MINIREVIEW

Physiology of Alpha-Fetoprotein as a Biomarker for Perinatal Distress: Relevance to Adverse Pregnancy Outcome

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The many physiologic roles of human alpha-fetoprotein (HAFP) and its correlation with perinatal distress/pregnancy outcome are rarely addressed together in the biomedical literature, even though HAFP has long been used as a biomarker for fetal birth defects. Although the well being of the fetus can be monitored by the measurement of gestational age–dependent HAFP in biologic fluid levels (serum, amniotic fluid, urine, and vaginal fluids) throughout pregnancy, the majority of clinical reports reflect largely second trimester and (less likely) first trimester testing due to regulatory clinical restrictions. However, reports of third-trimester and pregnancy term measurement of HAFP levels performed in clinical research and/or investigational settings have gradually increased over the years and have expanded our base knowledge of AFP-associated pregnancy disorders during these stages. The different structural forms of HAFP (isoforms, epitopes, molecular variants, etc.) detected in the various biologic fluid compartments have been limited by antibody recognition of specific epitopic sites developed by the kit manufacturers based on antibody specificity, sensitivity, and precision. Concomitantly, the advances in elucidating the various biologic actions of AFP are opening new vistas toward understanding the physiologic roles of AFP during pregnancy. The present review surveys HAFP as a biomarker for fetal distress during the perinatal period in view of its structural and functional properties. An attempt is then made to relate the AFP fluid levels to adverse pregnancy complications and outcomes. Hence, the present review was divided into two major sections: (I) AFP structure and function considerations and (II) the relationship of AFP levels to the distressed fetus during the third trimester and at term. Exp Biol Med 232:993–1004, 2007

Key words: alpha-fetoprotein; birth defects; pregnancy outcome; fetal distress; biomarker

Introduction

Human alpha-fetoprotein (HAFP) is a tumor-associated fetal glycoprotein involved with both ontogenic and oncogenic growth (1, 2). The fetal protein is a 69-kDa single-polypeptide chain that contains 3%–5% carbohydrate and is produced in the yolk sac and fetal liver. It exhibits a triplicate domain structure configured by intramolecular loops dictated by disulfide bridging, resulting in a helical V- or U-shaped form observed in electron dot maps (3). Mammalian AFP has been classified as a member of a three-domain, cysteine-rich translated protein of the albuminoid gene family that currently consists of four members: albumin (ALB), vitamin D–binding protein, AFP, and α-ALB (4, 5). In the clinical laboratory, HAFP has long been employed both as a postoperational tumor monitoring agent and as a gestational age–dependent fetal defect marker demonstrating utility in screening for neural tube defects and aneuploidies (Table 1; Refs. 6–8). However, the main biologic role of AFP during pregnancy remains controversial to this day.

Presently, a vast biomedical literature has amassed concerning the use of HAFP during pregnancy as a biomarker in human maternal serum (MS) and amniotic fluid. Such studies have addressed the measurement of serum levels of AFP outside the normal levels in the sera of pregnant women; such values are indicative of multiple congenital malformations of the embryo and fetus. The first

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Table 1. Prenatal Screening Timeline for AFP Employed as a Biomarker Alone or in Combination with Other Analytes[a]

Year	Observation/event	Reference
1972	Elevated AFP in amniotic fluid for neural tube defects. Indication: potential biomarker.	Brock, DJH *et al*. Lancet 2: 191–194, 1972
1973	Elevated AFP in MS for neural tube defects. Indication: potential biomarker.	Leek, AE *et al*. Lancet 2: 385–386, 1973
1980	Early antenatal diagnosis of ventral wall defect using AFP.	Wald, NJ *et al*. Lancet 1: 368, 1980
1981	Maternal weight influence on MS-AFP level in prenatal screening.	Haddow, JE *et al*. Clin. Chem. Vol. 27: 133, 1981.
1981	Amniotic fluid acetylcholinesterase diagnoses for neural tube defects with elevated AFP levels.	Collaboration Study. Lancet 2: 321–323, 1981.
1984	Low maternal serum AFP levels discovered in prenatal Down syndrome pregnancy samples.	Merkatz, IR *et al*. Amer. J. Obstet. Gynecol. 148: 886–894, 1984.
1987	Combination of maternal age and AFP levels useful in Down syndrome pregnancies.	Cuckle, HS *et al*. Brit. J. Obstet. Gynecol. 94: 387, 1987.
1989	Screening for Down syndrome using AFP, μE3, and hCG (triple biomarker test).	Wald, NJ. Amer. J. Hum. Genet. 44: 586, 1989.
1990	Triple marker screen used to detect Trisomy-18 chromosomal disorders.	Canick, JA *et al*. Prenat. Diag. 10: 546, 1990.
1991	Low MS-AFP in congenital cardiac and diaphragmatic defects.	Resta, RG. Amer. J. Med. Genet. 40: 129, 1991.
1992	Prenatal screen in MS using multiple markers for fetal distress.	Haddow JE *et al*. New Eng. J. Med. 327: 588, 1992.
1994	Four-marker serum screening for Down syndrome (Quad test) using AFP, estriol, hCG, Inhibin-A	Wald, NJ. Prenat. Diagn. 14: 707–716, 1994.
2003	Comparison of triple vs. Quad test for Down syndrome using AFP, estriol, hCG, and Inhibin-A.	Wald, NJ *et al*. Lancet 361: 835–836, 2003.
2003	First- and second-trimester screening for Down syndrome, integrated testing (SURUSS trials).	Wald, NJ *et al*. Health Technol. Assess. 7 (11): 1–30, 2003.
2004	Combined (sequential) first- and second-trimester screening for Down syndrome using PAPP-A, B-hCG, followed by the AFP triple test.	Platt, LD *et al*. Obstet Gynecol. 104: 661–666, 2004.
2004	First-trimester PAPP-A and B-hCG, NT comparison levels for Down syndrome (FASTER trials).	Dugoff, L *et al*. Amer. J. Obstet. Gynecol. 191: 1446–1451, 2004.
2005	First and second combined screen for Down syndrome (FASTER trial follow-up).	Malone, FD *et al*. New Eng. J. Med. 353: 2001, 2005.

[a] MS, maternal serum; hCG, human chorionic gonadotrophin; estriol, unconjugated estriol; SURRUSS, prenatal screening clinical trials held in United Kingdom; PAPP-A, pregnancy-associated plasma protein-A; B-hCG, beta-hCG; NT, nuchal translucency; FASTER, prenatal screening clinical trials held in the United States.

developmental abnormalities to be associated with abnormal AFP levels were neural tube defects and brain/spinal cord malformations (9, 10). Later, other types of birth defects were found to reflect discordant AFP levels, including chromosomal abnormalities (anaploidies) and various anatomic congenital disorders (11, 12). It was subsequently determined that assaying additional analytes together with HAFP increased the prenatal screening detection rates (Table 2). While MS-AFP levels associated with neural tube fetal defects are elevated, the chromosomal disorders demonstrate low serum AFP concentrations. Following the association of increased AFP levels with neural tube defects, additional structural anomalies have been classified within the AFP-elevated level category (6, 13). AFP serum levels during pregnancy also have been used as an ancillary aid in the diagnosis of pregnancy-related hematologic disorders (anemias), placental abnormalities, fetal death, growth restriction/retardation, and preterm labor (14). However, there exists a paucity of reports that have attempted to correlate such pregnancy anomalies with the multiple biologic activities attributed to AFP in the last decade.

Objectives. The focus of the present report has addressed the increasing number of reports associated with abnormal AFP serum and amniotic fluid levels concommittant with perinatal complications and adverse pregnancy outcomes. This review has attempted to link the many functional roles of AFP with physicochemical stress/shock conditions of the fetus during the perinatal period of pregnancy. Moreover, the various physiologic roles attributed to AFP in the regulation of growth and differentiation during fetal/neonatal development have not kept pace with the increase of clinical research reports. Thus, the objectives of the present review are two-fold. First, the biologic activities of AFP during late pregnancy will be addressed, since many reviews have focused solely on AFP as a second-trimester fetal defect biomarker. Second, the various properties of AFP in light of its investigational utility as a clinical biomarker during perinatal development will be

surveyed. Thus, the multitude of fetal malformations, congenital anomalies, and genetic diseases with adverse pregnancy outcomes associated with AFP in late pregnancy will be addressed. For more intense accounts of the physicochemistry and genetics of AFP, the reader is directed to earlier reviews on AFP (15–19). Recently, a report on the functional mapping of the various domains and motifs of the HAFP molecule has been put forward (20). In view of the above discussion, the present review has been divided into two major sections. These will include the following: (I) structural and functional aspects of AFP and (II) the relevance of AFP fluid levels as a biomarker in the perinatal period to adverse pregnancy outcome.

I. Structural and Functional Aspects of AFP

Structural Variants. Molecular variants of mammalian AFP have been reported in the scientific literature since the 1970s. Some of these earlier variant forms were attributed to carbohydrate microheterogeneity and isoforms associated with varying isoelectric points (21, 22). Later reports demonstrated AFP forms that were genetic isoforms and lectin glycoforms demonstrable by electrophoretic and chromatographic procedures (23, 24). Still other variants were detected following high-pressure liquid chromatography using lectin, heavy metal, and hydrophobic solid-phase separation methodology (25). The advent of monoclonal antibodies permitted the detection and analyses of epitopic domains and subdomains that comprise the overall antigenic determinant sites on AFP (26, 27). Finally, the discovery and characterization of the molten globule forms of AFP have provided a new level of understanding regarding the various folding transition forms of this fetal protein (28).

Molecular variants of HAFP have further been reported as a result of clinical assays that detected aberrant molecular forms. Several such reports of aberrant AFP molecules first appeared in the clinical cystic fibrosis literature, resulting in confusion of the clinical usefulness of AFP for this genetic disorder. In the 1970s and 1980s, prior to the development of monoclonal antibodies, polyclonal antibody assays were not as precise and sensitive as today's immunoassays. Such factors resulted in disparate baseline levels of HAFP in the sera of nondisease, healthy adult patients, which ranged in concentrations from 5 to 20 ng/ml. In addition, a previously reported cationic form of HAFP has been confirmed to be HAFP complexed with immunoglobulin M molecules; this cationic form has now been described in several independent laboratories (1, 25, 29, 30). Aberrant forms of HAFP also have been detected in the reproductive and urinary tracts in various clinical patients, as well as in the sera of human patients (breast cancer, reproductive disorders, etc). A nonsecreted form of HAFP lacking the N-terminal signal sequence segment was recently reported in recombinant AFP studies employing yolk sac tumors (31). Truncated forms of HAFP (~50,000 Daltons) have further been detected in cell cultures comprising hepatomas, testicular embryonal carcinomas, and breast tumors (30). Variant forms of HAFP transcripts from nontranslated regions of the AFP mRNA have recently been reported in $CD34^+$ hematopoietic progenitor cells derived from mesodermal germ cells (32). These latter investigations described two variant forms of HAFP mRNA that are not expressed in mature cells. The variant AFP mRNAs differed from the authentic transcripts by incorporating exons from the 5′-untranslated region of the HAFP gene. The abnormal AFP transcript was found only in bone marrow, thymus, and brain tissue. The various folding intermediate forms of HAFP have recently been investigated using bacterial and yeast recombinant methodology (33, 34). The folding of both glycosylated (yeast) and nonglycosylated *(Escherichia coli)* forms of recombinant HAFP was studied following protein purification from aggregation-prone inclusion bodies. After AFP was denatured, it readily refolded under dilution, redox, reactions, and enzyme-linked immunoabsorbent assay (ELISA) conditions in both of the recombinant-produced AFP forms. In summary, the denaturation of recombinant-derived HAFP was found to be a reversible process independent of its starting source, fatty acid relationship, and glycosylated state.

Table 2. Detection Rate Percent (%) Produced by Increasing the Number of Analyte Screening Components in Prenatal Assays with the False-Positive Rate Constant at 5%[a]

Screening components (agents)[b]	% Detection rate[b]
1. Maternal age alone	35–40
2. Maternal age plus MS-AFP in second trimester	40–45
3. MS-AFP plus MS-hCG in second trimester	50–55
4. MS-AFP plus MS-hCG plus MS-E3 in second trimester (triple test)	65–70
5. MS-AFP, MS-hCG, MS-E3, and Inhibin-A in second trimester (Quad test)	70–80
6. Combined test: first-trimester maternal age, PAPP-A, β-hCG	80–85
7. Serum integrated test: PAPP-A plus Quad	85–90
8. Integrated: first trimester (PAPP-A, hCG) plus second trimester Quad	90–95

[a] Serum integrated test indicates maternal serum PAPP-A in first trimester followed by Quad test in second trimester. Integrated indicates maternal serum PAPP-A and hCG in first trimester followed by Quad test in the second trimester. MS-E3, maternal serum unconjugated estriol.
[b] Range of three to four studies each. See Table 1 for reference citations.

Biologic Roles. Determination of the biologic roles of mammalian AFP has been a research objective for many years. Similar to albumin, serum AFP is known to bind and transport a multitude of ligands, such as bilirubin, fatty acids, retinoids, steroids, heavy metals, dyes, flavonoids,

phytoestrogens, dioxin, and various drugs (35, 36). Indeed, AFP has been shown to bind *in vitro* many substances, some of which serve as ligands for members of the steroid/thyroid nuclear receptor superfamily (37–39). However, other ligands that bind to AFP (rodent and human) include metabolic stains, L-tryptophan, warfarin, triazine dyes, phenylbutazone, streptomycin, phenytoin, anilinonaphthaline sulfate, heavy metals, low–carbon-chain alcohols, and polyunsaturated fatty acids (1, 25). Although the physicochemical and structural properties of HAFP have been extensively described, it was mostly the *in vitro* functional roles that have been confirmed to date. Thus, the physiologic properties of HAFP have encompassed mainly ligand carrier/transport functions, but modulation of the immune response assays has been widely addressed (see below). Interestingly, it is growth regulation that has recently emerged as an important function of human AFPs as well as in other mammals (see below).

During the last decade, a multitude of studies have established AFP as a regulator of ontogenic and oncogenic growth (1, 25). In fact, it is the growth-modulating activity that distinguishes AFP from albumin, a major blood protein carrier/transport molecule of the albuminoid gene family. Reports now support the concept that native, full-length AFP is largely a growth-enhancing protein whose overall activity is enacted through a cyclic AMP–protein kinase A activation pathway (40, 41). However, growth is a process that requires fine tuning for both upregulation and downregulation to operate correctly over defined time periods, such as pregnancy. Although sustained growth of the fetus is required for full-term pregnancy, the fetus does encounter situations that require periods of temporary or prolonged growth cessation, such as differentiation, transformation, and the prevention of organ/tissue overgrowth (42). Furthermore, the fetus may experience pulses of stress/shock insults in the microenvironment compartments of both the extracellular and intracellular fetal mileux. Thus, fetal growth in a tissue or the extracellular matrix may require a temporary halt until fetal homeostasis is achieved and/or until compensated signal transduction pathways are reestablished via adaptor/scaffold protein–protein interactions. Such stress/shock encounters involving AFP include environmental extremes of osmolality, pH, oxygen tension, ischemia, glucose shock, osmotic pressure, anemia, anoxia, and excessively high ligand (steroids, fatty acids, etc) concentrations (25).

The growth regulatory properties of AFP have aroused investigational interest in studies of ontogeneic and oncogenic growth in both cell cultures and animal models. A multitude of reports have now documented that HAFP is capable of regulating growth in reproductive, hematopoietic, placental, hepatic, inflammatory, and lymphatic cells (25, 43–46). Since the late 1990s, AFP is viewed as a protein associated with modulating cell proliferation, differentiation, regeneration, and transformation in both ontogenetic and oncogenic growth processes (1, 6). Although an AFP gene knockout in mice resulted in infertile female offspring and not lethality or developmental arrest (47), a similar outcome may not necessarily be true for human beings. Although such an investigation cannot be ethically pursued in humans, clinical cases of AFP congenital deficiency have been reported in the literature (13, 48). Patients in such studies were asymptomatic and presented with normal development in their clinical histories.

In its native form, HAFP displays largely growth-enhancing properties, regardless of whether the tissue is of fetal or postnatal origin; it is the ligand-free form of HAFP at physiologic dose levels that has been shown to enhance tumor growth (49, 50). HAFP has further been shown to possess proangiogenic properties that promote neovascularization and growth in both fetal and tumor tissues (51, 52). Recent findings further indicate that HAFP can also stimulate the expression of certain oncogenes (c-Fos, c-Jun, and n-Ras) which, in turn, enhances the proliferation of human carcinoma cells (53). Finally, HAFP has been shown to functionally impair dendritic cells, inducing immune dysfunction and apoptosis of antigen-processing cells (54). In the latter report, the authors suggested a mechanism by which hepatoma cells could escape immunologic surveillance as a result of cells bearing AFP molecules on their cell surfaces.

The immunoregulatory functions of HAFP have long been studied (55). In brief, full-length HAFP has been found to be immunosuppressive in both B- and T-cell lectin blast cell stimulation (56, 57). However, recent studies have reported that not all self versus nonself AFP-specific T-cell clones are deleted during ontogeny, and that potential AFP antigenic sites persist and are recognized by both murine and human T cells. During the last several years, multiple research groups have succeeded in mapping T-cell immunodominant epitope sites on HAFP (58–62). These research groups have determined that four major human leukocyte antigen A epitotopic sites and several more minor epitopic determinants can be localized throughout the three-domain structure of HAFP.

The proposed concept that normal human pregnancy is actually a controlled state of inflammation has recently been validated in the biomedical literature (63). The human conceptus has classically been viewed as a foreign (nonself) object in the mother's body and has long been considered a tissue allograft residing in the maternal uterus. Investigators have recently shown that the conceptus resides in an immunologically privileged site situated in juxtaposition to the placental cells (barrier), which are in direct contact with uterine tissue containing natural killer cells of the maternal innate immune system. In turn, the maternal natural killer cells secrete cytokines that attract maternal lymphocytes to the placental boundary, causing the maternal tissues to view the foreign cell clusters as tissue inflammatory sites. Such lymphocytes wall off intruder cells from the maternal tissues at the placental/uterine interface, and the cells of the conceptus are viewed by maternal cells as sites of tissue

inflammation. Obviously, any fetal protein that contains cell surface proteins resembling the many cytokines of the immune system would have a definitive advantage in sustaining and maintaining the foreign tissue as a site of inflammation. AFP is such a protein consisting of a series of successive modular cassette-like peptides serving as immune system cytokine mimics, substitutes, and backup immunoregulatory peptides (1). Since the first reports emerged in the 1970s, AFP has long been recognized as both a B- and T-cell immunoregulatory protein (64).

AFP Influence on Genital Function. Mammalian AFP has been shown to affect postnatal and adult genital function, including the onset of puberty, menstrual cycling, and spermatogenesis. AFP concentrations are low in prepubertal mammals, but supplemental injections of purified rodent AFP have been shown to inhibit follicular maturation and ovulation in the ovary and spermatogenesis in the testis (65). Rodent AFP was found to reduce the number of gonocytes during fetal and postnatal life rather than stopping oocyte meiosis at the diplatene stage, as previously proposed (66). During pregnancy, follicular maturation could be blocked by administration of purified rodent AFP at the antral stage; the follicles at this stage contain degenerating oocytes that are AFP positive following immunofluorescent staining (67). AFP was first proposed and then confirmed to play a blocking role in genital cycling as well as the induction of follicular atreas (68). Prior to that report, AFP had been localized both in the ovary and hypophysis of prepubertal rats (69). During postnatal rat development, it is known that AFP serum levels drop to adult (low) concentrations coincident with achievement of ovarian maturity on Postnatal Day 35, and administration of supplemental AFP alters reproductive events at this time (70). Finally, studies now suggest that high fetal AFP levels during human pregnancy may contribute to congenital disorders, which can include cryptorchidism in term pregnancies (71).

Studies in rodents have further demonstrated that neonatal AFP prevents circulating estrogens from accessing the brain, thus defending it from masculinization and defeminization (72–74). Such events occur in the first week of life in rodents and in the late third trimester of humans. Rodent AFP, which binds E2 with high affinity, has been proposed to either prevent entry of the estrogens into the brain or to actively transport estrogens in the developing female brain (74). Using AFP gene knockout mice, investigators have demonstrated that these mice displayed both defeminization and masculization traits. Injections of the aromatose inhibitor 1,4,6-androstene-3,17-dione into the rat brain rescued the animals from defeminization, indicating that AFP may serve to protect the rodent female brain from such effects (75). These same investigators had earlier demonstrated that female AFP knockout mice were sterile at birth due to underexpressed genes of the gonadotrophin-releasing peptide hormone, resulting in downregulation of the pituitary hormone pathways (76). Since HAFP binds little or no E2 and rodent AFP is a high-affinity estrogen binder, it is difficult to reconcile the relevance of these results to the human state. However, if the basis of estrogen brain protection is based on cell regulatory signal transduction cascades rather than E2 binding to AFP, then steroid ligand binding is not the crucial event in these studies.

AFP and AFP Receptor Expression in the Normal Human Placenta. The authors of several studies have investigated the role of the placenta in the transport and possible synthesis of ATP *in situ* (77–80), since an earlier report showed that AFP was synthesized by the first-trimester human placenta (81). The objective of these investigations was to probe for the placental expression of AFP and its receptor in trophoblast cells of normal pregnancies at full term. Lafuste *et al.* had previously (82) reported that AFP was synthesized in the first-trimester placenta but not the term placenta; nonetheless, the presence of AFP could be histochemically localized in the villous tissue of the term placenta, presumably in the course of transplacental passage. Also, they found no evidence of mRNA AFP in the placental tissues or MS in term pregnancies. Since they also detected the presence of the AFP receptor in the placental villous tissue, the authors proposed a receptor-mediated transport mechanism for AFP placental transfer to maternal tissues. Similar to albumin, AFP transplacental passage might involve a temperature-sensitive process that does not depend on an intact cell cytoskeleton but is associated with a megalin/clathrin-mediated receptor endocytosis pathway localized to the villous trophoblast cells (83). Thus, the presence of the AFP receptor in the term placenta signified a means of transplacental passage of AFP to the maternal decidual tissues. Knowledge of the presence of AFP in the placenta is deemed important, since fetomaternal hemorrhages can occur during chorionic villus sampling in the first trimester (84).

II. Adverse Pregnancy Complications/Outcomes Related to HAFP Levels

Even though AFP was thought to be the "gold standard" biomarker for neural tube defects, elevated AFP levels had been used since 1976 as an indicator for additional perinatal distress conditions, such as bilateral renal agensis, fetomaternal transfusion, preeclampsia, intrauterine growth retardation (IUGR), and fetal demise (Table 3; Refs. 85–87). In many instances, AFP accumulates in a biologic compartment (such as amniotic fluid) by: (i) leakage from fetal serum and cerebrospinal fluid (NTD); (ii) exposure of blood vessels in extruding viscera, leading to transudation of AFP (exopthalmos); (iii) expedited protein filtration and passage into urea (congenital nephrosis); (iv) impaired fetal swallowing or digestion in amniotic fluid (GI anomaly); and (v) altered or obstructed transplacental passage, such as in placenta accreta (Table 3). The early developmental malformations reported in the

Table 3. Pregnancy Stages/Conditions with Abnormal Levels (High/Low) of HAFP[a]

I. Stage-specific disorders	
First and second trimester of pregnancy	Third trimester of pregnancy
1. Oligohydraminos	1. Severe preeclampsia
2. Renal agenesis	2. Intrauterine growth retardation
3. Gastrointestinal defects	3. Premature labor
4. Fetal growth restriction	4. Perinatal loss
5. Cystic hygroma	5. Fetal demise
6. Fetal-maternal bleed	6. Placental previa
7. Placental obstructions	7. Placental acrecia
8. Multiple gestation	8. Placental abruption
9. Incorrect gestational age levels	9. Prematurity
II. Fetal defect associated	
High AFP levels	Low AFP levels
1. Spina bifida	1. Blighted ova
2. Anencephaly	2. Polyhydraminos
3. Duodemal	3. Insulin-dependent diabetes
4. Omphalocoele	4. Diapharmatic hernia
5. Gastroschissis	5. Trisomy-21
6. Congenital nephrosis	6. Turner's syndrome/ hydrops
7. Neuroblastoma, hepatablastoma	7. Intrauterine growth retardation
8. Tyrosinemia	8. Hydrocephalus
9. Germ cell tumors	9. Trisomy-18
III. Pregnancy condition associated	
High HAFP levels	Low HAFP levels
1. Stillbirth	1. Trisomies/aneuploides
2. Premature labor	2. Stillbirth fetus
3. Neonatal death	3. Hydadiform mole
4. Fetal wastage	4. Long-standing fetal demise
5. Multiple pregnancy (twins)	5. Nonpregnancy
6. Low birth weight	6. Fetal death
7. Open spinal defect	7. Overestimated gestational age
8. Toxemia of pregnancy	8. HIV infection
9. Rh-isoimmunization	9. Spontaneous abortion

[a] Data were extracted and compiled from the following references: Mizejewski (6, 46); Walters, BNJ, Brit. J. Obstet. Gynecology 92: 341, 1985; Thomas, RL, Obstet Gynecol Surveys 45: 269, 1990.

literature were structural in nature, and late-pregnancy complications were directly life-threatening to the fetus and, oftentimes, the mother. Such conditions included severe preeclampsia, premature labor, intrauterine and/or perinatal death, preterm birth, fetal wastage, and trophoblast abnormalities, including placental previa and disruption (88–92). Nonpathologic elevations of AFP in pregnancy can be the result of physiologic or procedural phenomena such as twining or multiple pregnancy, low birth weight, prematurity, or incorrect gestational age dating (93–96). Fetal defects and malformations can also be parsed by classifying them according to high or low levels of AFP in biologic fluids (Table 3). Elevated serum and amniotic fluid AF–AFP levels are usually indicative of the presence of an anatomic lesion, such as those observed in NTD, anencephaly, ventral wall defects, gastrointestinal atresia, renal anomalies, polyhyramnios and oligohyramnios, cystic hygromas with fetal hydrops, teratomas, blastomas, and disruption of placental barriers (97–103).

At the opposite extreme, low AFP levels signify the presence of chromosomal abnormalities (aneuploides), such as trisomies, as well as fetal loss, hydatidiform mole, hydrocephalus, diaphragmatic hernias, Turner's syndrome, choroid plexus cyst, duodermal atresia, renal pyelectasis, and fetal growth restriction (104–108). Again, the fetal malformation and adverse conditions can be classified according to the calculated multiple of a population AFP median compared with the patient's AFP median value. Low-AFP disorders categorized by various investigators have included low birth weight, multiple pregnancies, fetal wastage, perinatal death, spontaneous abortions, stillbirths, and neonatal deaths (109–111). An association was also found between second-trimester low HAFP levels and subsequent Sudden Infant Death Syndrome (SIDS). The investigators in this latter study suggested that the risk of SIDS might be mediated in part through impaired fetal growth and occurrence of adverse preterm birth events (112). Finally, the measurement of MS-AFP together with the introduction of computer-assisted Doppler measurement, which is indicative of absent or altered diastolic arterial flow in critical tissue areas, has been a major advancement in prenatal monitoring technologies.

The application of Doppler velocimetry as an adjunct to perinatal screening programs has recently increased in clinical usage. Elevated MS-AFP has now been correlated with reduced uteroplacental blood flow observed in the uterine artery (113). A study for second-trimester screening for preeclampsia was recently reported which showed that higher MS-AFP levels of human chorionic gonadotrophin (hCG), Inhibin-A, Activin-A, and AFP were accompanied by increased rates of the Doppler prediastolic notch and the derived uterine artery resistance index (114). Even though a test sensitivity of 70%–93% and a specificity of 87%–98% was achieved, the addition of Doppler velocimetry only slightly improved the predictive efficiency of the total biomarkers when used alone. The measurement of fetal middle-cerebral artery Doppler velocity has also been employed, together with MS-AFP and fetal hemoglobin as biomarkers to predict the risk of fetal anemia (115). Investigators have indeed found significant correlations between MS-AFP and both Doppler arterial measurement ($r = 0.56$) and fetal hemoglobin ($r = 0.71$) levels. In cases of alloimmunized pregnancies with fetal anemia, measured MS-AFP elevations preceded the presence of increased Doppler velocity by nearly 3 weeks. In contrast to the previous report, another group reported that both first- and

Table 4. Summary of Univariate Data Analysis Showing the Prediction of Severe Placental Complications in Women with Unexplained Combined AFP and/or hCG for Term Pregnancies[a]

	Elevated MS-AFP + hCG		Elevated MS-AFP or hCG	
Condition	RR	RR range	RR	RR range
1. Pregnancy-induced hypertension	2.17	1.34–3.52	1.41	1.20–1.66
2. Abruption placentae	2.90	0.91–9.23	1.60	1.01–2.53
3. Intrauterine growth retardation	4.70	2.43–9.07	1.59	1.16–2.17
4. Fetal death	16.16	6.77–38.55	5.01	2.88–8.71
5. Preterm birth	8.67	3.94–19.10	2.42	1.59–3.68
6. Premature rupture of membranes	3.60	2.14–6.08	1.68	1.38–2.06

[a] Data extracted from Chandra *et al.* (120). Note the advantage of combining MSAFP with hCG. Screening was performed in the second trimester for prediction in the third trimester and term. Relative risk (RR) of 1.0 indicates no risk.

second-trimester biochemical markers of trisomies had no relationship to maternal hemoglobin concentrations; however, Doppler velocity measurements were not done in this instance (116). Finally, the application of Doppler methodology to the analysis of factors predicting antepartum stillbirth was studied in conjunction with MS-AFP and pregnancy-associated plasma protein-A (PAPP-A) levels (117). Antepartum stillbirth, the single most common cause of perinatal death, has been previously associated with fetal abnormalities, congenital infections, Rh-isoimmunization, and pregnancy complications, such as IUGR, preeclampsia, and placental abruption (118). Both MS-AFP and PAPP-A measurement used in conjunction with Doppler indices of resistance to flow were found predictive of antepartum stillbirth. Both MS-AFP and PAPP-A levels are involved in placental passage and functional dynamics, because the risk of stillbirth in late pregnancy may be a result of a placental dysfunction of the placenta in early pregnancy. The invasion of the trophoblast into the uterine vessels is associated with decreased resistance to flow in the uterus, and impaired placentation is reflected in high-resistance Doppler flow velocity waveforms recorded from the uteroplacental circulation (119).

The increased usage of uterine artery Doppler (UAD) and placental ultrasound has aided in the elucidation of unexplained discordant levels of MS-AFP in the perinatal period of pregnancy. However, in the third trimester, the root cause of an AFP-associated pregnancy disorder can often be attributed to some form of placental dysfunction. Pregnancy complications related to placental disease include: (i) preeclampsia, (ii) intrauterine growth restriction, (iii) placental abruption, (iv) fetal death, and (v) spontaneous preterm labor/birth (94). Both AFP and hCG alone or in combination with other analytes can increase these associations and their subsequent risks (Tables 2 and 4; Refs. 120, 121). Premature labor and subsequent preterm delivery constitute some of the major contributors of perinatal death in the world (119). Adverse perinatal complications, such as fetal death or preterm delivery, are often attributable to chronic uteroplacental vascular insufficiency and placental infarction. Elevated MS-AFP levels are frequently found to be associated with reduced uteroplacental blood flow detected by UAD measurements (114). Moreover, abnormalities such as placental shape and/or texture in conjunction with elevated MS-AFP have been correlated with poor pregnancy outcome.

In a recent Canadian study, combined elevations of AFP and hCG were employed to predict severe placental complications using UAD, ultrasound, and placental morphology at the 19- to 23-week period (Table 4; Ref. 120). Relative risk data from this combined analyte study revealed the presence of abnormalities in studies using both placental ultrasound and UAD correlated with multiple perinatal complications, such as preterm delivery, IUGR, intrauterine fetal death, and preeclamptic pathology. Their clinical results showed a 10-fold increase in abnormal UAD scans, underscored by a high rate of underlying chronic placental vascular pathology that limited the maternal blood supply and damaged the nutrient-exchanging placental villi. These investigators further reported instances of cases displaying severe preelcampsia in addition to patients exhibiting the HELP (Hemolysis, Elevated liver enzymes, and Low Platelets) syndrome. The authors stated that their test may be able to identify the majority of women destined to either lose the fetus or deliver a preterm baby due to uteroplacental vascular insufficiency.

Other investigators have attempted to correlate abnormal second-trimester MS-AFP levels with adverse pregnancy outcomes. One group determined that abnormally high MS-AFP levels were associated with low birth weight, prematurity, and antepartum hemorrhage, whereas abnormally low unexplained MS-AFP correlated with macrosomia (overgrowth disorder) and advanced gestational age at delivery (111). While the negative predictive value of this test was high (96%), the positive predictive value was disappointingly low (9%–12%). Thus, the use of this assay to formulate a treatment plan was not promising; however, the test might find value in reassuring women about their pregnancy outcome. Another group of investigators tested whether several amniotic fluid protein components (insulin-like growth factor 1 [IGF-1], IGFβP-1, leptin, AFP) correlated with the severity of IUGR (122). Among the

candidate proteins, only an association with AFP levels was found to be significant. Thus, only elevated AFP levels in amniotic fluid were useful in the early (14–18 weeks) detection of populations at risk for developing IUGR. The Quad test (AFP, hCG, UE3, and Inhibin-A) was also analyzed as a predictor of adverse pregnancy outcome in association with preterm birth, IUGR, preeclampsia, and fetal loss (123). Although it was determined that the use of multiple markers had a relatively low sensitivity and positive predictive value, it was superior to using an individual screening marker alone. Finally, the use of high MS-AFP and low amniotic fluid–AFP in the second trimester (15 weeks) indicated that diagnostic ultrasound imaging should be applied in the third trimester. Subsequent sonography at 33 weeks in this latter study revealed the presence of polyhydramios, echogenic amniotic fluid images, and gastric dilatation without gross anatomic malformations. The female karotype in this study was normal, as were the amniotic fluid Ache and AFP levels. However, at a term of 35 weeks, the newborn had pyloric atresia and displayed cutaneous blisters and erosions confirmed as epidermolysis bulbosa (124), which has previously been associated with elevated MS-AFP levels (125).

The combination of prolactin (PRL) and human placental lactagen (HPL) measurements together with an AFP determination has also been employed in the clinical diagnosis of premature rupture of membranes (PROM; Refs. 126, 127). Although such protein determinations have been measured in MS and amniotic fluid, greater sensitivity has been achieved using vaginal secretion specimens up to 41 weeks' gestation with the MS-AFP measurements determined by ELISA assay. Using 30 ng/ml as the cutoff level, researchers reported sensitivity and specificity at 98% and 99% confidence levels, respectively (128). The investigators of this latter report stated that the results obtained with AFP alone compared with other clinical tests and echographic (ultrasound) observations were significantly better than measurement of pH together with diamino-oxidase assays and determination of PRL levels. However, in an earlier study using vaginal fluids (VFs) compared to MS and urine, VF levels of PRL and AFP were found to be 2- to 10-fold and 5- to 50-fold higher, respectively, than in paired MS and/or AF specimens. Especially in the PROM state after the 33rd week of pregnancy, VF levels of MS-AFP were predominantly higher than those found in MS, extending up to 5500 μg/ml (129). There have also been attempts to employ AFP, HPL, and PRL biomarkers in early pregnancy, although they met with less success (130, 131). In summary, only PROM studies involving HPL and AFP have continued to display their usefulness when employed in late pregnancy or at term (132).

Concluding Statements

This review has focused on the physiologic roles of AFP and its utility as a biomarker to predict perinatal distress and adverse pregnancy outcomes. Since the discovery that AFP was tumor associated in the mid-1960s, the functional roles of AFP have slowly emerged concomitantly with its ever-growing use as a biomarker in the clinical laboratory. Even though the quantitative serum levels of AFP do not always correlate with increasing size of endodermally derived tumors, the use of AFP as a tumor marker has not abated, even to the present day. Its popularity as a fetal birth defect marker increased dramatically in the 1970s and 1980s and achieved prominence in the screening of neural tube defect and chromosomal anomalies. Although AFP is not employed as a biomarker for Down syndrome in the first trimester, its association with Trisomy-21 in the second trimester provided the underpinnings for the advances in other MS marker development in first-trimester Down syndrome pregnancies.

The discoveries of discordant AFP levels correlated with perinatal distress and adverse pregnancy outcomes slowly emerged in reports emanating from the late 1980s–1990s. With each passing decade, the physiologic roles of AFP had gradually increased, and only scant attempts were made to merge those functions with the multitude of congenital malformations that had been reported. Still prominent is the long association of AFP with immune function, which is now beginning to come into greater prominence, suggesting a role of AFP in maintaining the fetal/placental unit in a controlled state of inflammation. In the future, we can expect the role of AFP in maintaining the fetus as an allograft in the mother's body to become more clear as its relationships to the cytokines and the natural killer receptors are unraveled.

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1. Mizejewski GJ. Alpha-fetoprotein as a biologic response modifier: relevance to domain and subdomain structure. Proc Soc Exp Biol Med 215:333–362, 1997.
2. Abelev GI, Eraiser TL. Cellular aspects of alpha-fetoprotein reexpression in tumors. Semin Cancer Biol 9:95–107, 1999.
3. Luft AJ, Lorscheider FL. Structural analysis of human and bovine alpha-fetoprotein by electron microscopy, image processing, and circular dichroism. Biochemistry 22:5978–5981, 1983.
4. Mizejewski GJ. The phylogeny of alpha-fetoprotein in vertebrates: survey of biochemical and physiological data. Crit Rev Eukaryot Gene Expr 5:281–316, 1995.
5. Long L, Davidson JN, Spear BT. Striking differences between the mouse and the human alpha-fetoprotein enhancers. Genomics 83:694–705, 2004.
6. Mizejewski GJ. Levels of alpha-fetoprotein during pregnancy and early infancy in normal and disease states. Obstet Gynecol Surv 58: 804–826, 2003.
7. Dashe JS, Twickler DM, Santos-Ramos R, McIntire DD, Ramus RM. Alpha-fetoprotein detection of neural tube defects and the impact of standard ultrasound. Am J Obstet Gynecol 195:1623–1628, 2006.

8. Rozenberg P, Bussieres L, Chevret S, Bernard JP, Malagrida L, Cuckle H, Chabry C, Durand-Zaleski I, Bidat L, Lacroix I, Moulis M, Roger M, Jacquemot MC, Bault JP, Boukobza P, Boccara P, Vialard F, Giudicelli Y, Ville Y. Screening for Down syndrome using first-trimester combined screening followed by second-trimester ultrasound examination in an unselected population. Am J Obstet Gynecol 195: 1379–1387, 2006.
9. Drugan A, Weissman A, Evans MI. Screening for neural tube defects. Clin Perinatol 28:279–287, vii, 2001.
10. Muller F. Prenatal biochemical screening for neural tube defects. Childs Nerv Syst 19:433–435, 2003.
11. Benn PA, Ying J. Preliminary estimate for the second-trimester maternal serum screening detection rate of the 45,X karyotype using alpha-fetoprotein, unconjugated estriol and human chorionic gonadotropin. J Matern Fetal Neonatal Med 15:160–166, 2004.
12. Duric K, Skrablin S, Lesin J, Kalafatic D, Kuvacic I, Suchanek E. Second trimester total human chorionic gonadotropin, alpha-fetoprotein and unconjugated estriol in predicting pregnancy complications other than fetal aneuploidy. Eur J Obstet Gynecol Reprod Biol 110: 12–15, 2003.
13. Sharony R, Amiel A, Bouaron N, Kidron D, Itzhaky D, Fejgin M. Congenital deficiency of alpha-fetoprotein and associated chromosomal abnormality in the placenta. Am J Med Genet A 121:113–117, 2003.
14. Bartha JL, Comino-Delgado R, Arce F, Alba P, Broullon JR, Barahona M. Relationship between alpha-fetoprotein and fetal erythropoiesis. J Reprod Med 44:689–697, 1999.
15. Chen H, Egan JO, Chiu JF. Regulation and activities of alpha-fetoprotein. Crit Rev Eukaryot Gene Expr 7:11–41, 1997.
16. Johnson PJ, Poon TC, Hjelm NM, Ho CS, Blake C, Ho SK. Structures of disease-specific serum alpha-fetoprotein isoforms. Br J Cancer 83: 1330–1337, 2000.
17. Moldogazieva NT, Terent'ev AA, Shaitan KV. [Relationship between structure and function of alpha-fetoprotein: conformational status and biological activity]. Biomeditsinskaia khimiia 51:127–151, 2005.
18. Lazarevich NL. Molecular mechanisms of alpha-fetoprotein gene expression. Biochemistry (Mosc) 65:117–133, 2000.
19. Gillespie JR, Uversky VN. Structure and function of alpha-fetoprotein: a biophysical overview. Biochim Biophys Acta 1480: 41–56, 2000.
20. Terentiev AA, Moldogazieva NT. Structural and functional mapping of alpha-fetoprotein. Biochemistry (Mosc) 71:120–132, 2006.
21. Lamerz R. AFP isoforms and their clinical significance (overview). Anticancer Res 17:2927–2930, 1997.
22. Ichikawa E, Kuriyama S, Yuji J, Masaki T, Uchida N, Nishioka M, Taketa K. Further resolution of alpha-fetoprotein glycoforms by two-dimensional isoelectric focusing and lectin affinity electrophoresis. Electrophoresis 27:3480–3487, 2006.
23. Taketa K. Characterization of sugar chain structures of human alpha-fetoprotein by lectin affinity electrophoresis. Electrophoresis 19:2595–2602, 1998.
24. Taketa K. Multimodal application of lectin affinity electrophoresis of alpha-fetoprotein. Electrophoresis 19:1774–1779, 1998.
25. Mizejewski GJ. Alpha-fetoprotein structure and function: relevance to isoforms, epitopes, and conformational variants. Exp Biol Med (Maywood) 226:377–408, 2001.
26. Kang Y, Matsuura E, Sakamoto T, Sakai M, Nishi S. Analysis of epitopes of mouse monoclonal antibodies against human alpha-fetoprotein. Tumour Biol 22:254–261, 2001.
27. Yakimenko EF, Yazova AK, Goussev AI, Abelev GI. Epitope mapping of human alpha-fetoprotein. Biochemistry (Mosc) 66:524–530, 2001.
28. Uversky VN, Narizhneva NV, Kirschstein SO, Winter S, Lober G. Conformational transitions provoked by organic solvents in beta-lactoglobulin: can a molten globule like intermediate be induced by the decrease in dielectric constant? Fold Des 2:163–172, 1997.
29. Beneduce L, Castaldi F, Marino M, Tono N, Gatta A, Pontisso P, Fassina G. Improvement of liver cancer detection with simultaneous assessment of circulating levels of free alpha-fetoprotein (AFP) and AFP-IgM complexes. Int J Biol Markers 19:155–159, 2004.
30. Mizejewski GJ. Biological role of alpha-fetoprotein in cancer: prospects for anticancer therapy. Expert Rev Anticancer Ther 2: 709–735, 2002.
31. Fukasawa H, Iwamoto H, Hirata S, Shoda T, Yokota S, Nishi S, Hoshi K. Novel human alpha-fetoprotein mRNA isoform lacking exon 1 identified in ovarian yolk sac tumor. J Soc Gynecol Investig 12:456–462, 2005.
32. Kubota H, Storms RW, Reid LM. Variant forms of alpha-fetoprotein transcripts expressed in human hematopoietic progenitors. Implications for their developmental potential towards endoderm. J Biol Chem 277:27629–27635, 2002.
33. Yazova AK, Goussev AI, Christiansen M, Kushlinsky NE, Stogova E, Norgaard-Pedersen B, Abelev GI. Human fetal and tumor alpha-fetoproteins differ in conformationally dependent epitope variants expression. Immunol Lett 85:261–270, 2003.
34. Leong SS, Middelberg AP. The refolding of different alpha-fetoprotein variants. Protein Sci 15:2040–2050, 2006.
35. Arsenov DV, Golubeva MB, Kisel MA, Konoplya NA, Lyubin GS, Kuz'mitskii BB, Strel'chenok OA. Modification of humoral immune response in C57Bl/6 mice with a complex of alpha-fetoprotein and retinoid acid derivatives. Bull Exp Biol Med 132:963–965, 2001.
36. Milligan SR, Khan O, Nash M. Competitive binding of xenobiotic oestrogens to rat alpha-fetoprotein and to sex steroid binding proteins in human and rainbow trout (Oncorhynchus mykiss) plasma. Gen Comp Endocrinol 112:89–95, 1998.
37. Mizejewski GJ. An apparent dimerization motif in the third domain of alpha-fetoprotein: molecular mimicry of the steroid/thyroid nuclear receptor superfamily. Bioessays 15:427–432, 1993.
38. Dauphinee MJ, Mizejewski GJ. Human alpha-fetoprotein contains potential heterodimerization motifs capable of interaction with nuclear receptors and transcription/growth factors. Med Hypotheses 58:453–461, 2002.
39. Bois-Joyeux B, Chauvet C, Nacer-Cherif H, Bergeret W, Mazure N, Giguere V, Laudet V, Danan JL. Modulation of the far-upstream enhancer of the rat alpha-fetoprotein gene by members of the ROR alpha, Rev-erb alpha, and Rev-erb beta groups of monomeric orphan nuclear receptors. DNA Cell Biol 19:589–599, 2000.
40. Wang XW, Xu B. Stimulation of tumor-cell growth by alpha-fetoprotein. Int J Cancer 75:596–599, 1998.
41. Li MS, Li PF, Yang FY, He SP, Du GG, Li G. The intracellular mechanism of alpha-fetoprotein promoting the proliferation of NIH 3T3 cells. Cell Res 12:151–156, 2002.
42. Butterstein G, Morrison J, Mizejewski GJ. Effect of alpha-fetoprotein and derived peptides on insulin- and estrogen-induced fetotoxicity. Fetal Diagn Ther 18:360–369, 2003.
43. Oertel M, Menthena A, Chen YQ, Shafritz DA. Properties of cryopreserved fetal liver stem/progenitor cells that exhibit long-term repopulation of the normal rat liver. Stem Cells 24:2244–2251, 2006.
44. Schnater JM, Bruder E, Bertschin S, Woodtli T, de Theije C, Pietsch T, Aronson DC, von Schweinitz D, Lamers WH, Kohler ES. Subcutaneous and intrahepatic growth of human hepatoblastoma in immunodeficient mice. J Hepatol 45:377–386, 2006.
45. Butterstein GM, Mizejewski GJ. Alpha-fetoprotein inhibits frog metamorphosis: implications for protein motif conservation. Comp Biochem Physiol A Mol Integr Physiol 124:39–45, 1999.
46. Mizejewski GJ. Biological roles of alpha-fetoprotein during pregnancy and perinatal development. Exp Biol Med (Maywood) 229: 439–463, 2004.
47. Gabant P, Forrester L, Nichols J, Van Reeth T, De Mees C, Pajack B,

Watt A, Smitz J, Alexandre H, Szpirer C, Szpirer J. Alpha-fetoprotein, the major fetal serum protein, is not essential for embryonic development but is required for female fertility. Proc Natl Acad Sci U S A 99:12865–12870, 2002.
48. Sharony R, Zadik I, Parvari R. Congenital deficiency of alpha fetoprotein. Eur J Hum Genet 12:871–874, 2004.
49. Dudich E, Semenkova L, Dudich I, Denesyuk A, Tatulov E, Korpela T. Alpha-fetoprotein antagonizes X-linked inhibitor of apoptosis protein anticaspase activity and disrupts XIAP-caspase interaction. FEBS J 273:3837–3849, 2006.
50. Uversky VN, Narizhneva NV, Ivanova TV, Tomashevski AY. Rigidity of human alpha-fetoprotein tertiary structure is under ligand control. Biochemistry 36:13638–13645, 1997.
51. Liang OD, Korff T, Eckhardt J, Rifaat J, Baal N, Herr F, Preissner KT, Zygmunt M. Oncodevelopmental alpha-fetoprotein acts as a selective proangiogenic factor on endothelial cell from the fetomaternal unit. J Clin Endocrinol Metab 89:1415–1422, 2004.
52. Takahashi Y, Ohta T, Mai M. Angiogenesis of AFP producing gastric carcinoma: correlation with frequent liver metastasis and its inhibition by anti-AFP antibody. Oncol Rep 11:809–813, 2004.
53. Li MS, Li PF, Chen Q, Du GG, Li G. Alpha-fetoprotein stimulated the expression of some oncogenes in human hepatocellular carcinoma Bel 7402 cells. World J Gastroenterol 10:819–824, 2004.
54. Um SH, Mulhall C, Alisa A, Ives AR, Karani J, Williams R, Bertoletti A, Behboudi S. Alpha-fetoprotein impairs APC function and induces their apoptosis. J Immunol 173:1772–1778, 2004.
55. Murgita RA, Tomasi TB Jr. Suppression of the immune response by alpha-fetoprotein on the primary and secondary antibody response. J Exp Med 141:269–286, 1975.
56. Mizejewski G, Muehlemann M, Dauphinee MJ. Update of alpha-fetoprotein growth inhibitory peptides as chemotherapeutic agents for tumor growth and metastasis. Chemotherapy 52:83–90, 2006.
57. Mizejewski GJ, Eisele L, Maccoll R. Anticancer versus antigrowth activities of three analogs of the growth-inhibitory peptide: relevance to physicochemical properties. Anticancer Res 26:3071–3076, 2006.
58. Bui LA, Butterfield LH, Kim JY, Ribas A, Seu P, Lau R, Glaspy JA, McBride WH, Economou JS. In vivo therapy of hepatocellular carcinoma with a tumor-specific adenoviral vector expressing interleukin-2. Hum Gene Ther 8:2173–2182, 1997.
59. Vollmer CM, Jr., Eilber FC, Butterfield LH, Ribas A, Dissette VB, Koh A, Montejo LD, Lee MC, Andrews KJ, McBride WH, Glaspy JA, Economou JS. Alpha-fetoprotein-specific genetic immunotherapy for hepatocellular carcinoma. Cancer Res 59:3064–3067, 1999.
60. Butterfield LH, Meng WS, Koh A, Vollmer CM, Ribas A, Dissette VB, Faull K, Glaspy JA, McBride WH, Economou JS. T cell responses to HLA-A*0201-restricted peptides derived from human alpha fetoprotein. J Immunol 166:5300–5308, 2001.
61. Mizukoshi E, Nakamoto Y, Tsuji H, Yamashita T, Kaneko S. Identification of alpha-fetoprotein-derived peptides recognized by cytotoxic T lymphocytes in HLA-A24+ patients with hepatocellular carcinoma. Int J Cancer 118:1194–1204, 2006.
62. Hanke P, Rabe C, Serwe M, Bohm S, Pagenstecher C, Sauerbruch T, Caselmann WH. Cirrhotic patients with or without hepatocellular carcinoma harbour AFP-specific T-lymphocytes that can be activated in vitro by human alpha-fetoprotein. Scand J Gastroenterol 37:949–955, 2002.
63. Sargent IL, Borzychowski AM, Redman CW. NK cells and human pregnancy–an inflammatory view. Trends Immunol 27:399–404, 2006.
64. Ritter M, Ali MY, Grimm CF, Weth R, Mohr L, Bocher WO, Endrulat K, Wedemeyer H, Blum HE, Geissler M. Immunoregulation of dendritic and T cells by alpha-fetoprotein in patients with hepatocellular carcinoma. J Hepatol 41:999-1007, 2004.
65. Seralini GE, Stora C. Inhibition of murine genital function by alpha-fetoprotein. Tumour Biol 8:292–293, 1987.
66. Lambert JC, Seralini GE, Stora C, Vallette G, Vranckx R, Nunez EA. Effects of alphafetoprotein on isolated mouse oocytes. Tumour Biol 7: 91–97, 1986.
67. Seralini GE, Lafaurie M, Krebs B, Stora C. Alphafetoprotein and atretic follicles in the ovary of the pregnant rat. Tumour Biol 7:1–8, 1986.
68. Seralini GE, Lafaurie M, Castelli D, Krebs B, Stora C. [Alpha-fetoprotein and blockade of the sexual cycle]. C R Acad Sci III 298: 397–402, 1984.
69. Castelli D, Aussel C, Lafaurie M, Ayraud N, Stora C. Immunolocalization of alpha-fetoprotein in the ovary and hypophysis of immature female rats. Histochem J 14:879–887, 1982.
70. Aussel C, Castelli D, La Faurie M, Krebs B, Ayraud N, Stora C, Masseyeff R. Role of alpha-fetoprotein (AFP) in the regulation of ovarian activity during hepatocarcinogenesis and post-natal development. Oncodev Biol Med 3:65–71, 1982.
71. Boyd HA, Myrup C, Wohlfahrt J, Westergaard T, Norgaard-Pedersen B, Melbye M. Maternal serum alpha-fetoprotein level during pregnancy and isolated cryptorchidism in male offspring. Am J Epidemiol 164:478–486, 2006.
72. Chung WC, Pak TR, Weiser MJ, Hinds LR, Andersen ME, Handa RJ. Progestin receptor expression in the developing rat brain depends upon activation of estrogen receptor alpha and not estrogen receptor beta. Brain Res 1082:50–60, 2006.
73. Puts DA, Jordan CL, Breedlove SM. Defending the brain from estrogen. Nat Neurosci 9:155–156, 2006.
74. Bakker J, De Mees C, Douhard Q, Balthazart J, Gabant P, Szpirer J, Szpirer C. Alpha-fetoprotein protects the developing female mouse brain from masculinization and defeminization by estrogens. Nat Neurosci 9:220–226, 2006.
75. De Mees C, Laes JF, Bakker J, Smitz J, Hennuy B, Van Vooren P, Gabant P, Szpirer J, Szpirer C. Alpha-fetoprotein controls female fertility and prenatal development of the gonadotropin-releasing hormone pathway through an antiestrogenic action. Mol Cell Biol 26:2012–2018, 2006.
76. Wiwanitkit V. Interaction between alpha-fetoprotein gene and gonadotropin-releasing hormone and effects on brain sexual differentation: molecular function and biological process. Sex Disabil 24: 169–173, 2006.
77. Newby D, Dalgliesh G, Lyall F, Aitken DA. Alphafetoprotein and alphafetoprotein receptor expression in the normal human placenta at term. Placenta 26:190–200, 2005.
78. Brownbill P, Edwards D, Jones C, Mahendran D, Owen D, Sibley C, Johnson R, Swanson P, Nelson DM. Mechanisms of alphafetoprotein transfer in the perfused human placental cotyledon from uncomplicated pregnancy. J Clin Invest 96:2220–2226, 1995.
79. Butler EL, Dashe JS, Ramus RM. Association between maternal serum alpha-fetoprotein and adverse outcomes in pregnancies with placenta previa. Obstet Gynecol 97:35–38, 2001.
80. Koster EL, Dashe JS, McIntire DD, Ramus RM. Association of maternal serum alpha-fetoprotein with persistent placenta previa. J Matern Fetal Neonatal Med 16:3–7, 2004.
81. Duc-Goiran P, Mignot TM, Robert B, Machavoine F, Mondon F, Hagnere AM, Vacher-Lavenu MC, Danan JL, Vaiman D, Benassayag C, Ferre F. Expression and localization of alpha-fetoprotein mRNA and protein in human early villous trophoblasts. Placenta 27:812–821, 2006.
82. Lafuste P, Robert B, Mondon F, Danan JL, Rossi B, Duc-Goiran P, Mignot TM, Nunez EA, Benassayag C, Ferre F. Alpha-fetoprotein gene expression in early and full-term human trophoblast. Placenta 23: 600–612, 2002.
83. Lambot N, Lybaert P, Boom A, Delogne-Desnoeck J, Vanbellinghen AM, Graff G, Lebrun P, Meuris S. Evidence for a clathrin-mediated recycling of albumin in human term placenta. Biol Reprod 75:90–97, 2006.

84. Pelikan DM, Kanhai HH, De Groot-Swings GM, Mesker WE, Tanke HJ, Scherjon SA. Fetomaternal hemorrhage in relation to chorionic villus sampling revisited. Prenat Diagn 26:201–205, 2006.
85. Pham TT, Benirschke K, Masliah E, Stocker JT, Yi ES. Congenital pulmonary airway malformation (congenital cystic adenomatoid malformation) with multiple extrapulmonary anomalies: autopsy report of a fetus at 19 weeks of gestation. Pediatr Dev Pathol 7: 661–666, 2004.
86. Odibo AO, Sehdev HM, Stamilio DM, Macones GA. Evaluating the thresholds of abnormal second trimester multiple marker screening tests associated with intra-uterine growth restriction. Am J Perinatol 23:363–367, 2006.
87. Wald NJ, Morris JK, Ibison J, Wu T, George LM. Screening in early pregnancy for pre-eclampsia using Down syndrome quadruple test markers. Prenat Diagn 26:559–564, 2006.
88. Zarzour SJ, Gabert HA, Diket AL, St Amant M, Miller JM, Jr. Abnormal maternal serum alpha fetoprotein and pregnancy outcome. J Matern Fed Med 7:304–307, 1998.
89. Smith GC, Shah I, Crossley JA, Aitken DA, Pell JP, Nelson SM, Cameron AD, Connor MJ, Dobbie R. Pregnancy-associated plasma protein A and alpha-fetoprotein and prediction of adverse perinatal outcome. Obstet Gynecol 107:161–166, 2006.
90. Benn PA, Craffey A, Horne D, Ramsdell L, Rodis JF. Elevated maternal serum alpha-fetoprotein with low unconjugated estriol and the risk for lethal perinatal outcome. J Matern Fet Med 9:165–169, 2000.
91. Smith GC, Shah I, White IR, Pell JP, Crossley JA, Dobbie R. Maternal and biochemical predictors of spontaneous preterm birth among nulliparous women: a systematic analysis in relation to the degree of prematurity. Int J Epidemiol 35:1169–1177, 2006.
92. Mulch AD, Stallings SP, Salafia CM. Elevated maternal serum alpha-fetoprotein, umbilical vein varix, and mesenchymal dysplasia: are they related? Prenat Diagn 26:659–661, 2006.
93. Entezami M, Runkel S, Sarioglu N, Hese S, Weitzel HK. Diagnostic dilemma with elevated level of alpha-fetoprotein in an undiagnosed twin pregnancy with a small discordant holoacardius acephalus. Am J Obstet Gynecol 177:466–468, 1997.
94. Crandall BF, Chua C. Risks for fetal abnormalities after very and moderately elevated AF-AFPs. Prenat Diagn 17:837–841, 1997.
95. Lei U, Wohlfahrt J, Christens P, Westergaard T, Lambe M, Norgaard-Pedersen B, Melbye M. Reproductive factors and extreme levels of maternal serum alpha-fetoprotein: a population-based study. Acta Obstet Gynecol Scand 83:1147–1151, 2004.
96. Shenhav S, Gemer O, Volodarsky M, Zohav E, Segal S. Midtrimester triple test levels in women with severe preeclampsia and HELLP syndrome. Acta Obstet Gynecol Scand 82:912–915, 2003.
97. Killam WP, Miller RC, Seeds JW. Extremely high maternal serum alpha-fetoprotein levels at second-trimester screening. Obstet Gynecol 78:257–261, 1991.
98. Nadel AS, Norton ME, Wilkins-Haug L. Cost-effectiveness of strategies used in the evaluation of pregnancies complicated by elevated maternal serum alpha-fetoprotein levels. Obstet Gynecol 89: 660–665, 1997.
99. Waller DK, Lustig LS, Cunningham GC, Feuchtbaum LB, Hook EB. The association between maternal serum alpha-fetoprotein and preterm birth, small for gestational age infants, preeclampsia, and placental complications. Obstet Gynecol 88:816–822, 1996.
100. Chen CK, Chang YL, Jou ST, Tseng YT, Lee YC. Treatment of mediastinal immature teratoma in a child with precocious puberty and Klinefelter's syndrome. Ann Thorac Surg 82:1906–1908, 2006.
101. Spencer K. Second-trimester prenatal screening for Down syndrome and the relationship of maternal serum biochemical markers to pregnancy complications with adverse outcome. Prenat Diagn 20: 652–656, 2000.
102. Sari N, Yalcin B, Akyuz C, Haliloglu M, Buyukpamukcu M. Infantile hepatic hemangioendothelioma with elevated serum alpha-fetoprotein. Pediatr Hematol Oncol 23:639–647, 2006.
103. Kovacs T, Csecsei K, Szabo M, Veress L, Toth Z, Papp Z. Outcome of mid-trimester pregnancies complicated by oligohydramnios. Quantitative evaluation of the prognostic value of maternal serum alpha-fetoprotein level. J Perinat Med 19(Suppl 1):397–402, 1991.
104. Cuckle H. Biochemical screening for Down syndrome. Eur J Obstet Gynecol Reprod Biol 92:97–101, 2000.
105. Rausch DN, Lambert-Messerlian GM, Canick JA. Participation in maternal serum screening for Down syndrome, neural tube defects, and trisomy 18 following screen-positive results in a previous pregnancy. West J Med 173:180–183, 2000.
106. Kiran TS, Bethel J, Bhal PS. Correlation of abnormal second trimester maternal serum alpha-fetoprotein (MSAFP) levels and adverse pregnancy outcome. J Obstet Gynaecol 25:253–256, 2005.
107. Aagaard-Tillery KM, Buchbinder A, Boente MP, Ramin KD. Beckwith-Wiedemann syndrome presenting with an elevated triple screen in the second trimester of pregnancy. Fetal Diagn Ther 22:18–22, 2007.
108. Sritippayawan S, Vachirasrisoontra C. Adverse pregnancy outcomes after a false-positive second trimester serum screen for Down syndrome in Thai pregnant women. J Med Assoc Thai 88:449–454, 2005.
109. Muller F, Dreux S, Sault C, Galland A, Puissant H, Couplet G, Lemay C, Larcher ME, Renom G. Very low alpha-fetoprotein in Down syndrome maternal serum screening. Prenat Diagn 23:584–587, 2003.
110. Cho S, Durfee KK, Keel BA, Parks LH. Perinatal outcomes in a prospective matched pair study of pregnancy and unexplained elevated or low AFP screening. J Perinat Med 25:476–483, 1997.
111. Baschat AA, Harman CR, Farid G, Chodirker BN, Evans JA. Very low second-trimester maternal serum alpha-fetoprotein: association with high birth weight. Obstet Gynecol 99:531–536, 2002.
112. Smith GC, Wood AM, Pell JP, White IR, Crossley JA, Dobbie R. Second-trimester maternal serum levels of alpha-fetoprotein and the subsequent risk of sudden infant death syndrome. N Engl J Med 351: 978–986, 2004.
113. Hershkovitz R, de Swiet M, Kingdom J. Mid-trimester placentation assessment in high-risk pregnancies using maternal serum screening and uterine artery Doppler. Hypertens Pregnancy 24:273–280, 2005.
114. Ay E, Kavak ZN, Elter K, Gokaslan H, Pekin T. Screening for pre-eclampsia by using maternal serum inhibin A, activin A, human chorionic gonadotropin, unconjugated estriol, and alpha-fetoprotein levels and uterine artery Doppler in the second trimester of pregnancy. Aust N Z J Obstet Gynaecol 45:283–288, 2005.
115. Bartha JL, Illanes S, Abdel-Fattah SA, Harrison G, Soothill PW. Maternal serum alpha-fetoprotein, fetal middle cerebral artery blood flow velocity and fetal haemoglobin in pregnancies at risk of fetal anaemia. Prenat Diagn 26:101–104, 2006.
116. Cowans NJ, Spencer K. First and second-trimester biochemical markers of chromosomal anomalies and their relationship to maternal haemoglobin levels. Prenat Diagn 25:693–699, 2005.
117. Yigiter AB, Kavak ZN, Bakirci N, Gokaslan H. Effect of smoking on pregnancy-associated plasma protein A, free beta-human chorionic gonadotropin, and nuchal translucency in the first trimester of pregnancy. Adv Ther 23:131–138, 2006.
118. Barkehall-Thomas A, Wilson C, Baker L, ni Bhuinneain M, Wallace EM. Uterine artery Doppler velocimetry for the detection of adverse obstetric outcomes in patients with elevated mid-trimester beta-human chorionic gonadotrophin. Acta Obstet Gynecol Scand 84:743–747, 2005.
119. Pasupathy D, Smith GC. The analysis of factors predicting antepartum stillbirth. Minerva Ginecol 57:397–410, 2005.
120. Chandra S, Scott H, Dodds L, Watts C, Blight C, Van Den Hof M. Unexplained elevated maternal serum alpha-fetoprotein and/or human

chorionic gonadotropin and the risk of adverse outcomes. Am J Obstet Gynecol 189:775–781, 2003.

121. Alkazaleh F, Chaddha V, Viero S, Malik A, Anastasiades C, Sroka H, Chitayat D, Toi A, Windrim RC, Kingdom JC. Second-trimester prediction of severe placental complications in women with combined elevations in alpha-fetoprotein and human chorionic gonadotrophin. Am J Obstet Gynecol 194:821–827, 2006.
122. Roig MD, Sabria J, Valls C, Borras M, Miro E, Ponce J, Vicens JM. The use of biochemical markers in prenatal diagnosis of intrauterine growth retardation: insulin-like growth factor I, Leptin, and alpha-fetoprotein. Eur J Obstet Gynecol Reprod Biol 120:27–32, 2005.
123. Dugoff L, Hobbins JC, Malone FD, Vidaver J, Sullivan L, Canick JA, Lambert-Messerlian GM, Porter TF, Luthy DA, Comstock CH, Saade G, Eddleman K, Merkatz IR, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, D'Alton ME. Quad screen as a predictor of adverse pregnancy outcome. Obstet Gynecol 106:260–267, 2005.
124. De Jenlis Sicot B, Deruelle P, Kacet N, Vaillant C, Subtil D. Prenatal findings in epidermolysis bullosa with pyloric atresia in a family not known to be at risk. Ultrasound Obstet Gynecol 25:607–609, 2005.
125. Leschot NJ, Treffers PE, Becker-Bloemkolk MJ, van Zanten S, de Groot WP, Verjaal M. Severe congenital skin defects in a newborn. Case report and relevance of several obstetrical parameters. Eur J Obstet Gynecol Reprod Biol 10:381–388, 1980.
126. Norman M, Eriksson CG, Eneroth P. A comparison between the composition of maternal peripheral plasma and plasma collected from the retroplacental compartment at caesarean section. A study on protein and steroid hormones and binding proteins. Arch Gynecol Obstet 244:215–226, 1989.
127. Sarandakou A, Kassanos D, Phocas I, Kontoravdis A, Chryssicopoulos A, Zourlas PA. Amniotic fluid hormone profiles during normal and abnormal pregnancy. Clin Exp Obstet Gynecol 19:180–188, 1992.
128. Gaucherand P, Guibaud S, Rudigoz RC, Wong A. Diagnosis of premature rupture of the membranes by the identification of alpha-feto-protein in vaginal secretions. Acta Obstet Gynecol Scand 73:456–459, 1994.
129. Phocas I, Sarandakou A, Kontoravdis A, Chryssicopoulos A, Zourlas PA. Vaginal fluid prolactin: a reliable marker for the diagnosis of prematurely ruptured membranes. Comparison with vaginal fluid alpha-fetoprotein and placental lactogen. Eur J Obstet Gynecol Reprod Biol 31:133–141, 1989.
130. Harrison RF, Biswas S. Maternal plasma, human placental lactogen, alpha-fetoprotein, prolactin and growth hormone in early pregnancy. Int J Gynaecol Obstet 17:471–476, 1980.
131. Bremme K, Eneroth P, Nilsson B. On the use of alpha-fetoprotein and prolactin in prenatal diagnosis of fetal abnormalities in early pregnancies. Int J Gynaecol Obstet 20:293–300, 1982.
132. Rochelson BL. Are vaginal fluid concentrations of prolactin, alpha-fetoprotein and human placental lactogen useful for diagnosing ruptured membranes? Br J Obstet Gynaecol 92:427, 1985.