ADAM12-s in coelomic fluid and maternal serum in early pregnancy

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Objectives ADAM12-s is a placental protein. In early pregnancy, reduced maternal levels of ADAM12-s have been reported in association with foetal trisomy 21 or 18 and in cases that subsequently develop pre-eclampsia and foetal growth restriction. The aim of this study is to investigate the distribution of ADAM12-s in early pregnancy by comparing its concentration in maternal serum, amniotic fluid and coelomic fluid.

Methods Coelomic fluid was obtained by coelocentesis from 13 singleton pregnancies with live foetuses at 6.9–9.3 weeks of gestation. Maternal serum was also obtained in all cases and in six cases amniotic fluid was also obtained. The concentration of ADAM12-s was measured by dissociation enhanced lanthanide fluoro-immunoassay.

Results The median concentration of ADAM12-s in maternal serum was 132.7 (range 33.8–254.5) ng/mL and in coelomic fluid it was 10.5 (range 1.3–15.8) ng/mL; there were no detectable levels in five of the six amniotic fluid samples. The concentration of maternal serum ADAM12-s increased significantly with gestation ($r = 0.862$, $p < 0.0001$). There was no significant association between coelomic fluid ADAM12-s and either gestation ($r = 0.255$, $p = 0.401$) or maternal serum ADAM12-s ($r = 0.302$, $p = 0.316$).

Conclusion The distribution of ADAM12-s in maternal serum and the early embryonic fluid compartments is consistent with its syncytiotrophoblastic origin. Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS: coelocentesis; ADAM12; first trimester; trisomy 21; pre-eclampsia

INTRODUCTION

The A Disintegrin And Metalloproteinase (ADAM) proteins are a novel family of more than 15 molecules likely to be involved in proteolysis, cell adhesion, cell fusion and signalling (Gilpin *et al*., 1998). ADAM12 was first described in mice, where it was implicated in cell fusion *in vitro* (Yagami-Hiromasa *et al*., 1995). Human ADAM12 exists in two forms resulting from alternative splicing of a single gene. The soluble form of (ADAM12-s) is expressed only in the placenta, whereas the long form of (ADAM12-*l*) is expressed in several tissues and is believed to originate from smooth muscle cells (Gilpin *et al*., 1998). ADAM12-s has a strong cleaving action on the insulin-like growth factor binding proteins 3 and 5 (IGFBP-3 and IGFBP-5) and it may be therefore implicated in the regulation of foetal growth (Loechel *et al*., 2000). In normal pregnancy, maternal serum concentration of ADAM12-s increases with gestation (Laigaard *et al*., 2003). In early pregnancy, reduced maternal levels of ADAM12-s have been reported in association with foetal trisomy 21 or 18 and in cases that subsequently develop pre-eclampsia and foetal growth restriction (Laigaard *et al*., 2003, 2005a,b).

The coelomic cavity surrounds the amniotic cavity in the first trimester of pregnancy, occupying the space between the amniotic membrane and the inner surface of the trophoblast (Figure 1). As there is no real anatomical barrier between the coelomic cavity and the trophoblast, the concentration of cytotrophoblastic products is substantially higher in the coelomic fluid than in the maternal blood or amniotic fluid, thus the coelomic fluid represents a 'liquid extension' of the placenta. In contrast, embryonic/foetal products are generally found at the highest level in the amniotic fluid, and maternal products in the maternal serum (Jauniaux and Gulbis, 2000).

Coelocentesis has been used in both animal (Santolaya-Forgas *et al*., 1998) and human studies (Jurkovic *et al*., 1993; Jauniaux and Gulbis, 2000) and it may prove to be a useful technique for early prenatal diagnosis of genetic disorders (Makrydimas *et al*., 2004).

The aim of this study is to investigate the distribution pattern of ADAM12-s in early pregnancy by comparing its concentration in maternal serum, amniotic fluid and coelomic fluid.

MATERIALS AND METHODS

Coelomic fluid and amniotic fluid samples were obtained by coelocentesis and amniocentesis, which was performed immediately before elective suction termination

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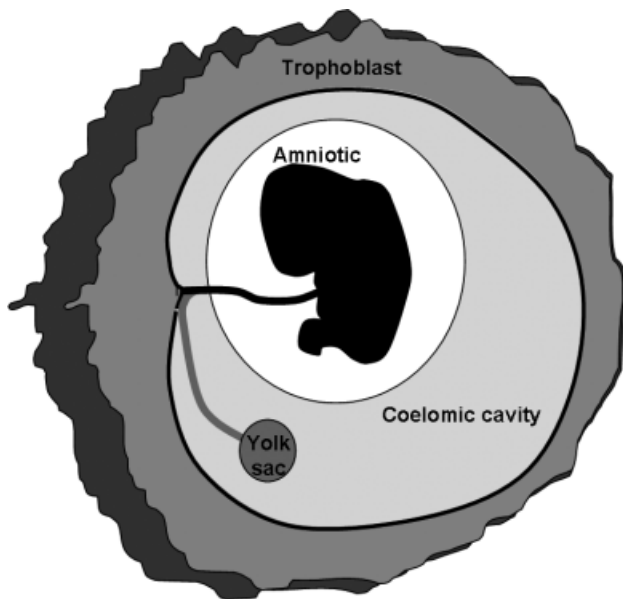
Figure 1—Diagram illustrating the placenta, amniotic and coelomic cavities, yolk sac and embryo at 7–10 weeks of gestation

of pregnancy for psychosocial indications, from 13 singleton pregnancies with live foetuses at 6.9–9.3 weeks of gestation. All participants were examined at the Department of Obstetrics and Gynaecology, University Hospital of Ioannina, Greece. The study was approved by the Hospital Ethics Committee and written consent was obtained from all participants.

The external genitalia and the vagina were carefully cleansed with an antiseptic solution. Transvaginal sonography with a 5 MHz transducer covered with a sterile rubber was then performed. The foetal crown-rump length (CRL) was measured and the amniotic membrane, coelomic cavity and yolk sac were identified. A 20G needle was introduced transvaginally into the coelomic cavity, through a guide attached to the transducer, and coelomic fluid was aspirated. Subsequently, a new 20G needle was used to aspirate amniotic fluid. The first 0.2 mL of the retrieved coelomic and amniotic fluids was discarded in order to avoid contamination. Maternal blood was also obtained in all cases just before the induction of anaesthesia. The samples were centrifuged and stored at $-80\,^{\circ}$C until assayed. The placenta was sent for cytogenetic studies and a normal karyotype was confirmed in all cases.

The concentration of ADAM12-s was measured, using a *Dissociation Enhanced Lanthanide Fluoro-Immunoassay* (DELFIA) kit (Perkin Elmer, Turku, Finland), on the basis of previously described ELISA and Auto DELFIA assays (Laigaard *et al.*, 2003, 2005a). Essentially, the samples, standards and controls (second-trimester pooled serum diluted with male serum that is free of ADAM12) were loaded in duplicate onto microtiter plates coated with anti-ADAM12 6E6 monoclonal antibody. After incubation with europium labelled anti-ADAM12 8F8 monoclonal antibody and washing, a dissociation solution was added and the plates were read for fluorescence (Victor, Wallac, Turku, Finland).

The analytical range of the assay is 0.02 to 820 ng/mL. The researcher was unaware about the samples being analysed. The precision of the duplicate measurements was within 10% and the precision between this assay and the assay from a previous study was of the order of 7%. ADAM12 has been shown to be stable through at least eight freeze/thaw cycles (Laigaard *et al.*, 2003).

Statistical analysis

Non-parametric methods were used for statistical analysis. The Friedman test was carried out for the comparison of concentrations among the three compartments and Spearman's rho test was used to test for correlations between the levels of ADAM12-s in the different compartments. All analyses were carried out in SPSS (SPSS 12.0, SPSS Inc., Chicago, Il, USA).

RESULTS

The median gestational age was 8.3 (range 6.9, 9.3) weeks. We analysed 13 matched samples of maternal serum and coelomic fluid and six samples of amniotic fluid. Retrieval of amniotic fluid before 8 weeks was not possible because of the very small size of the amniotic cavity.

The median concentration of ADAM12-s in maternal serum was 132.7 (range, 33.8–254.5) ng/mL and in coelomic fluid it was 10.5 (range, 1.3–15.8) ng/mL; there were no detectable levels in five of the six amniotic fluid samples (Friedman's test for related samples, $p = 0.002$; Figure 2). The maternal serum ADAM12-s concentration increased significantly with gestation ($r = 0.862$, $p < 0.0001$; Figure 3). There was no significant association between coelomic fluid ADAM12-s and either gestation ($r = 0.255$, $p = 0.401$) or maternal serum ADAM12-s ($r = 0.302$, $p = 0.316$).

DISCUSSION

The finding of this study, that maternal serum ADAM12-s increases significantly with gestation at 7–10 weeks, is compatible with the results of a previous study that reported a linear increase from a median of 180 ng/mL at 8 weeks to 12 000 ng/mL at 38 weeks (Laigaard *et al.*, 2003). Additionally, we found that although there is essentially no detectable ADAM12-s in amniotic fluid, it is always present in coelomic fluid, but the levels are 13 times less than those found in maternal serum.

Immunohistochemical studies in both early and term human placentas have recently localised ADAM12-s expression in syncytiotrophoblasts (Ito *et al.*, 2004; Huppertz *et al.*, 2006). Our observations provide the first evidence for the distribution pattern of this substance in the maternal and embryonic compartments, and are in complete agreement with its *in vitro* demonstrated origin. Syncytiotrophoblastic products (e.g. human placental lactogen (hPL) and pregnancy-associated plasma
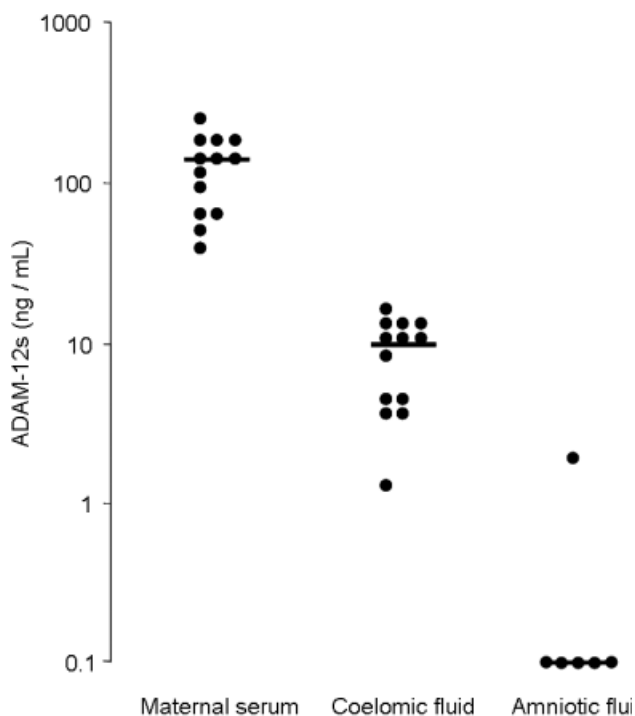
Figure 2—ADAM12-s concentration in maternal serum, coelomic fluid and amniotic fluid
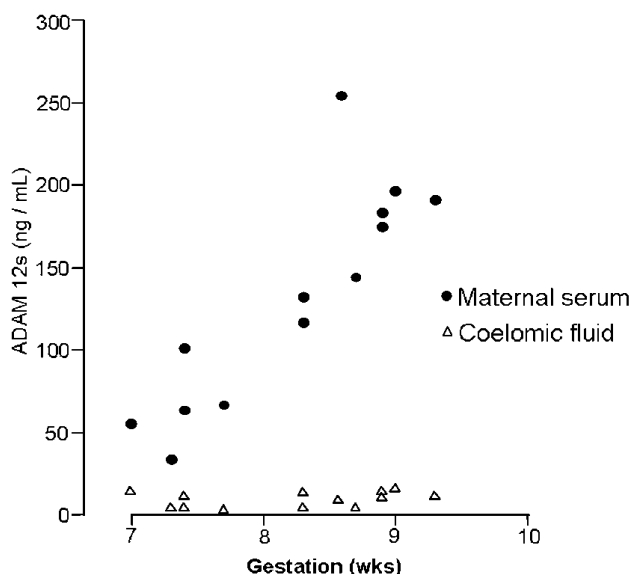

Figure 3—Relationship between maternal serum and coelomic fluid concentration of ADAM12-s with gestation

protein A (PAPP-A)) are present at the highest levels in the maternal serum, presumably because the syncytiotrophoblast is in closer proximity to the maternal circulation rather than to the coelomic fluid (Wathen *et al.*, 1992; Iles *et al.*, 1994) (Jauniaux and Gulbis, 2000). The relative concentration of maternal serum to coelomic fluid (MS : CF) levels depends on the size of the molecule, with a higher transfer to the coelomic cavity for smaller molecules. Thus, the median ratio of MS : CF concentration of hPL, ADAM12-s and PAPP-A is 3 (Wathen *et al.*,

1992), 13 and 45 (Iles *et al.*, 1994) respectively, which is consistent with their sizes of 20, 80 and 180 KDa, respectively.

According to this, the increase in serum levels of ADAM12-s with gestation presumably reflects its increased production, as well as its improved transport due to the establishment of the uteroplacental circulation. Given the large fluid volume of the maternal serum, the immediacy of its secretion in the maternal blood becomes more striking. In contrast, there was no significant association between ADAM12-s levels in coelomic fluid and gestational age, probably because ADAM12-s is transferred through the placenta to reach the coelomic cavity, and its increased production with gestation is counterbalanced by the increasing distance between the syncytiotrophoblast and coelomic cavity. The lack of detectable ADAM12-s in the amniotic fluid can be attributed to the impermeability of the amniotic membrane to large molecules, and a similar pattern has been described for PAPP-A (Iles *et al.*, 1994).

Our observations reflect the syncytiotrophoblastic origin of ADAM12-s from at least 7 weeks of gestation and provide a distribution pattern that is quite similar to the one reported for PAPP-A (Iles *et al.*, 1994). Both substances belong to the same broad family of proteases and they share a syncytiotrophoblastic origin and similar kinetics. PAPP-A has been used as a marker for foetal aneuploidies and placental dysfunction; similarly, the levels of ADAM12-s also appear altered in cases of trisomy and pre-eclampsia (Laigaard *et al.*, 2003, 2005a,b). Trisomic pregnancies and those that develop pre-eclampsia are characterised by impaired syncytiotrophoblastic function (Frendo *et al.*, 2000; Wright *et al.*, 2004; Huppertz and Kingdom, 2004), and the localisation and distribution of ADAM12-s appears to be consistent with its altered maternal serum levels in these conditions.

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