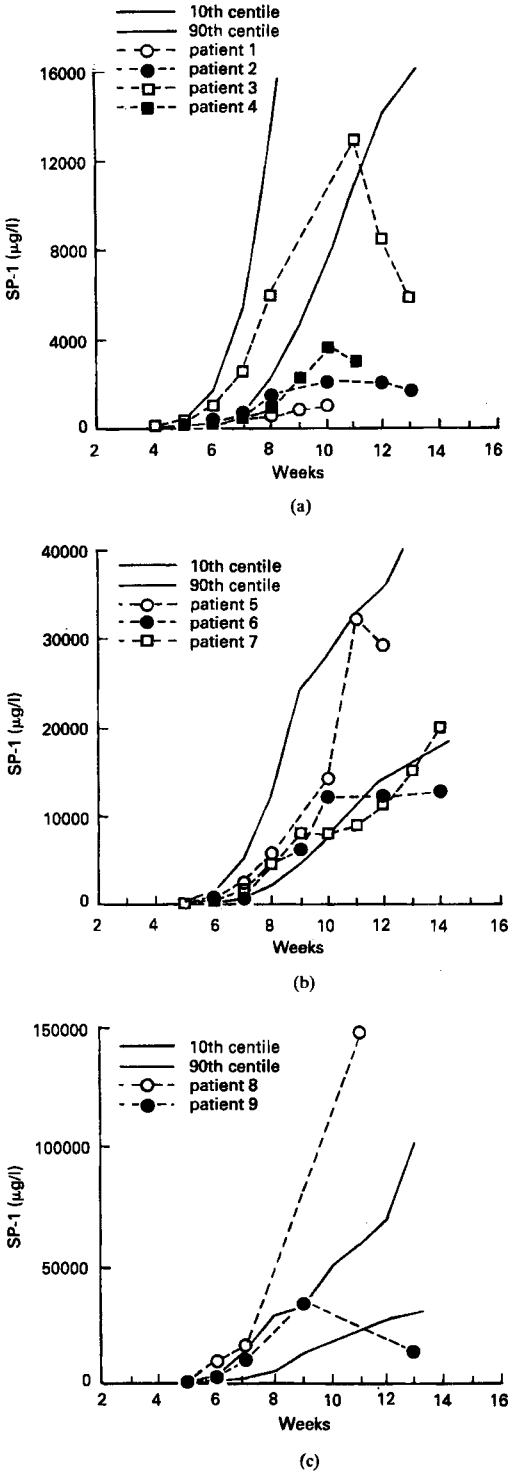
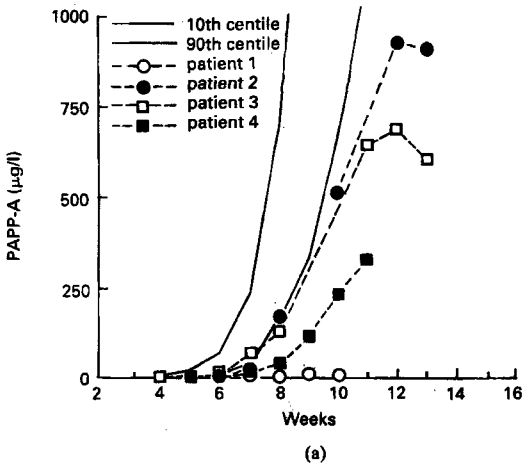
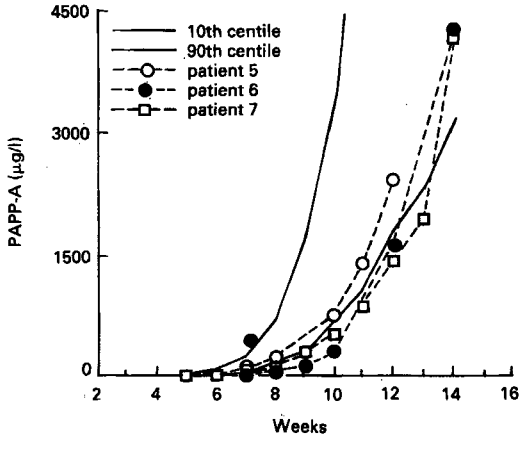
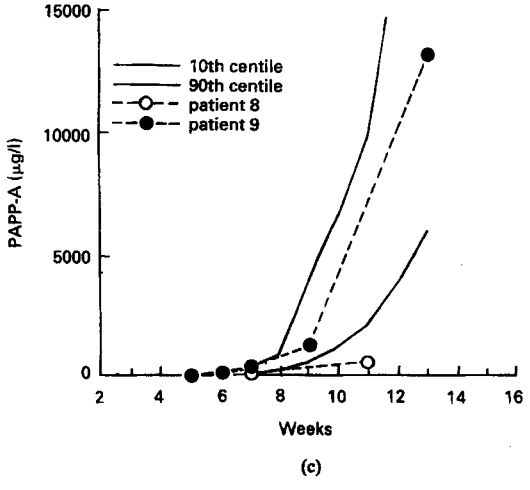
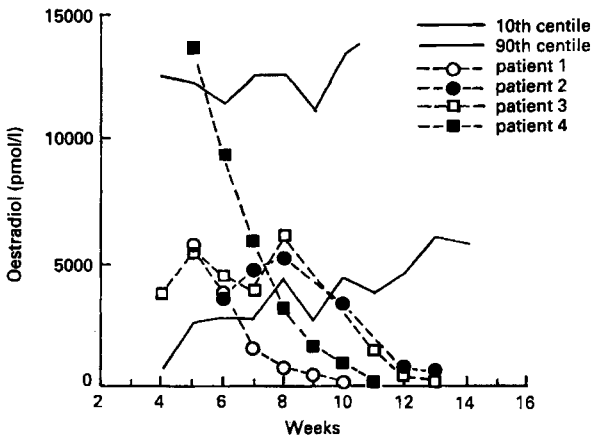


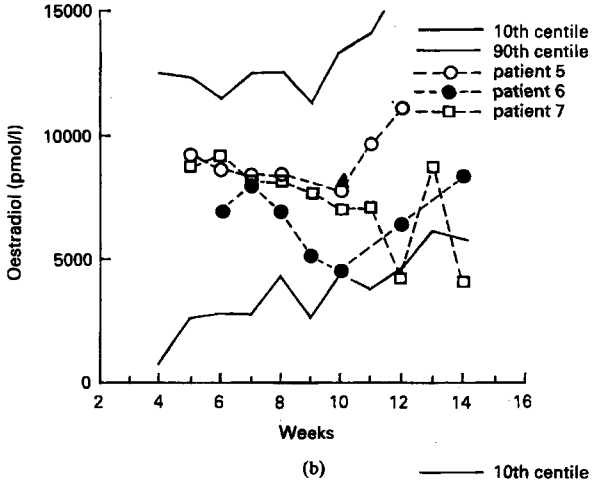
Fig. 3. Serum Schwangerschaft-protein (SP-1) levels of each pregnancy which miscarried after the demonstration of fetal heart activity, plotted against the 10th and 90th centiles of serum SP-1 levels of the control groups. Fig. 3a, singleton pregnancies which miscarried between 10 and 12 weeks; patient 1, at 10 weeks; patient 2, at 12 weeks; patient 3, at 12 weeks; and patient 4, at 10 weeks; Fig. 3b, singleton pregnancies which miscarried between 16 and 20 weeks gestation; and Fig. 3c, twin pregnancies which miscarried between 16 and 20 weeks gestation.











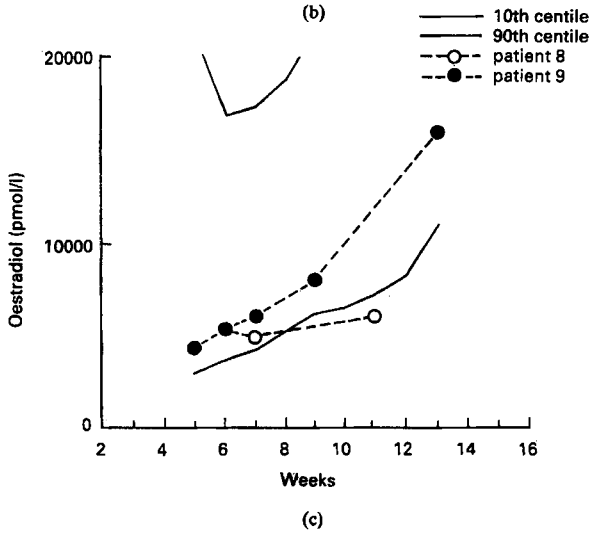


Fig. 4. Serum pregnancy-associated plasma protein A (PAPP-A) levels of each pregnancy which miscarried after the demonstration of fetal heart activity, plotted against the 10th and 90th centiles of serum PAPP-A levels of the control groups. Fig. 4a, singleton pregnancies which miscarried between 10 and 12 weeks; patient 1, at 10 weeks; patient 2, at 12 weeks; patient 3, at 12 weeks; and patient 4, at 10 weeks; Fig. 4b, singleton pregnancies which miscarried between 16 and 20 weeks gestation; and Fig. 4c twin pregnancies which miscarried between 16 and 20 weeks gestation.

Fig. 5. Serum oestradiol (E_2) levels of each pregnancy which miscarried after the demonstration of fetal heart activity, plotted against the 10th and 90th centiles of serum E_2 levels of the control groups. Fig. 5a, singleton pregnancies which miscarried between 10 and 12 weeks; patient 1, at 10 weeks; patient 2, at 12 weeks; patient 3, at 12 weeks; and patient 4, at 10 weeks; Fig. 5b, singleton pregnancies which miscarried between 16 and 20 weeks gestation; and Fig. 5c, twin pregnancies which miscarried between 16 and 20 weeks gestation.

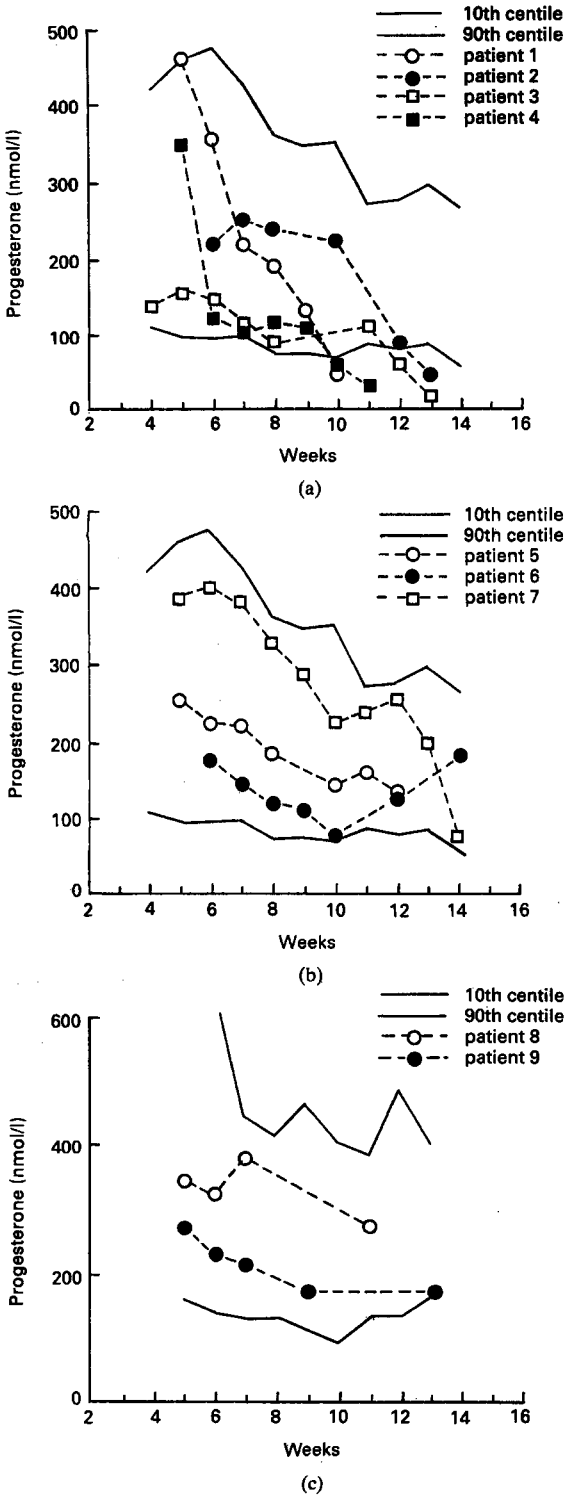


Fig. 6. Serum progesterone (P) levels of each pregnancy which miscarried after the demonstration of fetal heart activity, plotted against the 10th and 90th centiles of serum P levels of the control groups. Fig. 6a, singleton pregnancies which miscarried between 10 and 12 weeks; patient 1, at 10 weeks; patient 2, at 12 weeks; patient 3, at 12 weeks; and patient 4, at 10 weeks; Fig. 6b, singleton pregnancies which miscarried between 16 and 20 weeks gestation; and Fig. 6c, twin pregnancies which miscarried between 16 and 20 weeks gestation.

but one. That fetal growth is maintained in some despite the concomitant reduction in the circulating level of some of the placental factors suggests that the placenta possesses a degree of functional reserve. In addition, the ultrasound data in this group, in which the timing of conception is well defined, illustrate that the common practice of estimating gestational age by embryonic or fetal size may be misleading. If this practice had been followed here then the circulating levels of the analytes in two of the four women in Group 1 could have been classified as normal prior to miscarriage because of the smaller embryonic size, and consequently, would have altered the interpretation of the biochemical measurements.

It has been suggested that the reduced rate of implantation following superovulation and IVF–ET may be due to elevated oestrogen levels (Forman *et al.* 1988). If the process of implantation is impaired by high circulating levels of oestrogens, then those women with higher levels would be expected to miscarry more frequently. However, high serum oestradiol levels in the normal control population were not associated with lower circulating levels of the placental proteins (Johnson *et al.* 1993); and in the present study only one woman had circulating levels greater than the 90th centile at five weeks gestation, suggesting that oestradiol levels during early pregnancy are not a factor in the aetiology of miscarriage. However, whether or not they are important during the luteal phase has not been examined.

The presence of vaginal bleeding bore no relation to any of the other parameters assessed in this study. It occurred when progesterone levels were greater and less than the 10th centile, and thus its aetiology is unlikely to be only that of endometrial breakdown, although an alteration in the sensitivity of the endometrium to progesterone may be of importance.

In pregnancies achieved following IVF the circulating levels of hCG have been shown to correlate well with those of SP-1 and PAPP-A (Johnson *et al.* 1993). These changes were considered to indicate that the placental proteins have a common source or that their synthesis and release were controlled by the same factors. In the present study two patterns in the circulating levels of the placental proteins were observed. In three of four pregnancies in which the FH was lost during the study period, PAPP-A, SP-1 and hCG were low; in the fourth case (which miscarried last) PAPP-A alone was reduced. In women who miscarried after the study period, PAPP-A levels were beneath the 10th centile in three, SP-1 levels were low in two of these and fell in the remaining two and those of hCG were beneath the 10th centile in only one. Thus, pregnancies which miscarried in the first trimester were characterised by a reduction in the circulating levels of all placental proteins, but in those women miscarrying later, this was found in one case only. The synthesis of placental proteins may be localised to specific cell types within the placenta, although regulated by the same factors; thus, dysfunction of a given cell type may be associated with a reduction in the circulating level of one or other protein. Possibly miscarriages of different aetiologies may be associated with the dysfunction of specific cell types and

Table 1. The clinical data on nine patients that miscarried after demonstration of fetal heart activity (FHA). The first figure in the column headed fetal heart activity represents the last time that FHA was seen, and the second the time at which FHA was found to be absent. When ERPC was not performed the figure in parenthesis gives the date of miscarriage. The figures in parenthesis in the column headed serum hormone levels represent the gestational age at which the serum level first fell beneath the 10th centile of the control population.

Case no.	Onset and duration of bleeding (weeks)	Fetal heart activity last seen/not seen (weeks)	ERPC (weeks)	Serum hormones levels first beneath 10th centile
1	5–7	9/10	10	hCG (5), SP-1 (6), PAPP-A (6), P (10), E_2 (7)
2	8–13	10/12	13	hCG (5), SP-1 (6), PAPP-A (6), P (11), E_2 (11)
3	7–9	11/12	13	hCG (12), SP-1 (12), PAPP-A (8), P (12), E_2 (12)
4	None	9/10	11	hCG (5), SP-1 (6), PAPP-A (6), P (10), E_2 (8)
5	6–9	12/-	None (16)	hCG (-), SP-1 (-), PAPP-A (-), P (-). E_2 (-)
6	None	13/-	None (16)	hCG (-), SP-1 (12), PAPP-A (7), P (-), E_2 (-)
7	None	14/-	19	hCG (7), SP-1 (7), PAPP-A (6), P (-), E_2 (12)
8	7–8	11/-	18	hCG (-), SP-1 (-), PAPP-A (7), P (-), E_2 (11)
9	11–12	13/-	20	hCG (-), SP-1 (13), PAPP-A (-), P (-), E_2 (-)

hence, with different patterns of reduction in the circulating levels of placental proteins. These findings are similar to those seen in a series of women with spontaneous abortion following natural conception, in whom PAPP-A levels were reduced most frequently (Westergaard *et al.* 1985). However, whether the relationships between the circulating levels of placental proteins and fetal growth/demise is the same in pregnancies which miscarry following spontaneous conception is unknown, although the similarity in the placental protein levels in spontaneous miscarriage described above (Westergaard *et al.* 1985) suggests that this may be the case.

Trophoblast function has been shown to deteriorate prior to embryonic or fetal demise in this series of women with spontaneous abortion, and the pattern of the reduction in the circulating levels of placental proteins suggests that differential trophoblast dysfunction occurs in association with miscarriage.

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