ORIGINAL ARTICLE

# Potential Function of Amniotic Fluid in Fetal Development—Novel Insights by Comparing the Composition of Human Amniotic Fluid with Umbilical Cord and Maternal Serum at Mid and Late Gestation

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**Background:** Amniotic fluid (AF) is a dynamic and complex mixture. Up to now, little is known about the physiological functions of AF in the process of fetal development. We suppose that AF carries components such as proteins or peptides, which contribute to the regulation of fetal development.

**Methods:** Compositions including biochemical components and tumor markers were determined in human AF, umbilical cord serum (UCS) and maternal serum (MS) from the same subject in the range of 15–42 weeks of gestation.

**Results:** (1) The levels of primary electrolytes such as sodium, chloride, anion gap and osmotic pressure in AF was almost the same as in UCS and MS. (2) The levels of organic substances, including total protein, glucose, triglycerides, cholesterol and various enzymes, were markedly lower in AF than in UCS and MS, especially for total protein, which was 8- and 12.5-fold lower in AF than in UCS and MS, respectively. (3) The levels of tumor markers, including carcinoembryonic antigen, ferritin, cancer antigen 125 and 199, and α-fetoprotein in AF displayed different dynamic changes compared to UCS and MS as gestation advanced.

**Conclusion:** This study demonstrated that AF is not a result of simple filtration from the blood but an independent fluid. We speculate that proteins or peptides in the amniotic fluid modulate the process of fetus development since they possess potent bioactivity on cellular growth and proliferation. AF provides a pathway to transport these "regulators" to the fetus and thus plays a pivotal role in fetal development. [*J Chin Med Assoc* 2009;72(7):368–373]

**Key Words:** amniotic fluid, composition, fetus, protein, tumor marker

## Introduction

Amniotic fluid (AF), the protecting liquid contained in the amnion cavity, is an essential component for fetal development and maturation during pregnancy. In early embryogenesis, AF is the extension of the fetal extracellular matrix and free diffusion occurs bidirectionally between the fetus and the AF through extracellular compartment. By 8 weeks of gestation in humans, the urethra is formed and the fetal kidneys start to produce urine. Fetal swallowing begins shortly thereafter. Fetal skin is gradually keratinized from 19 to 25 weeks of gestation. Excretion from fetal urine, respiratory system, gastroenteric system, umbilical cord and surface of the placenta become the sources of AF.[1]

It is well accepted that AF constitutes a protective sac around the fetus that allows fetal movement and growth and prevents mechanical and thermal shock.

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It also plays a significant defensive role as a part of the innate immune system since AF has an organized pool of antimicrobial peptides against common bacterial and fungal pathogens.[2] AF is 98% water and electrolytes, proteins, peptides, carbohydrates, lipids and hormones. The levels of these components in AF have been determined in many studies to screen for potential biomarkers of pregnancy-associated abnormalities. In recent years, an increasing number of proteins or peptides have been isolated from AF. Among these proteins, more and more are identified as growth factors or cytokines,[3–5] the levels of which usually change as gestation advances and they also demonstrate a different dynamic pattern from the maternal plasma.[4,6,7] Our previous study has shown that proteins in human AF from specific gestational age inhibit tumor growth in tumor-bearing mice.[8] In addition, AF has attracted increasing attention in recent years as a possible reserve of stem cells.[9] The above evidence suggests that the bioactive components in AF may play an important role in fetal development and maturation. However, up to now, little is known about the physiological functions of the majority of the constituents of AF. We suppose that AF may play a pivotal role in fetal development in addition to its protective effect on the fetus. The aim of this study was to provide new evidence to support our assumption by comparing the composition of human AF with corresponding umbilical cord serum (UCS) and maternal serum (MS) at mid and late gestation.

## Methods

### *Collection of AF, UCS and MS*

Forty-six healthy pregnant women aged 22–37 years undergoing legal pregnancy terminations or normal labor were enrolled in this study. They all signed an informed consent form. The mean gestational age was 27 weeks (range, 15–42 weeks). AF samples were obtained transcervically or from the uterus during delivery by elective cesarean section. Umbilical cord blood was drawn from the middle part of the umbilical cord. Maternal blood was obtained from median cubital veins. From each pregnant woman, AF, umbilical cord blood and maternal blood were simultaneously collected. Serums were prepared according to routine procedure. AF samples were first filtered and then cleared of cells by centrifuge (3000 rpm/min for 10 minutes at 4°C), frozen, and stored in aliquots at –20°C. Exclusion criteria included: multiple pregnancies, diabetes, infection, fetal malformation, gestational hypertension, hepatitis B or C antigen positive, maternal exposure to alcohol, cocaine or tobacco smoke. The study protocol was approved by the ethics committee of the local hospital.

### *Measurement of biochemical components*

A total of 38 biochemical components and osmolality were simultaneously quantified in the AF, UCS, and MS samples from each pregnant woman using an automatic biochemical analyzer (Olympus AU5400; Olympus Corp., Tokyo, Japan) with assay kits. The biochemical components included total protein, albumin, globulin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-transpeptidase, cholinesterase, creatine kinase, creatine kinase isoenzymes, lactate dehydrogenase, hydroxybutyrate dehydrogenase, amylase, glucose, cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), apoprotein A1 and B, lipoprotein(a), total bilirubin, direct bilirubin, indirect bilirubin, total bile, sodium, potassium, chloride, calcium, phosphate, magnesium, bicarbonate, urea, creatinine, anion gap, uric acid, and osmolality.

### *Measurement of tumor markers*

Levels of tumor markers including α-fetoprotein (AFP), carcinoembryonic antigen (CEA), ferritin, cancer antigen 125 (CA-125) and 199 (CA-199) were measured in the AF, UCS and MS from each pregnant woman with the UniCel™ DxI 800 Access Immunoassay System (Beckman Coulter Inc., Fullerton, CA, USA). For each gestational age in the range of 15–42 weeks, 2 pregnancies were subjected to the test.

### *Statistical analysis*

The data are presented as mean ± standard deviation. Statistical comparisons between AF and UCS or MS were carried out using GraphPad InStat version 3 (GraphPad Software Inc., La Jolla, CA, USA) with Student's paired *t* tests. A value of $p < 0.05$ was considered statistically significant.

## Results

### *Biochemical components in AF, UCS and MS*

Thirty-eight components including proteins, electrolytes, glucose, triglyceride and metabolites were simultaneously determined in the AF, UCS and MS samples from each pregnant woman. Matched samples were from 20 subjects. As shown in Table 1, proteins including total protein, albumin, globulin, and various enzymes were present in significantly lower concentrations in AF than in UCS or MS. The mean concentration of total protein in the matched samples

**Table 1.** Levels of proteins in amniotic fluid (AF), umbilical cord serum (UCS) and maternal serum (MS) at mid and late gestation in 20 subjects*

| Biochemical components | AF | UCS | MS |
|---|---|---|---|
| Total protein (g/L) | 4.87 ± 2.08†† | 39.46 ± 9.12 | 61.55 ± 6.36 |
| Albumin (g/L) | 2.24 ± 1.39†† | 27.35 ± 4.99 | 35.06 ± 4.39 |
| Globulin (g/L) | 2.63 ± 0.74†† | 12.11 ± 4.97 | 26.84 ± 3.58 |
| ALT (U/L) | 0.80 ± 0.70†† | 4.50 ± 2.65 | 6.25 ± 2.49 |
| AST (U/L) | 8.40 ± 4.28†† | 34.50 ± 15.36 | 17.95 ± 5.12 |
| Alk-P (U/L) | 19.80 ± 18.14†† | 161.10 ± 65.15 | 82.65 ± 49.08 |
| Cholinesterase (U/L × $10^3$) | 0.04 ± 0.06†† | 4.49 ± 1.31 | 5.58 ± 0.98 |
| γ-transpeptidase (U/L) | 148.30 ± 179.01†† | 123.25 ± 61.47 | 17.10 ± 20.56 |
| CK (U/L) | 1.00 ± 1.21†† | 171.25 ± 125.75 | 70.75 ± 70.16 |
| CK isoenzymes (U/L) | 2.75 ± 2.77†† | 71.35 ± 83.55 | 14.90 ± 7.00 |
| LDH (U/L) | 17.60 ± 18.02†† | 376.75 ± 173.40 | 208.65 ± 64.03 |
| Hydroxybutyrate dehydrogenase (U/L) | 14.90 ± 14.46†† | 296.75 ± 141.16 | 169.40 ± 53.45 |
| Amylase (U/L) | 74.20 ± 16.26† | 48.40 ± 18.15 | 82.80 ± 14.92 |
| HDL (mmol/L) | 0.016 ± 0.015†† | 0.645 ± 0.20 | 1.71 ± 0.47 |
| LDL (mmol/L) | 0.32 ± 0.02†† | 1.18 ± 0.50 | 2.58 ± 0.98 |
| VLDL (mmol/L) | 0.001 ± 0.003†† | 0.21 ± 0.20 | 1.20 ± 0.76 |
| Apoprotein A1 (g/L) | 0.012 ± 0.013†† | 0.59 ± 0.18 | 2.02 ± 0.52 |
| Apoprotein B (g/L) | 0.120 ± 0.002†† | 0.31 ± 0.086 | 0.93 ± 0.27 |
| Lipoprotein(a) (mg/L) | 84.53 ± 15.41† | 86.03 ± 13.42 | 286.72 ± 229.86 |

**Data presented as mean ± standard deviation; †p < 0.01 vs. UCS; ‡p < 0.01 vs. MS. ALT = alanine aminotransferase; AST = aspartate aminotransferase; Alk-P = alkaline phosphatase; CK = creatine kinase; LDH = lactate dehydrogenase; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very-low-density lipoprotein.*

was 8 times higher in UCS and 12.5 times higher in MS than in AF. As shown in Table 2, the concentration of the dominant cation, sodium, in AF was the same as in UCS, but slightly lower than in MS. Other minor cations, including potassium, calcium and magnesium, were present in lower concentrations in AF than in UCS and MS. No difference was found for the levels of the primary anion, chloride, and anion gap between AF and UCS or MS. There was a much lower level of glucose in AF than in UCS or MS. Triglyceride and cholesterol were almost undetectable. The levels of total bilirubin and bile acid were much lower in AF than in UCS and MS. However, some metabolites including urea, creatinine, and uric acid occurred at higher concentrations in AF than in UCS or MS. The osmolality of AF was the same as that of UCS, but slightly lower than that of MS.

### *Levels of tumor markers*

The levels of tumor markers including CEA, ferritin, CA-125, CA-199 and AFP were quantified at the different gestational ages and shown in Figure 1. The concentrations of these proteins in AF displayed dynamic changes as gestation advanced. Compared to UCS and MS, AF showed a different dynamic pattern of tumor markers at mid and late gestation.

## Discussion

There are few reports on the comparison of compositions among AF, UCS and MS since it is difficult to obtain human AF, UCS and MS samples at mid and late gestation from the same subject. By comparing the biochemical components, electrolytes and tumor-related proteins in human AF, UCS and MS, we found that: (1) the levels of primary electrolytes such as sodium, chloride, anion gap and osmotic pressure in AF were almost the same as in UCS and MS; (2) the levels of organic substances in AF, including total protein, glucose, triglyceride, cholesterol and various enzymes, were markedly lower than in UCS and MS, especially total protein, which was 8- and 12.5-fold lower in AF compared to UCS and MS, respectively; (3) the levels of tumor markers, including CEA, ferritin, CA-125, CA-199 and AFP, in AF displayed different dynamic changes from that in UCS and MS as gestation advanced. These data suggest that the inorganic components in AF may be derived from blood, but other organic components such as protein, enzymes and tumor markers are present in AF at significantly different levels than in UCS and MS. The results imply that the fetus is the main source of these components in AF and that AF is an independent fluid.

**Table 2.** Levels of biochemical components in amniotic fluid (AF), umbilical cord serum (UCS) and maternal serum (MS) at mid and late gestation in 20 subjects*

| Biochemical components | AF | UCS | MS |
|---|---|---|---|
| Total bilirubin (μmol/L) | 2.23 ± 1.28†† | 24.57 ± 8.04 | 8.10 ± 5.54 |
| Direct bilirubin (μmol/L) | 1.13 ± 0.66†† | 8.06 ± 2.75 | 2.24 ± 1.36 |
| Indirect bilirubin (μmol/L) | 1.10 ± 0.73†† | 16.52 ± 6.12 | 5.86 ± 4.32 |
| Total bile acid (μmol/L) | 1.48 ± 0.80†† | 5.98 ± 2.34 | 2.30 ± 1.08 |
| Sodium (mmol/L) | 129.9 ± 11.65§ | 133 ± 5.39 | 136 ± 2.06 |
| Potassium (mmol/L) | 3.80 ± 0.41†† | 7.32 ± 2.48 | 5.94 ± 2.20 |
| Chloride (mmol/L) | 106.18 ± 7.82 | 103.03 ± 2.01 | 103.17 ± 2.27 |
| Calcium (mmol/L) | 1.42 ± 0.26†† | 2.45 ± 0.16 | 2.22 ± 0.12 |
| Phosphate (mmol/L) | 1.12 ± 0.23†† | 2.19 ± 0.59 | 1.53 ± 0.82 |
| Magnesium (mmol/L) | 0.59 ± 0.09†† | 0.92 ± 0.19 | 0.85 ± 0.12 |
| Bicarbonate (mmol/L) | 14.00 ± 2.97†† | 18 ± 3.76 | 17 ± 3.18 |
| Glucose (mmol/L) | 1.29 ± 0.72†† | 3.45 ± 1.16 | 3.58 ± 1.79 |
| Cholesterol (mmol/L) | 0.04 ± 0.02†† | 1.95 ± 0.61 | 5.37 ± 1.22 |
| Triglyceride (mmol/L) | 0.010 ± 0.007†† | 0.47 ± 0.44 | 2.66 ± 1.67 |
| Urea (mmol/L) | 3.34 ± 0.94† | 2.81 ± 0.57 | 3.07 ± 0.84 |
| Creatinine (μmol/L) | 100.15 ± 54.91†† | 55 ± 9.95 | 57 ± 9.17 |
| Anion gap (mmol/L) | 9.75 ± 6.46 | 11.80 ± 6.45 | 15.60 ± 5.29 |
| Uric acid (μmol/L) | 289.40 ± 97.84†§ | 238.5 ± 64.12 | 253.6 ± 67.49 |
| Osmolality (mosm/L) | 246.85 ± 21.51§ | 254.2 ± 10.14 | 259.8 ± 3.74 |

**Data presented as mean ± standard deviation; †$p < 0.01$ vs. UCS; ‡$p < 0.01$ vs. MS; §$p < 0.05$ vs. MS.*

It is generally accepted that AF serves as an extension of the fetal extracellular matrix in early gestation. After the fetal skin is fully keratinized, fetal respiration, swallowing, and excretion of urine constitute the main pathways of exchange between the fetus and AF.[1] Our study revealed that much higher concentrations of metabolites including urea, creatinine, and uric acid were found in AF, representing the excretion of urine from the fetus. Compared to umbilical blood, AF contained significantly fewer nutritional components such as proteins, glucose, triglyceride and cholesterol, which may suggest that AF does not have an important role in fetal nutrition. What is the potential function of AF in fetal development? In this study, the mean total protein in human AF was 4.9 g/L at mid and late gestation, which was significantly lower than in UCS and MS; this finding is consistent with a previous report.[10] Since both electrolytes and proteins are key factors in maintaining osmotic pressure, it is expected that the proteins found in AF have low molecular weights if a low level of total proteins in AF is required to maintain the balance of osmotic pressure between AF and blood. There is evidence that most of the proteins or polypeptides in AF are of low molecular weight.[11] Among these proteins or peptides, more and more are being identified as tumor markers, growth factors and cytokines, which possess potent bioactivity.[3–5,12] A study has demonstrated that growth factors in AF are able to be absorbed by the fetal gastrointestinal tract and transported to the whole fetal body.[13] Thus, proteins in AF may play an important role in fetal development. It has been demonstrated that AFP is capable of regulating growth in ovarian, placental, uterine, phagocytic, bone marrow and lymphatic cells.[14,15] Our previous study showed that proteins or peptides with small molecular weight in human AF at specific gestational ages displayed opposite effects,[8] i.e. suppression or acceleration on mouse tumor growth. More recently, AF composition at 19–20 weeks of gestation was determined by proteomics analysis and a total of 842 proteins and peptides were identified, the functions of which included cellular movement, development of organs, cellular growth and proliferation.[16] The above data suggest that proteins or peptides in AF modulate the process of fetal development in addition to its protective effect. Together with other reports,[8,16] we hypothesize that these proteins act as regulatory messengers for cellular growth and proliferation and that AF provides a pathway to transport these messengers to the whole fetal body. In this way, AF plays a pivotal role in fetal development.

In conclusion, the present study demonstrated that AF is not a result of simple filtration from the blood but an independent fluid. We speculate that proteins or peptides in AF modulate the process of fetus development since they possess potent bioactivity

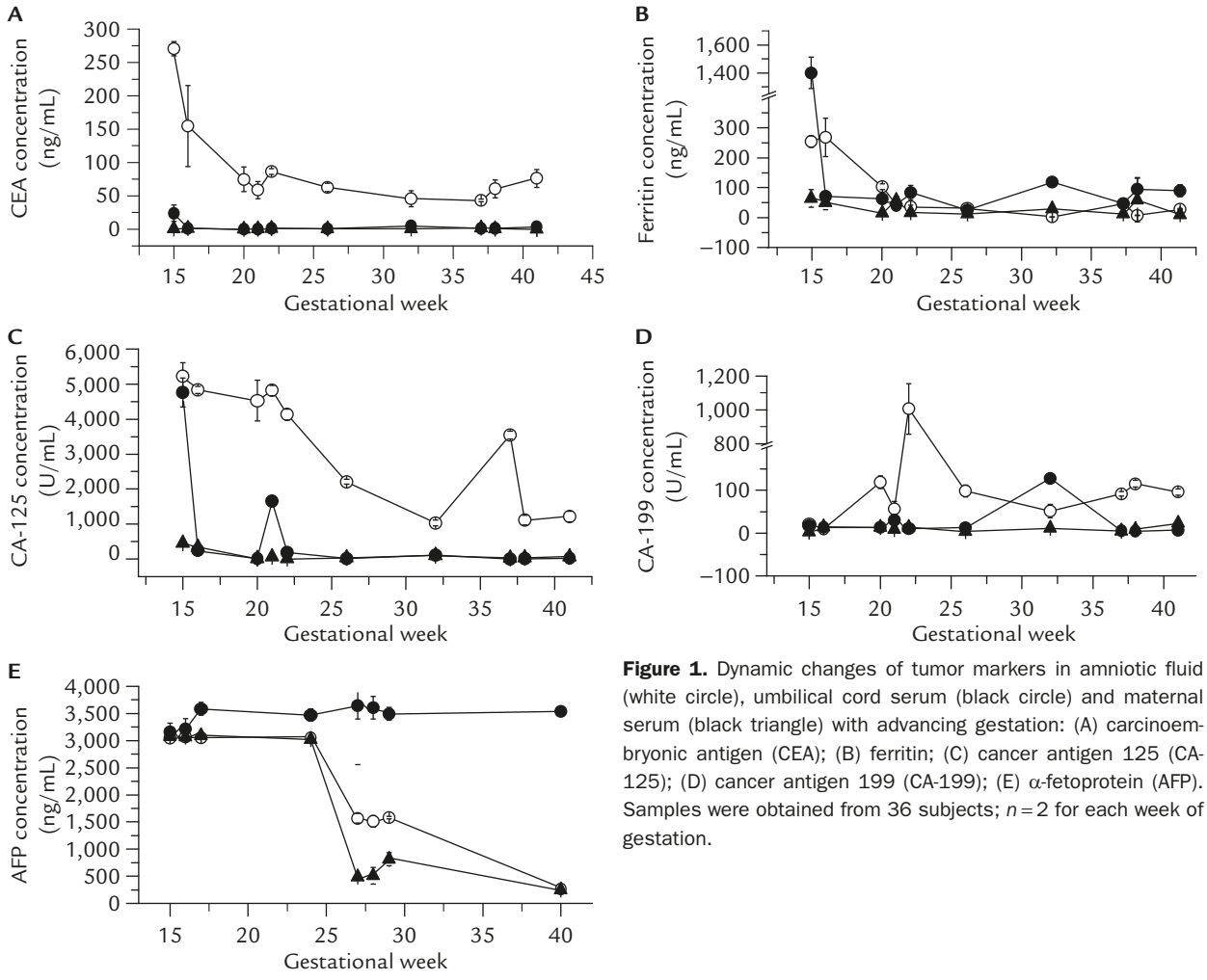


**Figure 1.** Dynamic changes of tumor markers in amniotic fluid (white circle), umbilical cord serum (black circle) and maternal serum (black triangle) with advancing gestation: (A) carcinoembryonic antigen (CEA); (B) ferritin; (C) cancer antigen 125 (CA-125); (D) cancer antigen 199 (CA-199); (E) α-fetoprotein (AFP). Samples were obtained from 36 subjects; $n = 2$ for each week of gestation.

on cellular growth and proliferation. AF provides a pathway to transport these "regulatory messengers" to the whole fetal body and thus plays a pivotal role in fetal development. To define this novel function of AF would potentially be significant for better understanding of embryonic development, prevention of genetic diseases and developing new therapeutics for human malignancies.

## References


1. Underwood MA, Gilbert WM, Sherman MP. Amniotic fluid: not just fetal urine anymore. *J Perinatol* 2005;25:341–8.
2. Akinbi HT, Narendran V, Pass AK, Markart P, Hoath SB. Host defense proteins in vernix caseosa and amniotic fluid. *Am J Obstet Gynecol* 2004;191:2090–6.
3. Blahovec J, Kostecka Z, Mester J, Cavaille F. Peptide-like substances in sheep amniotic fluid which regulate proliferation of BP-A31 cells. *Vet Med* 1997;42:185–9.
4. Blahovec J, Kostecka Z, Cavaille F, Lacroix MG, Mester J. Insulin-like growth factor binding proteins and mitogenic activity of partially fractionated sheep amniotic fluid. *Acta Vet Hung* 2001;49:65–70.
5. Ackerman WET, Rovin BH, Kniss DA. Epidermal growth factor and interleukin-1beta utilize divergent signaling pathways to synergistically upregulate cyclooxygenase-2 gene expression in human amnion-derived WISH cells. *Biol Reprod* 2004;71:2079–86.
6. Chow SS, Craig ME, Jones CA, Hall B, Catteau J, Lloyd AR, Rawlinson WD. Differences in amniotic fluid and maternal serum cytokine levels in early midtrimester women without evidence of infection. *Cytokine* 2008;44:78–84.
7. Tisi DK, Liu XJ, Wykes LJ, Skinner CD, Koski KG. Insulin-like growth factor II and binding proteins 1 and 3 from second trimester human amniotic fluid are associated with infant birth weight. *J Nutr* 2005;135:1667–72.
8. Tong XL, Zhang YF, Xu YF. Effect of human amniotic fluid on tumor-bearing mice. *Chin J Appl Physiol* 1992;8:182–4.
9. Perin L, Sedrakyan S, Da Sacco S, De Filippo R. Characterization of human amniotic fluid stem cells and their pluripotential capability. *Methods Cell Biol* 2008;86:85–99.
10. Bala S, Seth S, Seth PK. Total proteins in human amniotic fluid. *Aust N Z J Obstet Gynaecol* 1986;26:141–4.
11. Burdett P, Lizana J, Eneroth P, Bremme K. Proteins of human amniotic fluid. II. Mapping by two-dimensional electrophoresis. *Clin Chem* 1982;28:935–40.
12. Drohse H, Christensen H, Myrhoj V, Sorensen S. Characterisation of non-maternal serum proteins in amniotic fluid at weeks 16 to 18 of gestation. *Clin Chim Acta* 1998;276:109–20.

13. Bloomfield FH, Breier BH, Harding JE. Fate of (125)I-IGF-I administered into the amniotic fluid of late-gestation fetal sheep. *Pediatr Res* 2002;51:361–9.
14. Mizejewski GJ. Alpha-fetoprotein as a biologic response modifier: relevance to domain and subdomain structure. *Proc Soc Exp Biol Med* 1997;215:333–62.
15. Bartha JL, Romero-Carmona R, Comino-Delgado R, Arce F, Arrabal J. Alpha-fetoprotein and hematopoietic growth factors in amniotic fluid. *Obstet Gynecol* 2000;96:588–92.
16. Cho CK, Shan SJ, Winsor EJ, Diamandis EP. Proteomics analysis of human amniotic fluid. *Mol Cell Proteomics* 2007;6: 1406–15.