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Placental and ovarian hormones in anembryonic pregnancy

M.R.Johnson[1,5], A.F.Riddle[3], V.Sharma[3], W.P.Collins[3], K.H.Nicolaides[2] and J.G.Grudzinskas[4]

[1]Neuroendocrinology Unit, Charing Cross and Westminster Medical School, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, [2]The Harris Birthright Research Centre for Fetal Medicine and [3]The Department of Obstetrics and Gynaecology, King's College School of Medicine and Dentistry, Denmark Hill, London SE5 8RX and [4]Academic Unit of Obstetrics and Gynaecology, The Royal London Hospital, Whitechapel, London E1 1BB, UK

[5]To whom correspondence should be addressed

The circulating levels of human chorionic gonadotrophin (HCG), pregnancy-associated plasma protein-A (PAPP-A), Schwangerschaft protein 1 (SP-1), oestradiol and progesterone were measured in 81 pregnant patients between 4 and 11 weeks gestation, following in-vitro fertilization and embryo transfer. The patients were divided as follows: singleton anembryonic pregnancies, $n = 22$; singleton pregnancies which spontaneously aborted following the demonstration of fetal heart activity, $n = 7$; and normal singleton pregnancies, $n = 52$. The levels of all substances measured were significantly reduced in women with anembryonic compared to those with singleton pregnancies which proceeded to term. The serum levels of SP-1, weeks 6–8 ($P < 0.01$); HCG, weeks 6–8 ($P < 0.05$); oestradiol, weeks 5–8 ($P < 0.05$) and progesterone, weeks 6–8 ($P < 0.05$), were lower in anembryonic pregnancies than in those of pregnancies which spontaneously aborted. These differences may be a reflection of the fact that miscarriage, after the demonstration of fetal heart activity, represents fetal demise at a later stage in pregnancy. In anembryonic pregnancies, significant associations were found between HCG and both oestradiol and progesterone levels from weeks 6 and 8, suggesting that in the absence of an embryo, HCG is the prime determinant of steroid synthesis by the corpus luteum.

Key words: anembryonic pregnancy/ovarian hormones/placental hormones/Schwangerschaft protein 1

Introduction

An anembryonic pregnancy is characterized by the failure to demonstrate a fetal pole or fetal heart activity on ultrasound and the absence of embryonic tissue on microscopic examination of tissues removed from the uterus (Rushton, 1988). These findings have led to the assumption that anembryonic pregnancies develop from a conceptus without any embryonic tissue, a view which was challenged by the demonstration of elevated levels of α-fetoprotein in the circulation of women with anembryonic pregnancies (Stabile *et al.*, 1989), and by the similarity in the circulating levels of human chorionic gonadotrophin (HCG), human placental lactogen, oestradiol and progesterone in anembryonic pregnancies compared to pregnancies which miscarried after the demonstration of fetal heart activity (Whittaker *et al.*, 1989). These studies suggested that embryonic development did occur, but that it was arrested in early pregnancy and embryonic tissues subsequently resorbed.

Our recent work has focused on the relationship between fetus, trophoblast and corpus luteum. In singleton pregnancies, achieved following in-vitro fertilization and embryo transfer (IVF–ET), we found that HCG had little or no relationship with the circulating levels of progesterone derived from the corpus luteum and suggested that other factors may be involved in the modulation of corpus luteum function (Johnson *et al.*, 1993a). In the same study we demonstrated the existence of associations between the circulating levels of the placental proteins, HCG, pregnancy-associated plasma protein-A (PAPP-A) and Schwangerschaft protein 1 (SP-1). By contrast, in ectopic pregnancies we observed that trophoblast maturation was impaired, possibly due to poor blood supply, or the absence of direct contact with normal endometrium (Riddle *et al.*, 1992).

In order to investigate the hypothesis that there is no difference in the endocrine status of women who will undergo a spontaneous abortion after fetal heart activity has been demonstrated and those with an anembryonic pregnancy, and to study the interactions between the corpus luteum and trophoblast, we have measured the circulating levels of HCG, SP-1, PAPP-A, oestradiol and progesterone between 4 and 11 weeks gestation in anembryonic pregnancies, in pregnancies which miscarried after the demonstration of fetal heart activity and in normal singleton pregnancies, all achieved following IVF–ET.

Materials and methods

Patients

A total of 81 patients who had become pregnant following IVF–ET were studied at weekly intervals from the diagnosis of pregnancy to 11 weeks gestation. The patients were divided as follows: anembryonic pregnancy, $n = 22$ (age range 26–38 years, median 32); singleton pregnancies which spontaneously aborted following the demonstration of fetal heart activity, $n = 7$ (age range 22–38 years, median 33); and normal singleton pregnancies, $n = 52$ (age range 22–39 years, median 32). The indications for IVF were tubal disease, unexplained infertility and male factor (or a combination); the distribution was equal between groups. The methods used for ovarian stimulation and IVF–ET have been described previously (Sharma *et al.*, 1988).

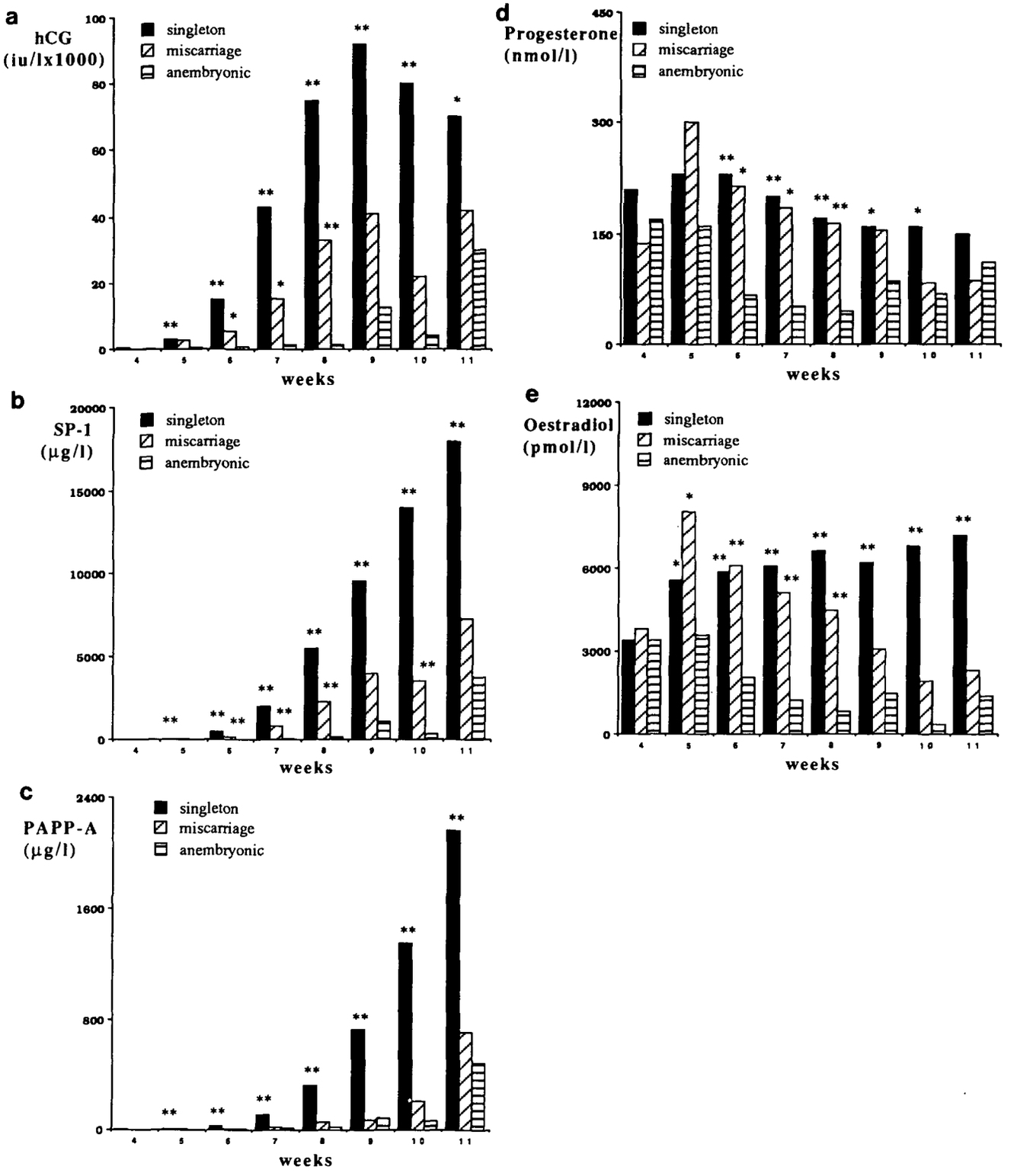

Fig. 1. The geometric means of the circulating human chorionic gonadotrophin (HCG) (**a**), Schwangerschaft protein 1 (SP-1) (**b**), pregnancy-associated plasma protein-A (PAPP-A) (**c**), progesterone (**d**) and oestradiol (**e**) levels in singleton and anembryonic pregnancies, and in pregnancies which miscarried after the demonstration of fetal heart activity, between 4 and 11 weeks of gestation. * Denotes a significant difference of $P < 0.05$ and ** of $P < 0.01$ between the serum levels in anembryonic pregnancies in comparison with either normal pregnancies or pregnancies destined spontaneously to miscarry.

Venous blood samples were obtained at one week intervals from 4 weeks gestation (oocyte retrieval plus 2 weeks) until week 11. Blood was collected into plain tubes, the serum separated by centrifugation and stored at −20°C within 2 h. Samples were analysed for HCG, PAPP-A, SP-1, oestradiol and progesterone.

An ultrasound was performed at each visit (Diasonics DRF-1, Bedford, UK, 3.5 MHz GPM-11 probe; Phillips SDR 1500, Hammersmith, London, UK, 3.5 and 5.0 MHz abdominal

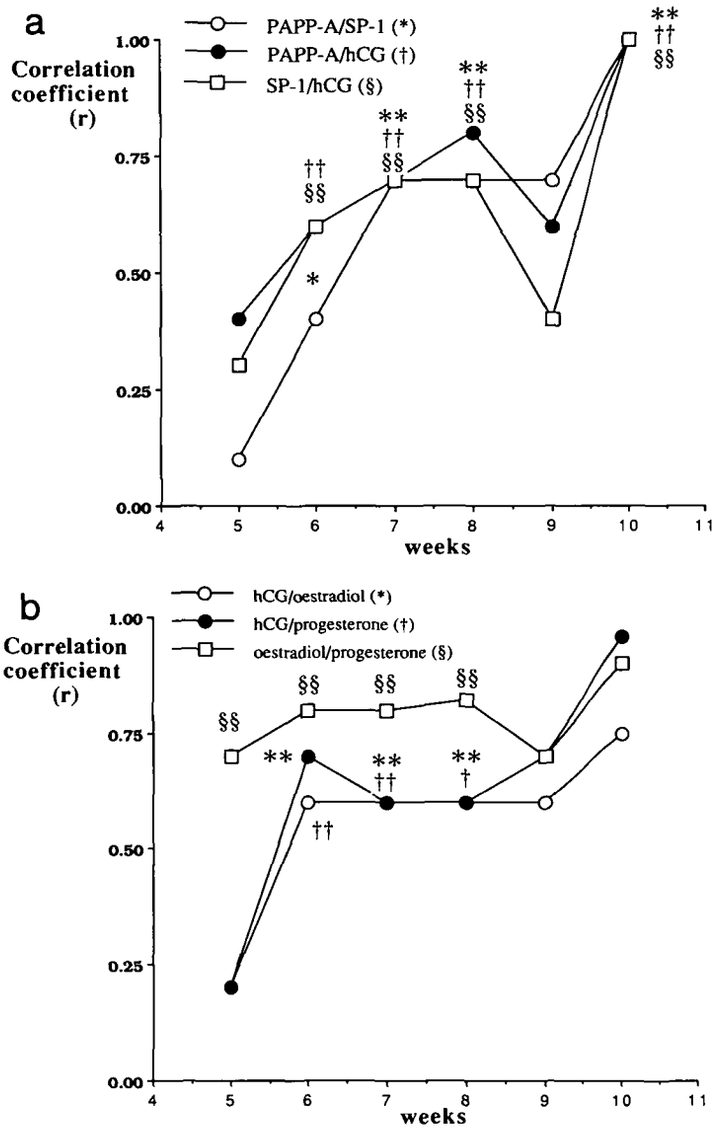

Fig. 2. (**a**) Correlation coefficient, *r*, plotted against time, between the circulating levels of HCG, PAPP-A and SP-1 (see legend to Figure 1), of each individual with anembryonic pregnancy at each time point. * Denotes a significant correlation of $P < 0.05$ and ** a significant correlation of $P < 0.01$ between PAPP-A and SP-1, † denotes a significant correlation of $P < 0.05$ and †† a significant correlation of $P < 0.01$ between PAPP-A and HCG, and § denotes a significant correlation of $P < 0.05$ and §§ a significant correlation of $P < 0.01$ between SP-1 and HCG. (**b**) Correlation coefficient, *r*, plotted against time, between the circulating levels of human chorionic gonadotrophin (HCG), oestradiol and progesterone, of each individual with anembryonic pregnancy at each time point. * Denotes a significant correlation of $P < 0.05$ and ** a significant correlation of $P < 0.01$ between HCG and oestradiol, † denotes a significant correlation of $P < 0.05$ and †† a significant correlation of $P < 0.01$ between HCG and progesterone, and § denotes a significant correlation of $P < 0.05$ and §§ a significant correlation of $P < 0.01$ between oestradiol and progesterone.

probes; International General Electric RT3000, Slough, Berkshire, UK, 3.5 and 5.0 MHz abdominal probes). An anembryonic pregnancy was suspected when a fetal pole could not be identified with a gestational sac volume of >2.5 ml, and the diagnosis was confirmed at subsequent ultrasound examinations. The protocol was approved by the Research Ethics Committee of King's College Hospital.

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 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 as described previously (Grudzinskas *et al.*, 1977; Sinosich *et al.*, 1982).

Statistical analysis

The data for each substance measured and the stage of gestation were log-normally distributed. Consequently, the concentrations were expressed as geometric means. Differences between the groups were assessed by the Mann–Whitney U-test. A simple regression analysis was used to determine the correlation between different substances measured at the same time points (weeks 5–10).

Results

The levels of all measured compounds were significantly lower in anembryonic compared to those in singleton pregnancies from week 5 for HCG, SP-1, PAPP-A and oestradiol, and week 6 for progesterone ($P < 0.01$) (Figure 1). The levels of SP-1, weeks 6–8 ($P < 0.01$), HCG, weeks 6–8 ($P < 0.05$), oestradiol, weeks 5–8 ($P < 0.05$) and of progesterone, weeks 6–8 ($P < 0.05$), were lower in anembryonic pregnancies than in those of pregnancies which spontaneously aborted after the demonstration of fetal heart activity (Figure 1).

In women with anembryonic pregnancies, correlations between the levels of individual substances at the same time points were high between the placental proteins SP-1, HCG and PAPP-A from weeks 6 and 8 (Figure 2a), between HCG and both oestradiol and progesterone from weeks 6 and 8 (Figure 2b), and between oestradiol and progesterone from weeks 5–8 (Figure 2b).

Discussion

In IVF pregnancies the predominant source of progesterone remains the corpus luteum until as late as 10–11 weeks (Johnson *et al.*, 1993a). We observed that while the serum placental proteins and oestradiol levels were reduced in pregnancies which spontaneously abort after the demonstration of fetal heart activity, those of progesterone were maintained and that there were no significant associations between the serum HCG levels and those of either oestradiol or progesterone (Johnson *et al.*, 1993b). From these and earlier data, in which we found no significant association between the levels of HCG and either oestradiol or progesterone in normal singleton IVF pregnancies (Johnson *et al.*, 1993a), we concluded that following rescue of the corpus luteum,

HCG did not seem to influence its function. In the present study the mean serum levels of SP-1, HCG, oestradiol and progesterone were lower in anembryonic pregnancies compared to a group of pregnancies which miscarried after demonstration of a fetal heart. This shows that the two groups are endocrinologically clearly distinct and in addition, that progesterone production by the corpus luteum may be enhanced by the presence of an embryo. The absence of any significant difference in the levels of PAPP-A between the two groups is of interest, and with the recent observation that PAPP-A is reduced in pregnancies with chromosomal abnormalities (Brambati *et al.*, 1993), suggests that either PAPP-A is a non-specific marker of a pathological pregnancy or that chromosomal abnormalities are of equivalent aetiological importance in both types of pregnancies.

In previous studies, the associations between assays of different substances in the first trimester have been investigated. In ectopic pregnancies (Riddle *et al.*, 1992), spontaneous abortions after the identification of a fetal heart (Johnson *et al.*, 1993b), and normal pregnancies achieved following IVF (Johnson *et al.*, 1993a), high correlations were found between HCG and SP-1. In normal pregnancies and spontaneous miscarriages, these associations were maintained throughout the first trimester. Associations between SP-1 and PAPP-A, from 6 weeks, and between HCG and PAPP-A, from 8 weeks, were found in normal pregnancies, but not in ectopic pregnancies (Johnson *et al.*, 1993a; Riddle *et al.*, 1992). In the present study, associations between all three substances (HCG, SP-1, PAPP-A) were found between weeks 6 and 8, suggesting that the development trophoblast of anembryonic pregnancy is normal.

In normal pregnancies and in pregnancies which miscarried after the demonstration of fetal heart activity, a relationship was not found between HCG and either oestradiol or progesterone until the placenta had become the dominant source of all three substances (Johnson *et al.*, 1993a,b). In contrast, in the present study correlations were found between HCG and both oestradiol and progesterone from 6 to 8 weeks gestation in anembryonic pregnancies. This suggests that at some point after corpus luteum rescue by HCG, the embryo takes over the control of corpus luteum steroid synthesis, but that in the absence of an embryo, HCG continues to determine corpus luteum function. Thus, the fact that no relationship was found between HCG and either oestradiol or progesterone at week 5 supports the idea that an embryo is present at an early stage in the development of an anembryonic pregnancy. An alternative explanation of the correlations found between HCG and both oestradiol and progesterone is that the corpus luteum has failed and that all three substances are derived from the trophoblast alone. However, since no associations were found between the circulating levels of either oestradiol or progesterone and those of either SP-1 or PAPP-A, this is unlikely. The presence of correlations between oestradiol and progesterone is probably a reflection of their common ovarian origin.

In anembryonic pregnancies occurring after spontaneous conception, the levels of progesterone are significantly reduced on day 16 post-conception compared with HCG-matched controls. No difference, however, was observed in anembryonic pregnancies achieved following IVF–ET (Lower *et al.*, 1992). The latter is confirmed in the present study and may occur because maturation of the conceptus is delayed in pregnancies achieved following IVF–ET, or because the process of ovulation induction with or without luteal support masks the difference.

These data support earlier suggestions that corpus luteum function is controlled by factors other than HCG. Furthermore they suggest that one of these factors may be derived from the embryo. In addition, they demonstrate that trophoblast and corpus luteum function is impaired more markedly in anembryonic pregnancies than in pregnancies which miscarry after the demonstration of fetal heart activity.

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