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Maternal serum concentrations of pregnancy associated placental protein A and pregnancy specific β-1-glycoprotein in multifetal pregnancies before and after fetal reduction

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Placental function in multifetal pregnancies before and after embryo reduction was investigated by measuring maternal serum concentrations of pregnancy associated placental protein-A (PAPP-A) and pregnancy specific β-1-glycoprotein (SP-1). Three groups of pregnant women were studied following assisted reproduction; groups 1 and 2, were 12 singleton and 12 twin pregnancies respectively, and group 3 comprised 12 women with multifetal pregnancies undergoing embryo reduction. PAPP-A and SP-1 were measured serially at 8–21 weeks gestation. In all pregnancies, maternal serum PAPP-A and SP-1 increased with gestation. In twin pregnancies the mean concentrations of SP-1 were significantly higher than in singletons at all gestations, whereas for PAPP-A, concentrations were similar between these groups. In multifetal pregnancies before embryo reduction, the serum concentrations of both proteins were significantly higher than in twin pregnancies. Following reduction, the concentrations of PAPP-A remained significantly higher than for twins throughout, whereas the concentrations of SP-1 gradually converged towards those of twins; by 19 weeks there was no difference between the means of the two groups. These findings suggest that circulating concentrations of SP-1 reflect total placental mass, which is proportional to the number of live fetuses, whereas the pattern of PAPP-A changes suggests that this protein is produced by the placenta, decidua and other tissues.

Key words: embryo reduction/multifetal pregnancy/pregnancy associated placental protein A/pregnancy-specific β-1-glycoprotein

Introduction

Pregnancy-specific β-1-glycoprotein (SP-1) is produced by the syncytiotrophoblast and is largely secreted into the maternal circulation, where it is detectable as early as 6 days after ovulation (Gordon *et al.*, 1977). Unlike SP-1, which is purely placental in origin, pregnancy associated placental protein A (PAPP-A) is both placental and decidual in origin and the maximum secretory capacity is determined by both sources (Lin and Halbert, 1976; Schindler *et al.*, 1984; Rosen, 1986). In the maternal circulation PAPP-A increases progressively from the seventh to the 40th week of pregnancy, whereas SP-1 reaches a plateau at around the 35th week, corresponding to the gestation when there is a diminishing rate of placental growth (Gordon *et al.*, 1977; Bischof, 1989). Recent studies also suggest that the concentrations of these proteins may be altered in pregnancies affected by fetal trisomies (Bersinger *et al.*, 1994).

The aim of the study was to investigate placental function in multifetal pregnancies before and after embryo reduction, by measuring maternal serum PAPP-A and SP-1.

Materials and methods

Three groups of women with pregnancies achieved by assisted reproduction were studied. Groups 1 and 2 included 12 singleton and 12 twin pregnancies achieved after in-vitro fertilization (IVF); blood samples were obtained on five occasions between 8 and 21 weeks gestation. Group 3 comprised 12 women with multifetal pregnancies (three fetuses, $n = 2$; four fetuses, $n = 5$; five fetuses, $n = 2$; six fetuses, $n = 2$; eight fetuses, $n = 1$). In this group ultrasound-guided embryo reduction to twins was performed by transabdominal injection of potassium chloride into the fetal heart. Maternal blood was obtained from all 12 cases immediately before embryo reduction at 8–11 weeks and at subsequent visits at 10–12 weeks, 15–17 and 19–21 weeks. Ultrasound demonstrated complete resorption of the dead fetuses and their placentas 8–12 weeks after reduction. All patients gave informed consent to participate in the study.

PAPP-A was measured by double sandwich time-resolved immunofluorometric assay (TR-IFMA) as described previously (Bischof, 1992) with chelated europium as a label and SP-1 was measured by an enzyme-linked immunosorbent assay (ELISA) method developed in our laboratory and using commercially available reagents. The sensitivity of the assay was 0.02 IU/l and the intra- and inter-assay variations were 8.2 and 11.6% respectively.

Serum concentrations of PAPP-A and SP-1 were logarithmically transformed to adjust for heterogeneity of variance and achieve a Gaussian distribution. The transformed values before and after embryo reduction were plotted against gestational age for each of the groups, singletons, twins and higher multiples, to show the trends with gestation. Grouped linear regression was used to determine whether a significant difference existed between the slopes of the lines, and analysis of covariance was performed to determine whether there was significant separation of the lines. In those cases where the lines were converging, intersection of the 95% confidence intervals was calculated to determine the point at which the two confidence intervals were no longer significantly different. Multiple regression analysis was performed to determine the significance of associations between the starting number of fetuses and PAPP-A and SP-1 concentrations.

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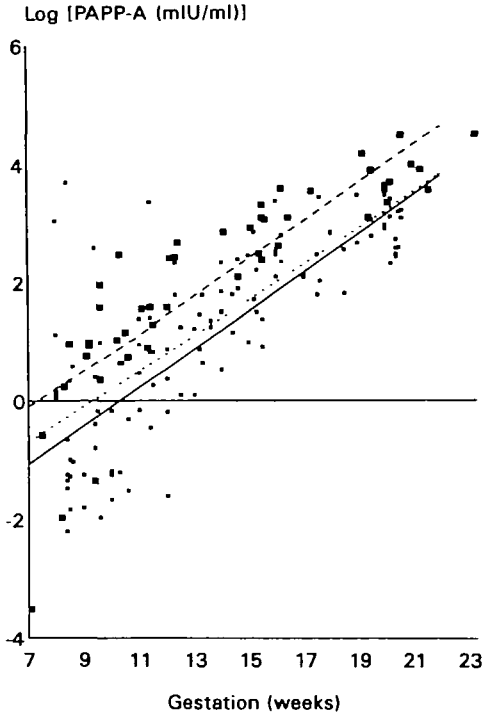

Figure 1. Individual values and regression lines of maternal serum pregnancy associated placental protein A (PAPP-A) with gestation in singleton (■—), twin (□—) and multifetal reduced to twin (⬚– – –) pregnancies.

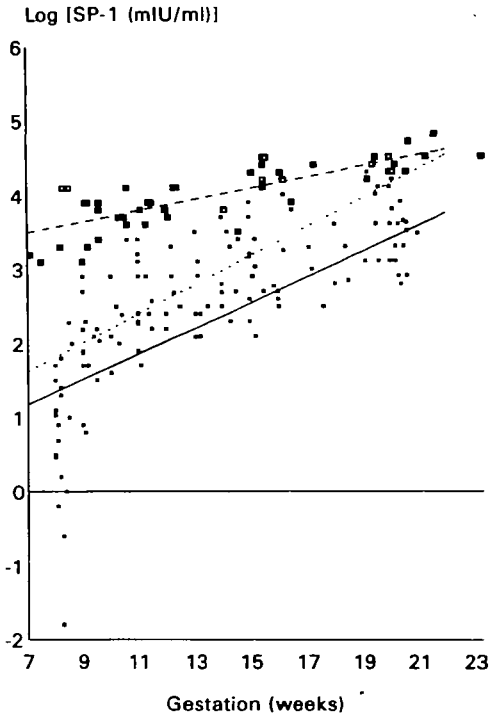

Figure 2. Individual values and regression lines of maternal serum pregnancy specific protein 1 (SP-1) with gestation in singleton (■—), twin (□—) and multifetal reduced to twin (⬚– – –) pregnancies.

Results

In singleton, twin and multifetal pregnancies, there were significant associations with gestation for maternal serum PAPP-A concentration (Figure 1; $r = 0.89$, $P < 0.0001$, $r = 0.72$, $P < 0.0001$ and $r = 0.86$, $P < 0.0001$ respectively) and serum SP-1 concentration (Figure 2; $r = 0.79$, $P < 0.0001$, $r = 0.74$, $P < 0.0001$ and $r = 0.80$, $P < 0.0001$ respectively). The concentration of SP-1 in twin pregnancies was significantly higher than that in singletons at all gestations examined ($t = -5.67$, $P < 0.0001$), whereas for PAPP-A there was no significant difference between concentrations in singletons and twins ($t = -1.26$, $P = 0.10$).

In the multifetal pregnancies, the serum PAPP-A and SP-1 concentrations before and after embryo reduction were significantly higher than in the singletons and twins (PAPP-A: $t = -4.84$, $P < 0.0001$; $t = -3.65$, $P = 0.0002$; SP-1: $t = -14.6$, $P < 0.0001$; $t = -9.0$, $P < 0.0001$ respectively). The original number of fetuses was significantly associated with the maternal serum concentration of SP-1 ($t = 2.16$, $P = 0.04$; Table I) but not PAPP-A ($t = 1.2$, $P = 0.24$; Table I). Following embryo reduction, maternal serum PAPP-A concentrations for multifetal pregnancies remained significantly higher than those for singleton or twin pregnancies (Figure 1; $t = -4.84$, $P < 0.0001$, and $t = -3.65$, $P < 0.0001$ respectively), and the concentration continued to rise in parallel for all three groups. In contrast, serum concentrations of SP-1 progressively converged towards those of non-reduced twins such that by 19 weeks there was no significant difference between the groups (Figure 2).

Table I. Maternal serum concentrations of SP-1 and PAPP-A at 8–11 weeks of gestation by starting number of fetuses

Number of fetuses	*n*	SP-1 concentration [median (range) mg/l]	PAPP-A concentration [median (range) mg/l]
⩾5	5	51.1 (46.6–6.3)	2.14 (1.27–7.12)
3–4	7	27.6 (21.3–58.5)	0.56 (0.03–4.95)
2	12	4.5 (0.7–11.5)	0.26 (0.06–3.10)
1	12	1.9 (0.5–9.9)	0.32 (0.11–1.80)

SP-1 = pregnancy specific β-1-glycoprotein; PAPP-A = pregnancy associated placental protein-A.

Discussion

The finding that in multifetal pregnancies maternal serum SP-1 concentration increases with the number of fetuses is compatible with the placental origin of this peptide, as placental mass is inevitably a major determinant of circulating concentrations (Stabile *et al.*, 1988). PAPP-A concentration is also higher in multifetal pregnancies than in singleton or twin pregnancies. However, unlike SP-1, PAPP-A does not increase in proportion to the number of fetuses, presumably because this protein is produced by the decidua as well as the placenta and the maximum secretory capacity of the endometrium is achieved with twin pregnancy (Lin and Halbert, 1976; Abbas *et al.*, 1995).

Multifetal pregnancy reduction to twins was associated with a relative decrease in maternal serum SP-1 concentration to that of the control twin group (group 2). In this respect, the pattern of change in SP-1 is similar to that of human chorionic gonadotrophin (HCG) and suggests that fetal reduction causes placental death and therefore decline in production of both

placental products (Johnson *et al.*, 1994a). These findings are different from those of maternal serum relaxin and α-feto-protein (α-FP) (Abbas *et al.*, 1994; Johnson *et al.*, 1994b). In multifetal pregnancies relaxin concentration increases with the number of fetuses but following reduction to twins, the level does not change; this is presumably because relaxin is a product of the corpus luteum whose function is unaffected by feto-placental death (Johnson *et al.*, 1994b). In the case of α-FP, embryo reduction was associated with an increase in maternal serum concentration which persisted for at least 10 weeks before decreasing to twin values; α-FP is stored in fetal tissues and after fetal death and tissue breakdown it is released into the amniotic fluid from where it is absorbed into the maternal circulation (Abbas *et al.*, 1994).

Relative concentrations of maternal serum PAPP-A did not change significantly following fetal reduction. Unlike SP-1, which has a half-life of ~30 h and disappears from the circulation soon after delivery, PAPP-A remains detectable in the maternal circulation for 4–6 weeks post-partum (Bohn, 1974; Lin *et al.*, 1976). This long half-life of PAPP-A could offer an explanation for the persistence of high concentrations following fetal reduction. Alternatively, PAPP-A is produced not only by the placenta, but by decidua and additional tissues, with the controlling mechanism set early in the pregnancy. This explanation is probably more likely in respect of the continued rise in PAPP-A values in parallel to those of non-reduced twins.

In multifetal pregnancies undergoing embryo reduction there is a procedure-related risk of spontaneous abortion, which usually occurs within 2 weeks of the intervention and is about 1–2%. However, there is a persisting risk of spontaneous abortion or severe preterm delivery which is much higher than in spontaneously conceived twins. For example the prevalence of preterm delivery before 32 weeks of gestation is about 1% in singleton pregnancies, 10% in twins, 30% in triplets (US Government, 1993) and 15% in multifetal pregnancies reduced to twins (Evans *et al.*, 1994). It is possible that the persistence of risk of miscarriage for several weeks after the procedure is due to the release of cytokines and stimulation of prostaglandins in response to the resorbing placental tissue of the dead fetuses. The slow relative decline in maternal SP-1 concentrations following embryo reduction presumably reflects the gradual degeneration of placental tissue and therefore, the persistence for several weeks of the stimulus for cytokine production.

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