This is to certify that the

thesis entitled

ABNORMAL TRIPLE TEST RESULTS
AND THE RELATION TO
ADVERSE PREGNANCY OUTCOMES

presented by

Robert D. Thomas

has been accepted towards fulfillment
of the requirements for

M.S. degree in Epidemiology

Claudia Holzman
Major professor

Date April 15, 1996

ABNORMAL TRIPLE TEST RESULTS AND THE RELATION TO ADVERSE PREGNANCY OUTCOMES

By

Robert D. Thomas

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Epidemiology

1996

ABSTRACT

ABNORMAL TRIPLE TEST RESULTS AND THE
RELATION TO ADVERSE PREGNANCY OUTCOMES

By

Robert D. Thomas

The triple test is routinely used as a prenatal screening procedure to detect an increased risk for neural tube defects or Down syndrome. We hypothesized that women who had high test results for both the AFP and hCG components of the triple test would be most at risk for adverse pregnancy outcomes when compared to women with normal test results. We retrospectively selected women based on their triple test results from the screening databases of the Henry Ford Hospital (Detroit) and Michigan State University prenatal screening programs. Four groups were assembled: those with elevated alpha-fetoprotein (AFP) (≥2.0 MoM), those with elevated human chorionic gonadatropin (hCG) (≥3.0 MoM), those with both elevated AFP and hCG, and a normal comparison group.

Our results indicated that women with elevated hCG levels were at increased risk for fetal defects. Women with elevated AFP levels were at significantly increased risk for stillbirth or spontaneous abortion (SAB), preterm delivery, and all outcomes examined combined. Women with elevated levels of both AFP and hCG were at an increased risk for stillbirth or SAB, fetal defects, preterm delivery, low birthweight, small for gestational age, and all adverse outcomes combined.

To my family and Joy

ACKNOWLEDGEMENTS

I'd like to acknowledge the work of the two screening centers at Henry Ford Hospital and Michigan State University for providing the raw data used in this study. I'd also like to thank the members of the research committee: Rachel Fisher, Jackie Roberson, Suzanne Diment, and Claudia Holzman for their input on the interpretation and cleaning of the raw data.

The final draft of this thesis would not have been possible without the feedback and constructive comments from my thesis advisor, Claudia Holzman, and the other members of my thesis committee: Rachel Fisher, Jack Lorenz, and Dorothy Pathak. Thank you for focusing me on areas in need of elaboration and clarification.

Finally, I'd like to acknowledge the faculty and staff of the Program in Epidemiology. I have thoroughly enjoyed my graduate education in this superb program. Your dedication to educating students and supporting their needs is commendable. As the first graduate of the program, I'd like to say, "We're on our way!".

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

BACKGROUND INFORMATION

Determining a method for detecting women with pregnancies at risk for chromosomal and structural abnormalities as well as other adverse outcomes has always been of primary importance to researchers in fetal development. By identifying women particularly at risk for certain abnormalities, medical care and interventions can be concentrated on those who need them. Today one of the most important of these screening procedures is the triple test which is used to screen for women with pregnancies at increased risk for trisomies 21 (Down syndrome) and 18 and neural tube defects (NTD's) such as anencephaly, encephalocele, and open spina bifida. The triple test measures three separate analytes found in maternal serum: alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), and unconjugated estriol (uE3).

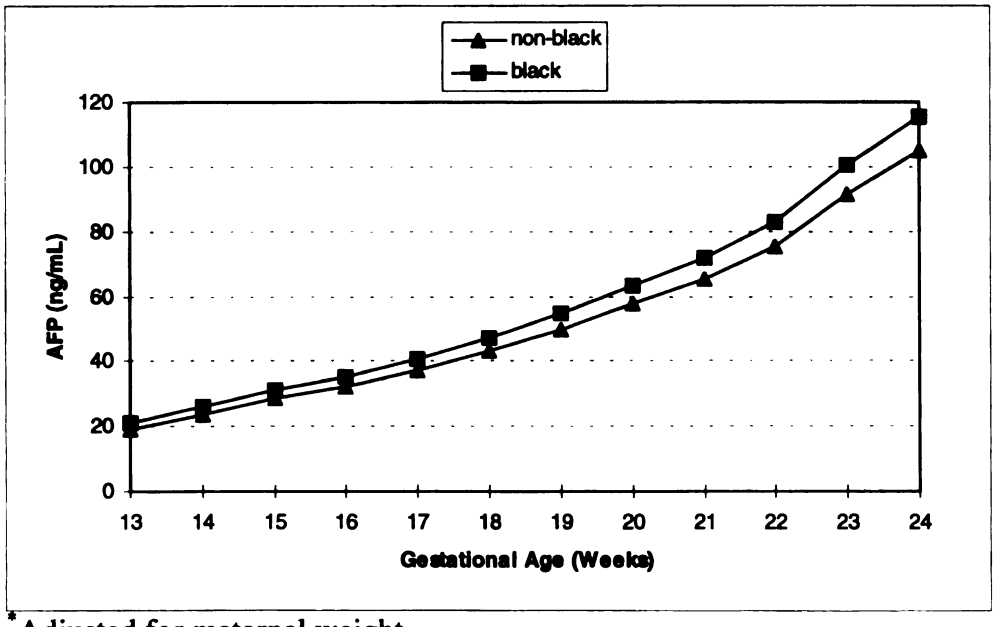
Alpha-fetoprotein

AFP was first identified independently by Bergstrand (2) and Gitlin (35) in 1956. This glycoprotein is primarily synthesized by the fetal liver and to a lesser extent the yolk sac and fetal gastrointestinal tract. AFP is a single polypeptide chain with several isoforms. AFP is analogous to albumin in the adult, although its function is not fully understood. Early in fetal life, AFP is the predominant circulating fetal protein (2). It reaches the maternal circulation via the placenta or diffusion across the fetal membranes.

AFP is measured by commercially prepared radioimmunoassay (RIA) or enzymeimmunoassay (EIA) kits using monoclonal or polyclonal antibodies to AFP. The typical non-pregnant woman might have a serum AFP concentration of 1 μg/L, with an

upper limit of 5 μg/L (48). However, during pregnancy AFP produced by the fetus passes into maternal circulation. The concentration of AFP in maternal serum is approximately 1/1,000 (48) of that found in the amniotic fluid in midtrimester. In normal pregnancies, about 2/3rds of the AFP in maternal circulation crosses transplacentally and the rest crosses transamnionally (44, 62). Measurable concentrations of AFP can be detected in maternal serum beginning at the end of the first trimester. Maternal serum levels rise steadily during pregnancy to a peak of approximately 500 μg/L (in normal pregnancies) at around 32 weeks of gestation (48). Thereafter levels decline steadily until term. During the typical screening period (15 to 20 weeks gestational age), normal median maternal AFP levels range from around 20 μg/L at 15 weeks to 45 μg/L at 20 weeks (106). The increase is about 15 percent per gestational week (48). Figure 1 presents cross-sectional

Figure 1. Median AFP values for the Michigan State University Prenatal Screening Program*.



*Adjusted for maternal weight.

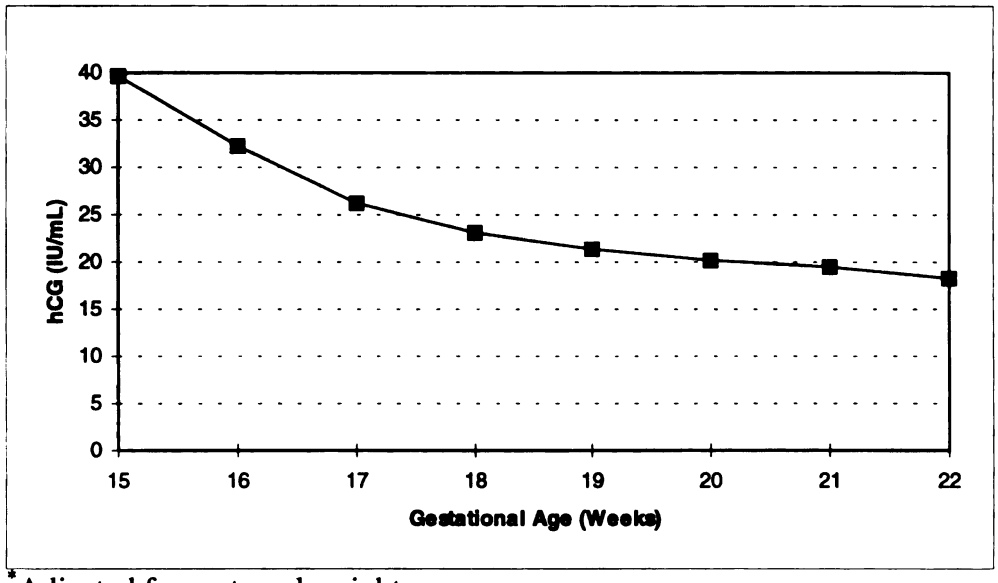
data from the Michigan State University Prenatal Screening Program showing weight-adjusted median maternal concentrations of AFP at each gestational week of testing.

Human chorionic gonadatropin

hCG is a sialoglycoprotein that is composed of two subunits of unequal size, α and β. The α-subunit is nearly identical to that of the pituitary hormones hFSH and hTSH, and the β-subunit is similar to that of the pituitary hormone hLH (98). These subunits exist either in free form or are noncovalently bonded to form intact hCG. This placental steroid is produced specifically by placental syncytiotrophoblasts which are in direct contact with maternal blood. The physiological action of hCG in early pregnancy is to sustain the corpus luteum beyond its normal lifetime. In this way, production of estrogen and progesterone, which are needed for the maintenance of the endometrium, continues, thus allowing the pregnancy to proceed.

A common method of measuring maternal serum hCG is to use an immunoradiometric kit which detects free-β as well as intact hCG (sometimes called total hCG). Detectable amounts of hCG may be found in maternal serum around the time of implantation. Levels in maternal serum rise rapidly to a peak during the third month of gestation and then decrease substantially until about 20 weeks of gestation, after which hCG levels remain relatively consistent until delivery (98). Levels in the second trimester range from approximately 5,000 to 50,000 mIU/mL (106). Figure 2 shows cross-sectional maternal weight-adjusted median serum hCG concentrations from the Michigan State University Prenatal Screening Program during the testing period.

Figure 2. Median hCG values for the Michigan State University Prenatal Screening Program*.



*Adjusted for maternal weight.

Unconjugated estriol

Estriol is one of the three major naturally occurring estrogens. It is produced almost exclusively during pregnancy, and it is the major estrogen produced in the human fetus (26). Unconjugated estriol is a steroid product of the fetoplacental unit and is synthesized in a series of steps beginning with the synthesis of dehydroepiandrosterone sulfate (DHEAS) from cholesterol in the fetal adrenal. Then, in the fetal liver, DHEAS is transformed into 16α-OH-DHEA and finally metabolized into uE3 in the placenta. During pregnancy, the production of uE3 depends on an intact maternal-placental-fetal unit, and therefore uE3 is a marker closely correlated with fetal and placental growth.

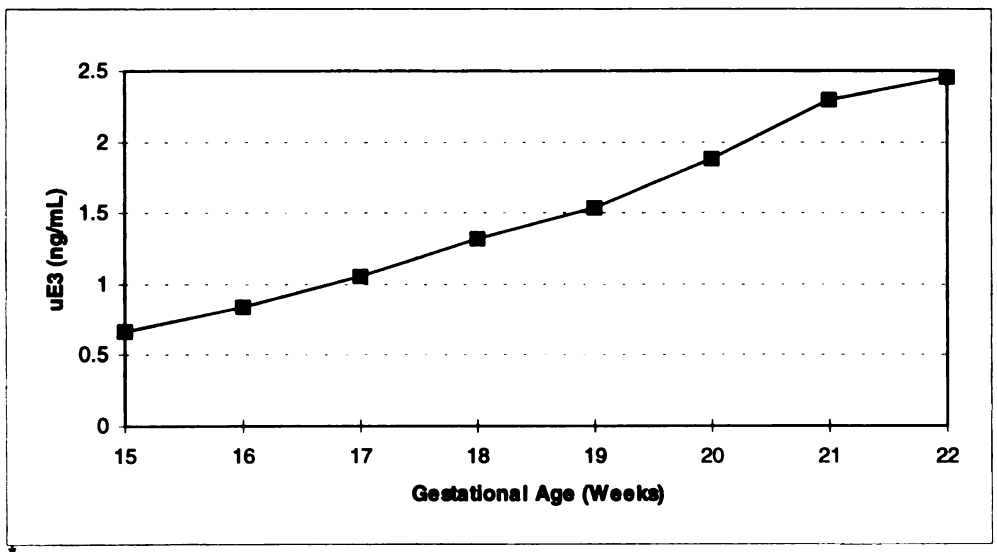
uE3 can be measured using radioimmunoassay (RIA) testing kits, and detectable amounts are found in maternal serum starting in the first trimester. Fetal-placental

production of uE3 leads to a progressive rise in maternal circulating uE3 levels during pregnancy. uE3 levels reach a peak which is about two to three orders of magnitude greater than non pregnant levels in late gestation (15, 16). During the second trimester, concentrations range from around 0.2 to 0.5 μg/L and levels increase approximately twenty percent per gestational week (106). Cross-sectional maternal weight-adjusted median uE3 values from the Michigan State University Prenatal Screening Program for each gestational week of screening are presented in Figure 3.

Evolution of the Triple Test

AFP screening was the first element of the triple test to come into place. It was brought about by the recognition in the early 1970's that significant AFP elevations in some women were associated with abnormal pregnancy outcomes such as fetal distress, fetal demise, anencephaly, and open spina bifida (10, 11, 96, 97, 109). A study by Brock

Figure 3. Median uE3 values for the Michigan State University Prenatal Screening Program*.



*Adjusted for maternal weight.

et al. (11) particularly ushered in the present era of prenatal screening with the discovery that anencephaly and open spina bifida were associated with increased levels of maternal AFP. The association between elevated maternal serum AFP and NTD's was particularly important because NTD's are relatively common and serious malformations. NTD's result from a failure in the closure of the developing neural tube during the first month of pregnancy. Depending on the cut-off used, 90 percent of anencephaly and 75-85 percent of spina bifida can be detected by elevated maternal serum AFP levels (48). NTD incidence varies by geographic location. The incidence in England is 2.95 per 1,000 (19), while in the United States the incidence is approximately 1 or 2 per 1,000 (92, 31, 78). The discovery of this association lead to a large collaborative study in the United Kingdom (116, 108) and a concurrent study by Macri et al. (69) in the United States. These studies unequivocally demonstrated the value of maternal serum AFP as a screening test for NTD's. Since there is overlap in AFP levels in normal and NTD pregnancies, however, AFP cannot be used alone as a diagnostic test. By the mid 1970's to early 80's, large screening programs were in place for the detection of NTD's, and the American College of Obstetrics and Gynecologists recommended in 1986 that the assessment of AFP levels be offered to all pregnant women (85).

The quest for a method of detecting women at increased risk for Down syndrome led to the remaining elements of the present triple test. The association between maternal age and the risk for Down syndrome formed the basis of the first diagnostic testing for Down syndrome. Women thirty-five or older are at an increased risk of having a Down syndrome fetus. At thirty-five years the Down's risk during midtrimester is 1 in 270, and

by 45 years the risk increases to 1 in 20 (48). From the late 1960's, when techniques for culturing cells became available, to the early 1980's, amniocentesis was routinely offered to women who would be thirty-five or older at delivery. This was an inefficient screening strategy, however, because women thirty-five or older only comprise approximately five percent of all pregnancies (80) and up to eighty percent of Down syndrome infants are born to women under thirty-five years of age (126). In addition, amniocentesis is an invasive procedure and in a small percentage can produce fetal loss. Using maternal age as a screening procedure for Down syndrome only allowed a detection rate of 20 to 45 percent (60, 80).

In 1984 Merkatz et al. (73) discovered that low values of AFP, which was already being used to screen for NTD's, were associated with an increased risk of Down syndrome in the fetus. They observed that 25 percent of pregnancies with autosomal trisomies had maternal serum AFP values less than or equal to 0.4 multiples of the median (MoM), whereas only 11.2 percent of the normal pregnancies had values below this level. This opened the door for the first screening program for all women to detect Down syndrome. Subsequent tests on this screening procedure confirmed these results and found that use of AFP in combination with maternal age allowed the detection of 20 to 33 percent of Down Syndrome cases in women less than 35 years at delivery (82, 25, 27). Since AFP is not related to maternal age (113), elevated levels would also detect 20-30 percent in all women regardless of age.

The recognition that maternal serum AFP levels were altered in the presence of a fetus with Down syndrome led to the investigation of a number of other potential serum

markers including hCG and uE3. A test even more effective than AFP for Down syndrome screening was discovered by Bogart et al. (4). In their study 11 out of 17 women (65 percent) with Down syndrome fetuses had elevated levels of intact hCG while only 1 among 74 unaffected pregnancies were elevated. They found that hCG levels were 2 to 2.5 times higher in women with a Down syndrome fetus at 18 to 22 weeks of gestation. Further studies followed confirming these results, and of 18 studies published between 1989 and 1991, the geometric mean hCG level was 2.05 times higher in Down pregnancies than that in unaffected pregnancies of the same gestational age (115).

Then, in 1988 the final element of the triple test came into use when Canick, et al. (17) noted that lower than average uE3 values in the second trimester were associated with Down syndrome. They found that of the 22 affected pregnancies, 16 (73 percent) had uE3 MoM values less than normal (1.0) with the median MoM being 0.79. Additionally, Haddow et al. (46) reported a 6 percent reduction in Down syndrome detection rates when uE3 was eliminated from the screening procedure.

Of the three biochemical markers, it is widely accepted that the best predictor of Down syndrome pregnancies is hCG, followed by uE3 and then AFP (113, 103). Though the three markers had each been shown to be associated with Down syndrome in the fetus, there was no evidence that combining them into one overall 'risk score' would be beneficial. In 1988, however, Wald et al. (113) proposed a combined risk assessment using AFP, hCG, uE3, and maternal age. They published a multivariate statistical model for evaluating levels of all three analytes to calculate a single patient-specific risk. The results of the three tests were combined into one overall 'risk score' which assessed the

chance of that woman delivering a Down syndrome infant. This was not a diagnostic test for Down syndrome, but rather the results indicated the level of risk for a particular pregnancy. Using the model they developed and a risk cut-off for Down syndrome of 1 in 250, Wald et al. retrospectively tested maternal serum samples from 77 affected pregnancies and 385 unaffected pregnancies. They were able to detect 67 percent of Down syndrome fetuses with a false positive rate of 5 percent. This was a significant improvement from the detection rates possible with only maternal age. Furthermore, they found that the levels of uE3, hCG, and AFP were independent of maternal age and only weakly correlated with each other. Using this information they were able to determine that the three measurements provided largely independent information, meaning that the three analytes used together in combination would be a more sensitive Down syndrome screening test than any of the individual analytes. Additionally, they found that with the detection rate remaining constant, the false-positive rate decreased with the addition of each marker, hCG causing the most dramatic reduction. Therefore the combined triple test had higher specificity as well as sensitivity.

The triple test methods developed by Wald et al. (113) have since been subjected to prospective trials. Wald et al. (119) performed their own prospective trial and found a detection rate of 48 percent with a false positive rate of 4.1 percent. The first trial in the United States by Haddow et al. (46) used a risk cut-off of 1 in 190 and detected 66 percent of Down syndrome births. When they considered the reported incidence of spontaneous abortion of Down syndrome infants between mid-trimester and term, the true detection rate proved to be 58 percent, with 6.6 percent of all women testing positive.

The second trial in the United States by Phillips et al. (84) restricted the study population to those women under 35 years and used a risk cut-off of 1 in 274. They had a screen positive rate of 7.2 percent and were able to identify 57 percent of Down syndrome cases. Additionally, a study by Burton et al. (12) used a cut-off risk of 1 in 270 (equal to that of a woman 35 years or older). Their study found a screen positive rate of 10.4 percent, and they were able to detect 83 percent of Down syndrome births. When accounting for spontaneous abortions, the detection rate they reported was 67 percent.

These studies confirmed the benefit of the triple test in Down syndrome detection. The major advantage of the triple test is that women of all ages can be screened without invasive procedures, thus allowing for prenatal identification of a greater proportion of Down syndrome cases. Chitty et al. (20), in their review of current techniques in screening, concluded that age alone can detect 30 percent of Down syndrome cases, age and hCG, 49 percent; age, hCG, and AFP, 56 percent; age, hCG, and estriol, 57 percent; and age, hCG, estriol, and AFP can detect 61 percent of Down syndrome cases. It is important to realize that even with a 61 percent detection rate, the triple test is not a diagnostic test for Down syndrome, but rather it is a screening test that defines a population of women who may require additional testing.

Current Debate in Triple Test Screening

Since the publication of Wald et al. (113), a number of studies have attempted to evaluate the extent to which the addition of hCG and uE3 measurements improve screening sensitivity. These works have lead to two controversies: first whether or not

the addition of uE3 adds to the sensitivity, and second whether free-β hCG is a superior marker compared to total hCG.

Since Canick et al. (17) and Wald et al. (113) reported that uE3 was useful in screening for Down syndrome, several studies have questioned those findings (66, 67, 64, 102, 24, 60). Some studies found no improvement in detection rates for Down syndrome with the addition of uE3 (66, 67), and one study (24) found a decrease in detection rate with the addition of uE3 to the screening procedure.

These studies are in the minority, however, and the findings could be due to a study population that is not representative of the screening population as a whole. Most research has shown that uE3 does improve detection rates. The debate lies in whether this small improvement in detection rates is cost effective given the correlation of uE3 with AFP levels (60). However, since uE3 variation within gestational week is smaller than that of the other markers, including it in the triple test can provide an additional check on gestational age accuracy. Therefore uE3 can be a useful measure regardless of detection rates.

The second triple test debate that has arisen is whether free-β hCG is a better predictor of risk than total hCG. Macri et al. (65) measured the free-β protein in maternal sera from 29 cases of Down syndrome and 450 unaffected pregnancies. They found a significant increase in the level of free-β hCG in Down syndrome pregnancies. 83 percent of the Down pregnancies exceeded the median of the controls, and 52 percent exceeded twice the median. When the free-β levels were combined with AFP levels and

maternal age in pregnancies under 17 weeks of gestation, a detection rate of 80 percent with a false positive rate of 5 percent was reported.

Spencer et al. (103) directly compared free-β hCG with total hCG univariately and in a multivariate risk analysis with AFP, uE3, and maternal age in 29 Down pregnancies and 145 controls. They found the median free-β subunit from affected pregnancies to be 2.06 times that of unaffected pregnancies, and total hCG to be 1.88 times higher. The univariate analysis found a 34 percent detection rate with the free-β subunit and 29 percent with total hCG. In the multivariate analysis, at a false positive rate of 5.9 percent, they found a detection rate of 66 percent for the free-β subunit compared with only 52 percent for total hCG measurement. The differences, however, were not statistically significant.

This led Spencer et al. to conduct the large multicenter study mentioned earlier (102). In addition to their findings that uE3 was not useful, this study confirmed their earlier findings regarding free-β hCG. Comparing the markers against one another for effectiveness, they found that at a false positive rate of 5 percent the relative detection rates were AFP, 7 percent; uE3, 18 percent; maternal age, 28 percent; total hCG, 38 percent; and free-β hCG, 45 percent. They determined that the best combination of the various markers was free-β hCG, AFP, and maternal age, with a 66 percent detection rate at a false positive rate of 6 percent. This finding was significant, and the authors concluded they had clear evidence to justify the claim that free-β hCG was a superior marker to total hCG.

Wald et al. (118) went even further by testing separately for both free-β hCG and free-α hCG. In their study of 75 singleton Down pregnancies and 367 unaffected singleton pregnancies using a four-marker serum screening test (AFP, uE3, free-β hCG, free-α hCG, and maternal age), they were able to detect 65 percent of Down syndrome pregnancies with a 5 percent false-positive rate. This was in comparison to a detection rate of 59 percent with the conventional triple test. The authors concluded that this four-marker test is the most effective method of prenatal screening for Down syndrome suitable for routine use. More research will be needed examining the four marker test before any conclusions about its effectiveness can be drawn, however.

Current techniques in triple test screening

Triple test screening is usually performed between 15 and 22 weeks gestation, with the optimal time being from 16 to 18 weeks gestation. Results of the test are commonly expressed as multiple of the median (MoM). This allows for comparison across different testing sites even though they may use different testing kits with varying levels of detection. It also allows for comparison of women tested at different gestational ages given that the normal values of the analytes change during pregnancy. The distribution of AFP, hCG, and uE3 values for women with uncomplicated pregnancies are analyzed at each testing center for each gestational week (or day) during the normal testing period. The median value from these distributions is identified, and it is assigned a value of 1.0. Every other value for that gestational week (or day) from that center is then expressed as a multiple of its median.

As previously mentioned, maternal age and accurate gestational age estimates are important covariates in determining the risk for Down syndrome and NTD's in a fetus, and they are therefore taken into account when performing the triple test. Other variables can also affect the triple test for singleton pregnancies and need to be taken into account when interpreting the results. The most important of these variables are maternal weight, maternal race, and maternal insulin-dependent diabetes.

All three markers are inversely associated with maternal weight (106). Heavier women have, on average, lower concentrations of AFP, hCG, and uE3 than lighter women. In other words, if no adjustment is made, too many lighter women and too few heavier women will have values above any given MoM risk cut-off value. This can be explained by the larger blood volume in heavier women (42, 110). It has been determined that this adjustment will reduce the false-positive rate by about 15 percent while not adversely affecting the detection rate (53).

Maternal race also affects the levels of the three analytes in maternal serum. Serum samples from black women contain AFP concentrations that are approximately 10 to 15 percent higher than measurements from other racial groups (52). In other words, when screening for NTD's and Down syndrome, too many blacks and too few whites would have results above the median if a single MoM scale was used. HCG levels appear to be higher in blacks, but this finding is not consistent among researchers (106). A study by Bogart et al. (3) found no significant differences in hCG levels between blacks, whites, and Hispanics; however the weight adjusted MoM's of blacks were 9.8 percent higher and Orientals 16 percent higher than those for whites. uE3, on the other hand, may be

reduced by 5 percent in blacks (106). Because of this, most testing centers calculate MoM's based on separate medians for blacks and non-blacks for all three analytes.

Insulin-dependent diabetes has also recently been found to affect test results, although gestational diabetes does not appear to be associated with variations in concentrations (48). Women with insulin-dependent diabetes have been found to have AFP values that average 20 percent lower at any given time in the second trimester than women who do not have diabetes (111, 39). uE3 and hCG values are also lower, by about 10 percent, in women with insulin-dependent diabetes (106). This means that when assessing results using MoM's, too few women with insulin-dependent diabetes will have values considered to be elevated if no adjustment is made. Because of this, it has now been recommended that corrections for insulin-dependent diabetes be instituted (106, 59).

Other factors have also been found to affect analyte levels. AFP levels in multiple pregnancies are higher than singleton pregnancies by approximately the number of fetuses (117). Additionally, some drugs can affect AFP levels, so it is advisable to have women stop taking medications at least 12 hours in advance of having their blood drawn, if possible (106).

Chapter 2

PREVIOUS LITERATURE

Since the establishment of the triple test as a screening procedure for NTD's and Down syndrome, many researchers have investigated the relationship between these analyte levels and other adverse pregnancy outcomes. To date, most research has focused on elevated AFP levels, although some research has been done on elevated hCG levels and to a lesser extent on pregnancies with both AFP and hCG elevated.

Elevated AFP and adverse pregnancy outcomes

The first studies associating unexplained AFP elevations with adverse pregnancy outcomes focused on low birthweight (LBW), which is a composite of both preterm delivery and fetal growth. Brock et al. (7, 9) first reported that women with elevated AFP levels not explained by multiple gestations, open neural tube defects, or demise were at increased risk of delivering a low birthweight infant. He found a LBW rate of 10.7 percent among infants born to women with AFP greater than 2.3 MoM compared to a background rate of 4.2 percent. Another early study by Wald et al. (114) used AFP greater than 3 MoM as a definition of elevated, and they too found an association with LBW (relative risk (RR) 4.7). In addition, Wald et al. found an increased risk of preterm birth (RR 5.8) and perinatal death (RR 3.5) among infants of women with elevated AFP. These reports initiated further investigation on the association of LBW and other adverse pregnancy outcomes with elevated AFP levels. Studies from 1977 to 1991 which assessed these associations are summarized in table 1.

Table 1. Summary of articles reporting on elevated AFP levels and adverse pregnancy outcomes.

Study	Year	AFP Cut-off	LBW	IUFD	Peri D	PTD (LB)
Brock et al. (7)	1977	2.3	+	+	+	NR
Wald et al. (114)	1977	3.0	+	NR	+	+
Macri et al. (68)	1978	2.0	+	NR	NR	+
Gordon et al. (37)	1978	2.0	-	NR	+	+
Brock et al. (6)	1979	2.0	+	+	NR	NR
Brock et al. (8)	1980	2.0	+	NR	NR	+
Wald et al. (112)	1980	2.0	+	NR	NR	NR
Smith et al. (100)	1980	2.0	+	NR	NR	+
Read et al. (87)	1980	2.0	+	+	NR	+
Macri et al. (70)	1982	2.0	NR	+	+	NR
Persson et al. (83)	1983	2.3	+	NR	+	+
Haddow et al. (41)	1983	2.0	+	+	NR	NR
Purdie et al. (86)	1983	2.5	+	NR	NR	-
Crandall et al. (22)	1983	2.0	+	+	+	NR
Evans et al. (29)	1984	3.0	+	NR	+	NR
Schnittger et al. (94)	1984	2.0	+	NR	NR	NR
Milunsky et al. (74)	1984	2.0	-	+	NR	NR
Hamilton et al. (49)	1985	2.5	+	+	+	+
Furhman et al. (33)	1985	2.5	+	+	NR	NR
Secher et al. (95)	1985	2.0	-	NR	NR	NR
Ghosh et al. (34)	1986	2.0	+	NR	+	NR
Haddow et al. (47)	1987	2.0	+	NR	NR	NR
Doran et al. (28)	1987	2.5	+	+	+	NR
Nelson et al. (81)	1987	2.5	NR	+	NR	NR
Burton et al. (13)	1988	2.5	+	+	+	NR
Robinson et al. (89)	1989	2.5	+	+	NR	NR
Milunsky et al. (75)	1989	2.0	+	+	+	NR
Crandall et al. (23)	1991	2.5	+	+	NR	+
Waller et al. (120)	1991	2.0	NR	+	NR	NR

LB = live births only.
+ = risk elevated.
- = no elevation in risk.
NR = not reported.

Of the twenty-nine studies examined, twenty-three found that elevated AFP was associated with an increased risk of LBW, while only three studies failed to find an increased risk. Twenty-one studies reported relative risks or presented data from which relative risks could be calculated. The relative risks found in these studies ranged from 2 to 10. The nine studies using a cut-off of 2.0 MoM had relative risks ranging from 2 to 5. When a cut-off of 2.5 MoM was used, relative risks for eight studies ranged from 2 to 10. In the four studies which examined a cut-off of 3.0 MoM, the relative risks ranged from 3 to 5.2.

Sixteen studies reported an association between elevated AFP and intrauterine fetal death (IUFD). In the eleven studies where relative risks were reported or could be calculated, risks for IUFD ranged from 3.2 to 21.0. Among the four studies which examined a cut-off of 2.0 MoM the relative risks ranged from 2 to 8. When a cut-off of 2.5 MoM was used, relative risks found in five studies ranged from 4.4 to 21. The two studies which examined a cut-off of 3.0 MoM found relative risks of 8.5 and 10.4. One of the problems, however, with evaluating IUFD is the timing of the death in relation to the triple test measurement, since unrecognized fetal demise could be the cause of the elevated analyte levels. Therefore in order to determine the true association of elevated levels with IUFD, studies must take this into account.

There were also twelve studies which reported on the association between elevated AFP levels and neonatal or perinatal death. Nine of these studies reported relative risks or provided data from which they could be calculated. In these nine studies, relative risk estimates ranged from 3 to 10. When a cut-off of 2.0 MoM was used, the

relative risks from three studies ranged from 4.5 to 5.8. In the four studies that examined a cut-off of 2.5 MoM, relative risks ranged from 6 to 10. The two studies which examined a cut-off of 3.0 MoM found relative risks of 3.5 and 8.

Nine studies reported on an association between elevated AFP levels and preterm delivery (PTD), while only one study failed to find an association. Four studies provided data from which relative risks were calculated. Gordon et al. (37) used a cut-off for elevated AFP of 2.0 MoM, and they reported a relative risk of 3.5. Persson et al. (83), who used a cut-off of 2.3 MoM found a relative risk of 2, and Hamilton et al. (49) reported a relative risk greater than 10 when a cut-off of 2.5 was used. Wald et al. (114) examined preterm delivery using an AFP cut-off of 3.0 MoM, and they found a relative risk of 5.8. The main concern in studies reporting on preterm delivery is the accuracy of gestational age reporting. If gestational age estimates are inaccurate at the time of testing, they can affect both the analyte test results and the estimated gestational age at birth.

Katz et al. (54) reviewed eighteen studies that examined adverse pregnancy outcomes in women with elevated maternal serum AFP levels. These studies combined reported on over 225,000 women with AFP screening. They determined that the risk of low birthweight was increased 2 to 4 times in women with unexplained AFP levels greater than 2 MoM. Additionally, they reported that the risk of stillbirth was increased 2 to 8 times. Approaching the data from another perspective, Katz et al. determined that women with an unexplained AFP elevation have between a 10 and 33 percent chance of delivering an infant weighing less than 2,500 grams and a 20 to 38 percent chance of having an adverse pregnancy outcome (LBW, IUFD, IUGR, or preterm delivery).

Another review of the literature regarding elevated AFP and adverse pregnancy outcomes was done by Barbara Burton (14). She found that most studies examining low birthweight and high AFP report relative risks of around 2-5. She also found that approximately 15 percent of pregnancies that will ultimately deliver low birthweight infants will have elevated midtrimester AFP levels. However, if the pregnancy is complicated by IUGR, this detection rate could fall as low as 7 percent. Associations with fetal loss can often be complicated by unrecognized fetal demise at the time of testing, however, since this could be the reason for the elevated analyte levels. Burton concluded, however, that the evidence indicates that even with a viable fetus at the time of testing, there is an increased risk for later fetal loss or stillbirth when AFP levels are elevated. The relative risk ranged from 4.7-12.6. Burton also concludes that the positive predictive value for low birthweight appears to be relatively low (8-30% depending on the cut-off used).

Waller et al. (121) published another review of the studies examining elevated AFP and adverse pregnancy outcomes. They focused their review on the question of whether pregnant women with high levels of AFP who have singleton fetuses without birth defects have an increased risk of low birthweight and perinatal death. Twenty-three studies were reviewed, and eighteen which had comparable measurements were reported on. In order to make these studies comparable on a consistent basis, they recalculated risk estimates in some studies which had failed to exclude conditions which could have explained the elevation in AFP, such as relevant birth defects, multiple gestations, missed abortions, and cases of underestimated gestational age. Waller et al. found eleven studies

that concurred in reporting an increased risk of fetal death in women with high levels of AFP. If a cut-off of 2.0 MoM was used, estimates of risk ranged from 3.2 to 4.4. For cut-offs of 2.5 and 3.0, relative risks ranged from 4.4 to 21 and 8.5 to 10.4, respectively. Fourteen studies reported an increased risk of low birthweight (<2,500 grams) in women with elevated AFP. For the cut-offs of 2.0, 2.5, and 3.0 the range of relative risks found were 2.8-5.0, 1.9-6.4, and 4.1-5.2, respectively. Estimates of risk in seven studies reporting an association between elevated AFP and neonatal death were 4.9 and 5.8 for a 2.0 MoM cut-off and 4.0 and 6.0 for a 2.5 MoM cut-off. In addition, all studies that examined more than one level of high AFP found a dose-response relation between AFP level and LBW incidence. The authors concluded that elevated AFP is predictive of women at risk for adverse pregnancy outcomes.

Since these reviews several studies have been published examining the outcome of having a small for gestational age (SGA) infant, and its association with elevated AFP levels. In 1992, Capeless et al. (18) prospectively followed 381 screened women. They identified 23 (6 percent) women with AFP levels greater than 2.0 MoM. Fifteen percent of the elevated women delivered a SGA infant compared to only 6 percent of the comparison population. This translated into a relative risk for delivering a SGA infant of 2.54 in women with elevated AFP levels. In addition to these findings, Capeless et al. also reported an increased risk for preterm delivery (RR 6.35) and IUFD (RR 5.38) in women with AFP elevated above 2.0 MoM. Their study also highlighted the fact that AFP is not an effective screening test specifically for adverse pregnancy outcomes by pointing out that the positive predictive value was 49 percent and the sensitivity was 16

percent. In multivariate regression, elevated AFP remained significant meaning that it is an independent variable in adverse pregnancy risk assessment. The authors concluded, however, that it was unclear whether elevated AFP identified a new population at risk for these outcomes because of the high prevalence of historical risk factors such as previous SGA infant, PTD, IUFD, and pregnancy complications in their population.

Morssink et al. (77) published a study in 1995 that examined SGA infants and preterm delivery in women who had AFP levels ≥ 2.5 MoM. They examined adverse pregnancy outcomes which included extremely SGA infant (< 2.3 percentile), SGA infant ($<10^{th}$ percentile), preterm delivery (< 37 weeks), and either SGA infant or preterm delivery. They excluded pregnancies with unknown outcome, a congenital anomaly, delivery before 25 weeks of amenorrhea, or known insulin-dependent diabetes. Separate analysis was done in those who had elevated hCG levels as well as elevated AFP levels. They found that 9.4 percent of women with elevated AFP levels had extremely SGA infants compared to 2.1 percent of the comparison population. The relative risk was 4.5 ($p<.01$). For SGA infant, 27.1 percent of the elevated population and 9.9 percent of the comparison population met the criteria, for a relative risk of 2.7 ($p<.01$). Fourteen percent of the elevated population versus 5.9 percent of comparisons had preterm delivery. The relative risk was 2.4 ($p<.01$). For either SGA infant or preterm delivery, 37.5 percent of the elevated population and 15.2 percent of the comparison population fell into this category. The relative risk of either having an SGA infant or a preterm delivery for a woman with elevated AFP was 2.5 ($p<.01$).

Additionally, Silver et al. (99) in 1994 studied elevated AFP specifically in women with antiphospholipid antibodies. In their cohort of sixty pregnancies in women with median to high levels of IgG anticardiolipin antibodies, lupus anticoagulant, or both they found 13 (22 percent) had elevated AFP. None of the elevated AFP levels were explained by fetal anomalies, current fetal death, multiple gestation, incorrect dates, or vaginal bleeding. They found that pregnancies with elevated AFP values had a significantly higher incidence of fetal death (62 percent) than those without elevated AFP values (6 percent). Also, 77 percent of elevated AFP pregnancies versus 15 percent of non-elevated pregnancies resulted in perinatal loss. Both of these findings were statistically significant ($p<.001$).

In addition to the adverse pregnancy outcomes discussed above, countless studies have also found associations between various pregnancy complications and elevated AFP levels. The complications that have been found in at least one study to be associated with elevated AFP levels include: abruptio placenta (18, 86, 75, 49, 83), maternal infection (48, 75), ologiohydramnios (63, 32, 55, 105), preeclampsia (122, 76), and pregnancy-induced hypertension (76, 49).

The mechanism by which unexplained AFP levels are elevated in abnormal pregnancies has not been conclusively proven. Maternal levels of AFP may result from transport across either chorioamniotic membranes or the placenta. Since problems associated with elevated AFP levels can be related to uteroplacental disease, most investigators believe that pathology within the placenta leads to an increase in transfer of AFP across the maternal-fetal interface in women with unexplained AFP elevations.

There are several lines of support for the hypothesis that elevated AFP is a result of leakage through a defective feto-maternal placental barrier. First of all, most women with unexplained maternal serum elevations of AFP have normal amniotic fluid AFP levels (108). Secondly, a strong association between elevated AFP levels and fetal-maternal hemorrhage has been reported (50, 56, 61). Another line of support for this theory is that a strong relationship between vaginal bleeding and elevated AFP has been demonstrated (43, 83, 57). The fourth piece of supporting evidence is that several placental lesions (chorioangiomas, hematomas, hemangianomas) as well as chronic villitis have been associated with elevated maternal serum AFP levels (71, 91, 104). Finally, elevated levels of fetal red blood cells have been demonstrated in maternal serum when AFP levels are elevated (48). Therefore, because of this evidence, it appears that in most cases of unexplained AFP elevations the integrity of the uteroplacental interface has been breached allowing more fetal AFP to enter the maternal serum.

Elevated hCG and adverse pregnancy outcomes

Although the research regarding AFP and adverse pregnancy outcomes still far outweighs that for elevated hCG, recently several studies have reported on hCG levels and adverse pregnancy outcomes. These studies have become more focused and reliable as knowledge of hCG and testing strategies have improved. In all, eight articles have examined various adverse fetal outcomes. Table 2 summarizes the most common outcomes examined in these studies.

In one of the first prospective studies of hCG as a marker for detecting Down syndrome, Bogart et al. (3) found that hCG was also a marker for other adverse pregnancy

Table 2. Summary of articles reporting on elevated hCG and adverse pregnancy outcomes.

Study	# of Cases	Case Def.	IUFD	LBW (LB)	PTD (LB)	SGA (LB)
Bogart et al. 1991 (3)	255	>2.0 MoM	11.6%	28.8%	NR	NR
Gravett et al. 1992 (38)	7	>5.0 MoM	NR	33%	50%	NR
Gonen et al. 1992 (36)	284	>2.5 MoM	RR 4.1 (0.5-34.8)	NR	RR 1.1 (0.6-2.2)	NR
		>4.0 MoM	NR	NR	RR 3.3 (1.3-8.2)	NR
Muller et al. 1993 (79)	657	>95th percentile	RR 1.61 (0.6-4.4)	NR	NR	NR
Lieppman et al. 1993 (58)	225	≥2.0	NR	RR 4.7 (1.8-12.2)	RR 2.9 (1.4-6.1)	RR 1.8 (1.0-3.2)
Tanaka et al. 1993 (107)	42	>2.0	RR 28 (2.6-306)	NR	NR	RR 4.9 (2.4-10.4)
Wenstrom et al. 1994 (124)	22	≥2.0	13.6%	NR	18.2%	NR
Morissink et al. 1995 (77)	366	≥2.5	NR	NR	RR 1.4 (0.8-2.0)	RR 1.5 (1.2-2.0)

LB = live births only.
NR = not reported.
RR = relative risk.

outcomes. In their study, 6 of 11 pregnancies with fetal aneuploidy (54.5 percent) had abnormal hCG levels (>2.5 MoM). Additionally, they found an 11.6 percent incidence of fetal death and 28.8 percent incidence of low birthweight in women with elevated hCG levels. Inclusion of aneuploidy pregnancies could have affected these results. Also, corresponding AFP values for these women were not provided, and therefore some of these women could have had elevated AFP as well.

Gravett et al.(38) published an article in which they identified seven women with hCG levels elevated above 5 MoM (one also had elevated AFP equal to 2.87 MoM). Of the six women with elevated hCG without elevated AFP, 3 (50 percent) had preterm

deliveries and 2 (33 percent) had low birthweight infants. This was in contrast to a preterm birth rate of 16.4 percent in a high risk comparison population. The authors concluded that since AFP was not elevated in these cases, hCG could represent an independent risk factor for adverse pregnancy outcome. The small number of cases reported on, however, limits the conclusions that can be drawn from this study.

Gonen et al. (36) published an article in which they examined unexplained elevations of hCG greater than 2.5 MoM. They excluded pregnancies with fetal abnormalities, abnormal karyotypes, and a maternal serum AFP level greater than 2.5 MoM. 284 (4.7 percent) of the women screened had elevated hCG levels. They found that 10.3 percent of women with elevated hCG had pregnancies with fetal growth restriction compared to 5 percent of the normal hCG population (RR 2.8 95% CI 1.0-7.0). They found no difference, however, between fetal loss rates (1.8 percent verses 0.5 percent), preterm delivery (7.0 percent verses 6.3 percent), Apgar score < 7 at five minutes (0.7 percent verses 1.8 percent), or neonatal death (0.7 percent verses 0.0 percent). However when the hCG cut-off was raised to >4.0 MoM, preterm delivery became significant (RR 3.3 95% CI 1.3-8.2). The authors concluded that women with unexplained elevations of hCG are at an increased risk for adverse pregnancy complications and therefore may require careful obstetric management to optimize pregnancy outcome.

Muller et al. (79) published the results of a cohort study examining the association of elevated maternal serum hCG levels with fetal chromosome anomalies and subsequent fetal death. The study was limited to singleton pregnancies screened between 14 and 20

weeks of gestation. hCG was evaluated as percentiles based on maternal age and gestational week at the time of testing. They found that women who had hCG elevations $\geq 95^{th}$ percentile were at high risk of fetal and perinatal deaths. The relative risk for miscarriage was 3.0 and for in utero fetal death was 1.61. These results, however, could be partially explained by the inclusion of Down syndrome and other aneuploidy cases in their analysis. Also, the previously mentioned problem of whether the fetal death led to the elevated level of the analyte was not considered.

Lieppman et al. (58) conducted a cohort study of the association of hCG and adverse pregnancy outcomes. Study participants were screened between 15 and 18 weeks of gestation, and they excluded women who had pregnancies with multiple gestations, insulin-dependent maternal diabetes, known fetal chromosomal abnormalities, and fetal anomalies detected by ultrasonography before screening. They examined LBW (<2500 grams), preterm delivery (<37 weeks), and SGA infant ($<10^{th}$ percentile) in 225 pregnancies with hCG $\geq$ 2.0 MoM and 235 pregnancies that screened positive for Down syndrome but had hCG < 2.0, AFP < 2.0, and uE3 > 0.5 MoM. Thirteen women with elevated hCG also had AFP $\geq$ 2.0 MoM, however excluding these women did not alter any of the analysis results, nor did differences exist when analysis was done separately for those with uE3 $\leq$ 0.5 MoM and with uE3 > 0.5 MoM. Approximately 10 percent of women with elevated hCG and 2.1 percent of those in the comparison group had a LBW outcome. The relative risk was 4.7 (95% CI 1.8-12.2). After adjusting for maternal age, race, payment status, and gravidity, the relative risk was 4.0 (95% CI 1.6-9.9). Eleven percent of the elevated population and 3.8 percent of the comparison population delivered

prematurely for a relative risk of 2.9 (95% CI 1.4-6.1). After adjustment, the relative risk was 2.8 (95% CI 1.4-5.8). SGA infant occurred in 13.3 percent of the elevated population and 7.2 percent of the comparison population. The relative risk was 1.8 (95% CI 1.0-3.2), and the adjusted relative risk was also 1.8 (95% CI 1.0-3.2). Trend analysis was significant for all three adverse outcomes, indicating that risk increased with increasing levels of hCG. If hCG levels were 2.0 to 3.9 MoM, 4.0 to 5.9 MoM, or ≥6.0 MoM the corresponding LBW risks were 2.9 (95% CI 1.0-8.4), 8.7 (95% CI 3.2-25.1), and 20.3 (95% CI 4.0-104.0). For preterm delivery in the same categories the risks were 2.1 (95% CI 0.9-4.8), 4.6 (95% CI 2.0-10.6), and 11.8 (95% CI 2.6-54.5) respectively. For SGA births the risks corresponding to the categories were 1.3 (95% CI 0.7-2.5), 3.2 (95% CI 1.8-6.4), and 4.6 (95% CI 0.9-24.3). The authors conclude that elevated hCG in the midtrimester appear to be associated with adverse pregnancy outcome, that the magnitude of the risk correlates with hCG level, and that this risk is independent of any risks that might be associated with unexplained elevations of AFP.

Tanaka et al. (107) published a study in which 638 consecutive pregnant women were screened. Forty-two women (6.6 percent) had hCG elevations ≥ 2.0 MoM. SGA infant was determined using fetal growth curves constructed from Japanese data, with SGA infants defined as those below the mean minus 1.5 standard deviations. This can approximately be considered equal to the 7^{th} percentile. They found that 19 percent of the elevated population delivered SGA infants compared to 3.9 percent of the population with hCG < 2.0 MoM. The relative risk was 4.9 ($p<.001$). Fetal death was also significantly associated with hCG ≥ 2.0 MoM. 4.8 percent of the elevated population and

0.34 percent of the comparison population experienced a fetal death for a relative risk of 28 ($p<.05$). Although this study failed to take into account AFP levels of the elevated hCG women, the authors concluded that an elevated hCG level may be used for selecting women at high risk for pregnancy complications.

A study by Wenstrom et al. (124) examined 126 women with poor pregnancy outcomes compared to 126 women with normal outcomes. All women in their study underwent amniocentesis because of elevated analyte levels, historical risk factors, or advanced maternal age. They found that 14 percent of complicated pregnancies versus 3 percent of normal pregnancies had elevated hCG levels ($p=.01$). The odds ratio associated with a poor obstetric outcome if hCG was elevated was 3.9 (95% CI 1.2-17.8). Of the 22 women with elevated hCG levels, 13.6 percent had a fetal death, and 18.2 percent delivered prematurely.

Morrsink et al. (77) published a cross-sectional study examining elevated hCG and its association with extreme SGA infant (<2.3rd percentile), SGA infant (< 10th percentile), preterm delivery (<37 weeks, with the exclusion of infants with a birth weight below the 10th percentile), and either SGA infant or preterm delivery. Elevated hCG was defined as ≥ 2.5 MoM, and women with unknown pregnancy outcome, a congenital anomaly, delivery before 25 weeks of amenorrhoea, or known insulin-dependent diabetes were excluded from analysis. Women with both hCG and AFP elevated were analyzed separately. They found that 4.4 percent of those with elevated hCG versus 2.1 percent of those < 2.5 MoM delivered extremely SGA infants. The relative risk was 2.1 ($p<.01$), and the detection rate was 2.7 percent. For SGA infant, they reported that 15.5 percent of

the elevated population and 10.1 percent of the comparison population had the outcome, yielding a relative risk of 1.5 ($p<.01$) and a detection rate of 7.1 percent. Preterm delivery was not significantly associated with elevated hCG, with 8.6 percent of the elevated population and 5.9 percent of the comparison population reported as delivering prematurely. They also reported that 22.7 percent of the elevated population and 15.4 percent of the comparison population had either SGA infants or preterm delivery. The relative risk was 1.5 ($p<.01$), and the detection rate was 6.8 percent. The authors concluded that women with elevated hCG are at increased risk for an adverse pregnancy outcome and should have some kind of increased surveillance.

Many of these above mentioned articles, as well as a few additional ones, also reported on the relationship between elevated hCG levels and various pregnancy complications. Elevated hCG was found to be associated with preeclampsia in both the study by Gravett et al. (38) and the study by Muller et al (79). Abruptio placenta and elevated hCG were associated in the Tanaka et al. (107) article and the Gravett et al. (38) article, but the Gonen et al. (36) article found no such relation. Gravett, et al.(38), Muller et al. (79), and Tanaka, et al. (107) all reported that elevated hCG was associated with preterm labor/premature rupture of membranes (PROM), but Gonen et al. (36) failed to find this association. Pregnancy induced hypertension (PIH) was associated with elevated hCG levels in the Gonen et al. (36) article as well as an article by Sorenson et al. (101), but not in the Tanaka et al. (107) article. Gonen et al. (36) failed to find an association between hCG elevation and either maternal diabetes or maternal bleeding. Additionally, an article by Los, et al. (63) found an association between elevated hCG and

oligohydramnios, and an article by Clark et al. (21) found that elevated hCG levels were associated with maternal lupus anticoagulant.

The specific mechanism for hCG elevation in abnormal pregnancies is unknown. One theory is that it results from the breakdown of the maternal placental barrier, much like with AFP (40). Another possible mechanism might involve decreased oxygen tension in the placenta. This could cause cytotrophoblastic hyperplasia (90) and increased trophoblast (which are responsible for hCG production) volume leading to increased production of hCG (36). This later thesis has also been supported by studies in animal models (30).

Both elevated AFP and elevated hCG and adverse pregnancy outcomes

Recently researchers have begun investigating the risks associated with adverse pregnancy outcomes in the presence of concurrent elevations of both AFP and hCG. Because this is a new topic of interest, the research is only in its early stages and much more research will need to be done in the future to confirm the findings of the early studies. Five studies have reported on a relationship between adverse pregnancy outcomes and elevated levels of both AFP and hCG. These studies are discussed below and summarized in Table 3.

The first study to report on this relationship was by Beekhuis et al (1). They noted that intrauterine fetal death (IUFD) occurred in four women from their screening program who were screen-positive for both NTD's and Down syndrome (i.e. elevated AFP causing a screen positive result for NTD's and elevated hCG driving a screen positive result for Down syndrome). This caused them to retrospectively evaluate all women who had been

Table 3. Summary of articles reporting on elevated AFP and elevated hCG with adverse pregnancy outcomes.

Study	# of Cases	Case Def.	IUFD	SGA (LB)	IUGR	PTD (LB)
Beekhuis et al. 1992 (1)	11	hCG≥2.0 AFP≥2.0	36%	NR	NR	50%
Walters et al. 1993 (123)	4	AFP>2.0 DS>1:270	50%	NR	NR	50%
Gross et al. 1994 (40)	5	AFP≥2.5 DS≥1:274	25%	100%	100%	50%
Wenstrom et al. 1994 (124)	8	AFP≥2.5 hCG≥2.0	37.5%	NR	12.5%	50%
Morssink et al. 1995 (77)	21	AFP≥2.5 hCG≥2.5	NR	38.1%	NR	15.4%

LB = live births only.
DS = Down syndrome risk.
NR = not reported.

screened by their program in whom both AFP and hCG had been found to be ≥ 2.0 MoM. Eleven women fulfilled the criteria.

Of these eleven women, only one had no pregnancy complications and only three delivered a healthy liveborn child, even though all the fetal karyotypes were normal. The pregnancy complications noted in the ten women included hemolysis, elevated liver enzymes and low platelet count (HELLP), eclampsia, oligohydramnios, hemorrhage, and PROM. Four women had an intrauterine death with subsequent histopathical examination showing no fetal congenital anomalies. Three women terminated their pregnancies because of severe fetal congenital anomalies (omphalocoele, cystic urachus and prune belly, and cystic kidneys). Of the four pregnancies that continued, one was uncomplicated and ended in normal delivery of a healthy full-term (40 week) infant, one was delivered by cesarean section at 38 weeks because of severe pre-eclampsia, and two were delivered prematurely at 36 and 26 weeks of gestation (the later baby died four days

later). Ten placentas were examined, and showed infarction in four cases, ischaemic changes in two, severe chorionitis in one, and a normal histology in three. These results led the authors to conclude that when levels of both AFP and hCG are elevated they no longer correspond to the risk of having a fetus with a NTD or Down syndrome, respectively, but rather indicate a population at increased risk for other adverse pregnancy outcomes.

Walters et al. (123) arrived at a similar conclusion relating adverse pregnancy outcome to elevated AFP and hCG, but only in the context of a positive triple analyte screen test for Down syndrome, whereby a decreased maternal serum uE3 level was also present. In their data from the Vermont Prenatal Screening Program, they identified four women (age 25-34) who met the double screen-positive criteria of AFP > 2.0 MoM and an increased second trimester risk for fetal Down syndrome of greater than 1 in 270 (hCG range: 1.93-4.62 MoM), and thirteen women (age 18-35) who had screening results of both AFP and hCG > 2.0 with a Down syndrome risk of less than 1 in 270. None of these pregnancies were affected by Down syndrome or NTD's. A comparison of mean biomarker levels in the two groups showed that the only difference between the two groups was that uE3 was significantly lower in those pregnancies with a Down syndrome risk greater than 1 in 270.

Their results showed that three of the four pregnancies in the double screen-positive group ended in an adverse outcome: two in IUFD and one in a preterm delivery at 29 weeks. In the thirteen women not screen-positive for both NTD and Down syndrome, however, all four completed pregnancies produced normal males. These

results led the authors to conclude that only those pregnancies in which the pattern of the three biochemical markers along with maternal age yielded an increased Down syndrome risk of greater than 1 in 270, while AFP was elevated, were associated with an adverse pregnancy outcome.

A study published by Gross et al. (40) reported on five women who were screen positive for both NTD, defined as AFP≥2.5, and Down syndrome, defined as risk ≥1 in 274 (hCG range: 2.62-3.65) from an original sample of 14,857 screened women. Four women elected to undergo amniocentesis, and all exhibited normal karyotype infants and normal amniotic fluid AFP levels. The Gross et al. (40) findings were consistent with those found previously by Walters et al (123). In their study, all five women had adverse pregnancy outcomes. IUGR occurred in all five pregnancies, and two were terminated because of this. One additional pregnancy resulted in intrauterine fetal demise, and the remaining two pregnancies resulted in live births. Of the two live births, one was delivered at 38 weeks by emergency Cesarean section for fetal distress, and the other was delivered by Cesarean section at 32 weeks for severe pre-eclampsia and fetal distress. Both live births had weights below the tenth percentile for their respective gestational ages. The authors concluded that although predictive value estimates cannot be offered regarding the incidence of adverse pregnancy outcomes in the context of both an elevated AFP and a positive serum triple screen test for Down syndrome because of the rarity of these cases, their experience suggests that there is an increased incidence of fetal demise, growth retardation, or infection in these cases.

Wenstrom et al. (124) examined analyte levels in 126 women with adverse pregnancy outcomes such as preterm delivery, stillbirth, and IUGR while excluding aneuploidy and structural abnormalities and compared them to 126 women with normal outcomes but who had an amniocentesis for other reasons (maternal age, family history of defects). They found that 37.5 percent of the women with both high had a fetal death, 50 percent had a preterm delivery, and 12.5 percent had IUGR. The results indicated both elevated hCG and AFP levels were significantly associated with preterm delivery and fetal death, and the odds ratio associated with any poor obstetric outcome was 7.4 (95% CI 1.1-168.2).

Morssink et al. (77) conducted a relatively large study in comparison to those previously discussed. Their study identified twenty-one women with both AFP and hCG level $\geq$ 2.5 MoM after excluding those with unknown pregnancy outcome, a congenital anomaly, delivery before 25 weeks of amenorrhoea, or known insulin-dependent diabetes. These women comprised 0.2 percent of the screened population of 8,892 singleton pregnancies. They found that 23.8 percent of the population with both markers elevated had an extremely (< 2.3 percentile) small for gestational age infant compared to only 2.2 percent of those with normal test results. This produced a relative risk of 10.9 ($p<.01$) and a detection rate of 2.7%. When SGA infant ($<10^{th}$ percentile) was examined, 38.1 percent of the pregnancies resulted in SGA infants compared to 10.3 percent of the normal population for a relative risk of 3.7 ($p<.01$) and a detection rate of 0.9 percent. For preterm delivery (<37 weeks), 15.4 percent of the population with both AFP and hCG elevated and 6.0 percent of the normal population resulted in a preterm birth. This result

was not found to be statistically significant. When SGA infant or preterm delivery were examined, 47.6 percent of the population with both AFP and hCG elevated and 15.7 percent of the normal population had these pregnancy outcomes. The relative risk was 3.0 ($p<.01$), and the detection rate was 0.8 percent. The authors concluded that a combined elevation of both AFP and hCG produced a higher positive predictive value and relative risk than AFP or hCG alone.

The mechanism for both markers being elevated is unknown. One possible explanation derives from a morphometric study by Boyd and Keeling (5). Their study showed an increased mean volume of placental parenchyma and a greater villous surface area in patients with raised AFP levels. This could lead to the elevated hCG since it is placental in origin (51).

Reduced perfusion could explain the relation of high levels of both AFP and hCG with adverse pregnancy outcome. Reduced perfusion is a stimulus for the formation of trophoblastic tissue which produces a larger amount of hCG (88). On the other hand, reduced perfusion causes infarction and ischaemic changes in the placenta (1) which could be a cause in the adverse pregnancy outcomes seen in these studies.

Chapter 3

OUR STUDY

Introduction

The purpose of this study is to examine the relationship between abnormal AFP and/or hCG levels and adverse pregnancy outcomes. We built on earlier studies by assessing elevations of AFP and hCG both separately and together in order to consider the effect of each marker on the various adverse pregnancy outcomes. In the process, we have constructed the largest study population of women with both elevated AFP and hCG levels to date to improve on the limited statistical power of previous small studies. We hypothesized that women who had high test results for both AFP and hCG would be most at risk for adverse pregnancy outcomes when compared to women with normal test results.

Methods

For this retrospective cohort study, women were selected from the screening databases of the Henry Ford Hospital (Detroit) and Michigan State University prenatal screening programs. These are two of the leading screening programs in Michigan, and together they provide the triple test for approximately 36,000 women annually in Michigan. Both programs use similar methods and protocols for their testing. The three analytes were assayed using FDA approved kits. The MSU program used the Kallestad AFP/OB for AFP detection, the Diagnostic Systems Laboratories kit for uE3, and the Serono kit for hCG detection. Henry Ford Hospital also used the Kallsted kit for AFP detection, however for uE3 they used the Amersham 3rd trimester kit until September

1991, the Amersham 2nd trimester kit until January 1992, and then the Diagnostic Systems Laboratories kit. For hCG they used the Serono kit until September 1991, the Amersham 2nd trimester kit until August 1992, and then the Corning Magic Lite kit. All of the hCG kits measured both free-β and intact hCG by using antibodies specific for the β-subunit. The MSU screening facility expresses results based on the median for the week of pregnancy the women were tested, and Henry Ford expresses results based on the median by day. Results were expressed as multiples of the median (MoM) to allow comparisons of women at different gestational ages and between the two sites. All results for AFP were adjusted for gestational age and maternal weight at the time of testing, and separate medians were used for black and non-black women. MoM's for the other two analytes were adjusted for gestational age at the time of testing, but were not adjusted for maternal weight nor calculated separately by race as the protocol for these markers did not require adjustment during the entire time period these women were tested.

Women were selected for participation in this study based on their triple test results. Eligible women were screened between January 1, 1991 and July 31, 1994, and all were screened between 15 and 22 weeks of pregnancy. Women were divided into four groups:

Group 1) Both high AFP and hCG: AFP≥2.0, hCG≥3.0
Group 2) High AFP only: AFP≥2.0, 0.70<hCG<1.30, 0.80<uE3<1.20
Group 3) High hCG only: hCG≥3.0, 0.75<AFP<1.25, 0.80<uE3<1.20
Group 4) Normal (referent): 0.75<AFP<1.25, 0.70<hCG<1.30, 0.80<uE3<1.20

In the general screened population, 5 to 6 percent of women had AFP values greater or equal to 2.0 MoM, 2 to 3 percent of women had hCG values greater or equal to 3.0 MoM, and approximately 4 per 1,000 had both elevated AFP and elevated hCG levels. All

group 1, both high women, and group 3, high hCG women, were sampled. Because of the large number of group 2, high AFP women, and group 4, normal women, the sampling strategy was to obtain a woman from these groups who had been tested closest to (either before of after) a both high woman. Women from the various groups were not matched to each other by any maternal variables. Women were excluded from the study if there was a multiple pregnancy or if further testing indicated Down Syndrome or open neural tube defects.

Follow-up information on women sampled was obtained from the prenatal screening follow-up data bases. Follow-up data was collected by contacting the referring physician's office after the woman delivered. Results were obtained either directly over the phone, by faxing the form for completion by the physician's staff at a later time, or abstracted from the pregnancy record by a staff member of the Henry Ford Hospital or Michigan State University screening program. The follow-up form requested information in the following categories: medical history prior to pregnancy, medical problems during pregnancy, date of delivery, problems during delivery, mode of delivery, birthweight and sex of the infant, and any abnormalities noted in the infant at birth. A copy of the form used by MSU and HFH can be found in Appendices A and B, respectively. If follow-up information was not available or if gestational age at birth, from the last menstrual period, was greater than 42 weeks, another woman from the same group was sampled.

Statistical methods

For analyses, the independent variable was the triple test results as defined in the four groups. Outcome variables examined included stillbirth or spontaneous abortion,

preterm birth, low birthweight, small for gestational age (SGA) infant, and fetal defects. Stillbirths and spontaneous abortions were combined because of the small numbers of events. Preterm birth was defined in two different ways, very preterm (<35 weeks gestation) and preterm (<37 weeks gestation). Low birthweight was defined as less than 2000 grams, and an SGA infant was defined as less than the 10^{th} percentile in birthweight for gestational age using the Williams (125) standards. For fetal defects, an attempt was made before analyzing the data to group these conditions. Defects were categorized by experts within the research group as to when they are typically detected (either pre- or post-natally) as the timing of the detection has implications for interventions and estimates of prevalence, and whether they were considered to be major or minor defects. Appendix C lists the defects included in each category. The distribution of various pregnancy complications in the four groups of women were also examined. The maternal complications prior to pregnancy that were examined are listed in Appendix D.

Initial analyses examined the distribution of demographic and maternal variables among the different triple test groups using the Chi Square test for dichotomous variables and the t-test for continuous variables. To determine odds ratios and confidence intervals for the outcomes we examined, logistic regression was used. We examined the risks of stillbirth or spontaneous abortion and the risks of fetal defects versus delivering a liveborn, defect-free infant. Assessments of preterm birth, low birthweight, and SGA infants were confined to live births only. We further broke this down in analysis by examining live births with and without defects, in order to examine the contribution of fetal defects to the risks of the other adverse outcomes examined. Finally, the risk for the

various groups of any adverse pregnancy outcome versus no adverse outcome was assessed. All analyses were performed using SAS statistical software (93).

Results

Groups with abnormal AFP and/or hCG levels were compared to the referent group of women with both normal AFP and hCG. Table 4 shows the distribution of selected variables of interest by triple test group. Women with high hCG only were more likely to be <20 years and Black when compared to the both normal group 4 women. Women with high AFP only and women with both high AFP and high hCG were more

Table 4. Maternal and Pregnancy Characteristics by screening status.

Characteristic	High AFP and high hCG (n=143)	High AFP only (n=151)	High hCG only (n=129)	Normal markers (referent) (n=150)
Maternal Age				
< 20	14.7%*	9.3%*	10.1%*	3.3%
20 - 34 (referent)	70.0%	82.8%	83%	86.7%
≥ 35	15.4%	8.0%	7.0%	10.0%
Maternal Race				
Black	19.7%*	14.1%	15.5%*	7.0%
Non-black	80.3%	85.9%	84.5%	93.0%
% Male	59.8%*	58.3%*	45.3%	46.3%
Mean Birthweight (Std Dev)	2847g (863.6)*	3243g (679.4)*	3406g (494.4)	3489g (582.2)
Mean Gestational Age (Std Dev)	37.2 (3.4)*	38.3 (2.7)*	39.3 (1.5)	39.2 (1.6)
Mode of Delivery				
C-section or Induced	11.2%	11.1%	12.5%	11.4%
Spontaneous Vaginal Delivery (referent)	88.8%	88.9%	87.5%	88.6%
Maternal Complications Prior to Pregnancy+	7.0%*	3.3%	3.9%	0.7%
Maternal Complications During Pregnancy				
Oligohydramnios	2.4%	1.4%	0.0%	0.0%
Eclampsia / Hypertension	4.8%	3.5%	5.5%	3.4%

+Complications include: Autoimmune, Endocrine, Renal, Asthma.
*$p \leq .05$ in comparisons with referent.

likely to be <20 years, to be Black, and to have maternal complications prior to pregnancy. In addition, infants born to women in these two groups had a lower mean gestational age at birth, a lower mean birthweight, and were more likely to be male.

Women with high AFP and high hCG were at significantly increased risk of having a stillbirth or SAB infant (OR 15.2, 95% CI 3.2-73.1) when compared to the referent group (Table 5). Women with high AFP only were also more likely to have a stillbirth or spontaneous abortion (OR 6.4, 95 percent CI 1.0-41.4). By contrast, women with high hCG only were not more likely to have a stillbirth or spontaneous abortion.

Fetal defects were broken down into three categories based on being either major or minor and, for major, depending on when they are typically first detected. A list of defects included in each group can be found in Appendix C. Women with both high AFP and high hCG were at significantly greater risk of having an infant with a major defect that can be detected pre-natally (OR 8.9, 95% CI 1.5-51.8). Infants of women with high AFP only or high hCG only were not at significantly greater risk for these major defects,

Table 5. Spontaneous abortion, stillbirth, and infant defects by screening status. Percentages and odds ratios* with 95% Confidence Intervals.

Outcome	High AFP and high hCG		High AFP only		High hCG only		Normal markers	
Stillbirth or SAB	9.4%	15.2+ (3.2, 73.1)	4.2%	6.4+ (1.0, 41.4)	0.8%	1.2 (0.1, 20.0)	0.7%	1.0 (referent)
Fetal Defects								
Major Dx Pre-natally	5.7%	8.9+ (1.5, 51.8)	2.1%	3.2 (0.4, 27.7)	0.0%	0.4 (0.0, 10.2)	0.7%	1.0 (referent)
Major Dx Post-natally	1.7%	6.3 (0.3, 133.1)	1.4%	5.3 (0.3, 111.9)	4.8%	16.0 (0.9, 287.7)	0.0%	1.0 (referent)
Minor Dx Post-natally	4.9%	7.6+ (1.2, 47.1)	1.4%	2.1 (0.2, 22.6)	2.5%	3.7 (0.4, 31.2)	0.7%	1.0 (referent)
Any Defect	11.5%	9.5+ (2.7, 33.2)	4.8%	3.7 (0.8, 16.5)	7.0%	5.6+ (1.4, 22.4)	1.3%	1.0 (referent)

*Unadjusted odds ratios calculated using live birth and defect free comparison group.
+Odds ratio significantly different from 1.0 ($p<.05$).

however the odds ratio for high AFP only was 3.2 and could indicate an elevated risk that is not detectable with the sample size of this study. The risk of having an infant with a defect detected postnatally was increased among all groups with abnormal analyte levels, most notably for women with high hCG only (OR 16.0). Because infants in the normal marker group did not have any of these defects, however, the confidence intervals for the estimated odds ratios were quite wide and inclusive of 1.0. Infants born to women with both high AFP and high hCG were more likely to have a minor defect (OR 4.9, 95% CI 1.2-47.1). Increased rates of minor defects were also observed among infants born to women with high hCG only or women with high AFP only (OR 2.1 and 3.7, respectively), but because of small numbers, the odds ratios were not statistically significant. The risks for any defect were significantly elevated for infants born to women with both high AFP and high hCG (OR 9.5, 95% CI 2.7-33.2), and infants born to women with high hCG only (OR 5.6 95% CI 1.4-22.4). The risk for infants born to women with high AFP only was elevated (OR 3.7) but the confidence interval included one.

We then examined adjusted odds ratios for risks of SAB, stillbirth, and fetal defects. Variables that differed by group (from Table 4) included maternal age, maternal race, and maternal complications prior to pregnancy. Data on maternal complications prior to pregnancy were not considered reliable because of data collection problems, therefore only maternal age and maternal race were available as possible confounders. Adjustment for maternal age did not alter the odds ratios significantly. Adjustment for maternal race produced slightly higher odds ratios for stillbirth or spontaneous abortion

among women with both high AFP and high hCG (OR 16.3, 95% CI 2.1-129.3) and women with high AFP only (OR 6.5, 95% CI 0.8-55.3). Adjustment for race did not affect the analyses of fetal abnormalities.

Next we assessed fetal growth and preterm delivery among the four groups of women. The results of these analyses are presented in Table 6. Women with both high AFP and high hCG were at increased risk of delivering a very preterm infant (<35 weeks gestation) compared to the referent (OR 11.2, 95% CI 4.0-31.2). There was no significantly increased risk for delivering a very preterm infant among women with high AFP only or women with high hCG only, however the odds ratio for high AFP only women was elevated to 2.8. The risk of delivering before 37 weeks was increased among both women with high AFP and high hCG (OR 8.2, 95% CI 3.9-17.5) and women with high AFP only (OR 4.3, 95% CI 1.9-9.7). There was no increase in risk seen for women with high hCG only.

Table 6. Fetal growth and preterm delivery for all live births by screening status. Percentages and odds ratios with 95% confidence intervals.

Outcome	High AFP and high hCG		High AFP only		High hCG only		Normal markers	
Preterm Birth*								
≤ 34 Weeks	19.1%	11.2+ (4.0, 31.2)	5.6%	2.8 (0.7, 10.5)	0.8%	0.4 (0.0, 3.5)	2.1%	1.0 (referent)
35-36 Weeks	14.4%	6.0+ (2.2, 16.6)	13.1%	5.4+ (2.0, 14.8)	4.7%	1.8 (0.5, 6.3)	2.7%	1.0 (referent)
Total < 37 Weeks	28.8%	8.2+ (3.9, 17.5)	17.4%	4.3+ (1.9, 9.7)	5.5%	1.2 (0.4, 3.4)	4.7%	1.0 (referent)
Birth Weight < 2,000g	18.4%	16.6+ (5.4, 51.2)	4.9%	3.8 (0.8, 16.6)	0.0%	0.2 (0.0, 4.8)	1.3%	1.0 (referent)
SGA < 10th Percentile	14.4%	3.0+ (1.3, 6.9)	9.7%	1.9 (0.8, 4.6)	5.5%	1.0 (0.4, 2.9)	5.4%	1.0 (referent)

*Unadjusted odds ratios of preterm verses term (≥ 37 weeks).
+Odds ratio significantly different from 1.0 (p<.05).

For low birthweight, defined as less than 2,000 grams, there was an increased risk for women with both high AFP and high hCG compared to women with normal markers (OR 16.6, 95% CI 5.4-51.2). Women with high AFP only had an elevated, although not statistically significant, odds ratio of 3.8. There were no low birthweight infants born to high hCG women only.

Women with both high AFP and high hCG were at an increased risk of an SGA infant (OR 3.0, 95% CI 1.3-6.9). Women with high AFP only were also at elevated risk (OR 1.9), but the results were not significant. There was no association between delivering an SGA infant and having high hCG only.

Analysis of preterm delivery, low birthweight, and SGA were repeated with adjustment for maternal age and race. Including these covariates had no effect on the results, but the inclusion of race slightly reduced the odds ratio of women with both high AFP and high hCG for delivery prior to 35 weeks gestation (OR 10.3, 95% CI 3.0-35.8).

To assess the extent to which the results were influenced by the inclusion of infants with fetal defects, analyses were repeated with defect-free live births only. The results are presented in Table 7. Although most of the odds ratios were slightly lower, the results remained robust.

Table 8 presents the findings for all adverse pregnancy outcomes combined (preterm delivery, SGA, low birthweight, fetal defects, SAB or stillbirth). For women with both high AFP and high hCG, the unadjusted odds ratios was 7.1 (95% CI 3.9-13.0). Women with high AFP only were also at increased risk for adverse pregnancy outcomes

Table 7. Fetal growth and preterm delivery for live births without fetal defects by screening status. Percentages and odds ratios with 95% confidence intervals.

Outcome	High AFP and high hCG		High AFP only		High hCG only		Normal markers	
Preterm Birth*								
≤ 34 Weeks	17.5%	9.9+ (3.4, 28.4)	5.8%	2.9 (0.8, 10.7)	0.9%	0.4 (0.0, 3.8)	2.1%	1.0 (referent)
35-36 Weeks	13.3%	5.4+ (1.9, 15.3)	13.0%	5.2+ (1.9, 14.5)	4.2%	1.5 (0.4, 5.9)	2.8%	1.0 (referent)
Total < 37 Weeks	26.7%	7.3+ (3.4, 15.8)	17.4%	4.2+ (1.8, 9.6)	5.0%	1.1 (0.4, 3.3)	4.8%	1.0 (referent)
Birth Weight < 2,000g	16.4%	14.2+ (4.4, 45.7)	5.1%	3.9 (0.9, 17.1)	0.0%	0.2 (0.0, 5.1)	1.4%	1.0 (referent)
SGA < 10th Percentile	14.7%	3.4+ (1.4, 8.2)	8.7%	1.9 (0.7, 4.9)	5.9%	1.3 (0.4, 3.7)	4.8%	1.0 (referent)

*Unadjusted odds ratios of preterm verses term (≥ 37 weeks).
+Odds ratio significantly different from 1.0 (p<.05).

(OR 3.3, 95% CI 1.8-6.1). The risk for women with high hCG only was elevated (OR 1.7, 95% CI 0.9-3.3), however the confidence interval included 1.0.

When race was entered into the model of any adverse pregnancy outcome, the adjusted odds ratio for women with both high AFP and high hCG was 6.0 (95% CI 3.3-11.1). Women with high AFP only also had an increased risk (OR 3.1, 95% CI 1.6-5.7). For women with high hCG only, the results were again elevated (OR 1.6, 95% CI 0.8-3.1) but not statistically significant. After inclusion of race as a covariate, further adjustment for maternal age did not alter the odds ratios for any adverse outcome in the logistic regression model.

Our study focused on reporting measures of association with adverse pregnancy outcomes because the questions posed involved attempting to better understand the underlying mechanisms of elevated marker levels. However, since the markers are used as a screening test for Down syndrome and neural tube defects, we decided to also report on screening measures. Using the results from our study, we calculated sensitivities,

Table 8. Any adverse outcome^ by screening status. Percentages and odds ratios with 95% confidence intervals.

	High AFP and high hCG	High AFP only	High hCG only	Normal markers
Percent	47.6%	29.8%	17.8%	11.3%
Unadjusted OR	7.1+ (3.9, 13.0)	3.3+ (1.8, 6.1)	1.7 (0.9, 3.3)	1.0 (referent)
OR Adjusted for Maternal Age	7.1+ (3.9, 13.0)	3.3+ (1.8, 6.1)	1.7 (0.9, 3.3)	1.0 (referent)
OR Adjusted for Race	6.0+ (3.3, 11.1)*	3.1+ (1.6, 5.7)	1.6 (0.8, 3.1)	1.0 (referent)
OR Adjusted for Maternal Age and Race	6.1+ (3.3, 11.2)*	3.1+ (1.7, 5.8)	1.6 (0.8, 3.1)	1.0 (referent)

^Includes: preterm delivery (GA<37), low birthweight (<2000g), SGA (<10th percentile), any fetal defect, and SAB or stillbirth.
+Odds ratio significantly different from 1.0 ($p<.05$).
*Adjusted model significantly improved compared to crude model using log likelihood statistic $p<.05$.

specificities, positive predictive values, and negative predictive values for the various testing results to detect any adverse pregnancy outcomes. We knew the prevalence of elevated markers in the screened population (high AFP, 5%; high hCG, 3%; both high AFP and hCG, 0.4%). To estimate the prevalence of adverse pregnancy outcomes in our screened population, we used data from our study. We took the prevalence of adverse pregnancy outcomes in study women with high markers (30%) and women with normal markers (11%) and weighed them according to the prevalence of high and normal markers in the previously mentioned screened population.

Using the cut-off values of AFP ≥ 2.0 MoM and hCG ≥ 3.0 MoM, the sensitivity of the both high AFP and high hCG test result in predicting adverse pregnancy outcome was 1.5%, with a specificity of 99.8%. The positive predictive value was 47.5%, and the negative predictive value was 87%. For elevated AFP only, the sensitivity was 11.5%

and the specificity 96%. The positive predictive value was 30% and the negative predictive value 88%. For high hCG only, the sensitivity was 4.2%, with a specificity of 97.2%. The positive predictive value was 18% and the negative predictive value 87%.

Discussion

Results from this study strongly suggest that women who have both high AFP and high hCG test results are at significantly increased risk for adverse pregnancy outcomes including preterm delivery, low birthweight, SGA infants, and most fetal defects. The one exception was major defects typically detected post-natally, for which the odds ratio was elevated, but due to the small numbers the confidence interval was very wide and included 1.0. For women with both high AFP and high hCG, our results are consistent with the relatively new research into this area (1, 123, 40, 124, 77). In particular, our study supports the smaller Morrsink et al. study (77) which found that women with both markers elevated were at higher risk for adverse outcomes than women with either high AFP or high hCG alone.

It appears from the results that elevated AFP only is also associated with adverse pregnancy outcomes. Odds ratios for these women were elevated for all outcomes examined, and were significant for stillbirth or SAB, preterm delivery, and any adverse pregnancy outcome. Our study supports those that found elevated AFP related to fetal death as well as preterm delivery (7, 114, 68, 37, 8, 100, 87, 83, 49, 23, 6, 70, 41, 22, 74, 33, 81, 89, 120, 99). Our results do not contradict previous reports of an association between high AFP and delivery of an SGA infant as well as low birthweight (7, 114, 68,

6, 8, 112, 100, 87, 83, 41, 86, 22, 29, 94, 49, 33, 34, 47, 28, 13, 89, 75, 23, 18, 77) though we lacked the power to confidently confirm these relationships.

Overall, our results seem to indicate that elevated hCG levels alone do not play as important a role for increased risk of adverse pregnancy outcomes as elevated AFP levels alone. Women with high hCG only were at a significantly increased risk for fetal defects. Our results for hCG do not support the results by previous studies that elevated hCG is associated with increased risk for fetal death, low birthweight, preterm delivery, or delivering a SGA infant (3, 38, 58, 107, 124, 77).

The low sensitivity of the triple test markers (both alone and in combination) indicate that they are not effective screening measures for adverse pregnancy outcomes. However, since the triple test is already in place to detect Down syndrome and neural tube defects, a more important question is what can be said about women with high analyte levels. To address this question, the focus should be on positive predictive value. The positive predictive value of the both high AFP and hCG test result, and to a lesser extent the positive predictive value of the high AFP only test result, indicate a high proportion of women who have these elevated markers are at risk for adverse pregnancy outcomes. At this time, little is known regarding possible interventions for some of the adverse outcomes included in this study. The identification, however, of a group of women particularly at risk that could be further studied in order to develop a better understanding of these outcomes is beneficial.

The most impressive finding, however, remains that when both AFP and hCG are elevated, women are at a significantly higher risk for all of the adverse outcomes we

examined, and that this risk was much higher than either elevated AFP or elevated hCG alone.

This study was initiated as a preliminary clinical investigation, and as a result there are several methodological drawbacks. First, the results could be affected by the strict criteria used for the various markers in the referent women with normal markers. As it turned out, the normal women were quite a select group. When the normal values for each of the three markers were considered individually, these cut-offs included 40 to 50 percent of the general screened population, however when considered together, women who were within the cut-offs for all three markers only comprised 10 percent of the general screened population. This could have affected the odds ratios for some of the outcomes examined since our referent group could be a group of women with a better than average estimate of gestational age.

There were also several methodological problems with data collection in this study. First there was a difference in protocols for data collection at the two screening sites. The instrument used to gather the follow-up information was much more specific at the MSU site than the HFH site (Appendix A and B). This could have led to underreporting of some of the outcomes we examined, and especially the maternal complications before and during pregnancy since the HFH instrument did not specifically ask for these. Additionally, some follow-up information was collected over the phone, some by fax, and some was abstracted from the medical records by a member of the screening staff. These different methods of collection could have led to different levels of completion of the forms and therefore an information bias. There is no evidence,

however, that the distribution of collection methods differed by group, so this bias is more likely to result in an underestimation of true odds ratios.

Second, since the follow up information was collected from the office of the reporting physician, for the most part the accuracy and completeness depended on how thoroughly the nurse or assistant who filled out the form searched the woman's pregnancy record. Follow-up data collection was not blinded with regards to testing status. If the baby tested normal, the nurses may be less likely to search the record for complications or minor defects. Similarly, if the baby appeared normal and healthy, then they may have been less likely to search through to find maternal health problems before and during pregnancy. The potential for information bias was especially evident in the forms completed by telephone interview of the clinic's staff. Some interviews only took five minutes to complete with the nurses reporting everything normal, while others could take up to 15 minutes as the nurse scoured the medical record in search of the information requested. It is possible, therefore, that the quality of the information varied depending on the reporting clinic and the time the nurse had to complete the form. Given these constraints, it is quite possible that they were less likely to thoroughly complete a form for a woman with a normal test result than for a woman with an abnormal one. The open ended questions asked in the follow-up form also introduced inconsistencies in data collection. Since the form did not ask about specific abnormalities, complications, etc., the nurse filling in the form was determining what was important enough to include. This also left it difficult to distinguish no response from a negative response.

In addition to possible inaccuracies in outcome measures, there are concerns about the data quality of other important variables. Since the serum marker levels change throughout pregnancy, accurate gestational age reporting is critical for correct interpretation of test results. In this study, gestational ages at the time of screening were calculated using the last menstrual period (LMP), and this method can sometimes be inaccurate since it depends on the woman's recall and knowledge of her menstrual cycle. If all pregnancies were dated routinely by ultrasound, the rate of false-positive screening results could be lowered by about 30 percent with no loss in sensitivity (48). When possible, the dates were confirmed by ultrasound; however, this could have led to another bias since the ultrasound rates differed by group with more of those with abnormal test results having ultrasounds performed. When this is coupled with the possibility of our study population having more accurate gestational ages due to the strict constraints on marker levels, however, this problem could be reduced or even eliminated.

In this study, hCG and uE3 MoM's were not calculated separately for Blacks and Whites as was done with AFP. Although using race specific medians for all three markers is now standard protocol (106), it was not for most of the period when these women were tested, so all results were left unadjusted. This might have affected the conclusions drawn since race is not distributed equally throughout the groups; however, the small changes that occur in marker distribution by race are unlikely to affect the large odds ratios found and the conclusions drawn in this study. Additionally, none of the tests results for the three markers were adjusted for insulin-dependent diabetes, therefore if some women had insulin-dependent diabetes, it is possible that they were false-positives.

Along this same line, during the study period the method of assaying the three analytes was changed due to the availability of kits. This should not have significantly affected any results, however, since all kits used were approved by the Foundation for Blood Research and CAP QA guidelines (45) and since the changes were instituted across all four groups. This would need to be confirmed by time trend analysis of our data, however, to be certain.

Because this study originated as a preliminary clinical investigation, many maternal variables of interest were unavailable. For example, there was no information on socioeconomic status or prenatal care. Additionally, information on previous pregnancy history and exposures such as alcohol, cigarettes, and drugs during pregnancy was unreliable. Ideally all of these should be taken into account when examining pregnancy outcomes.

Fetal defects and maternal complications had to be grouped into broad categories because individual defects and complications occur infrequently. These outcomes were combined into the various groups (Appendicies C and D) by the project members before analysis of the data. Although members of the group have expertise in adverse pregnancy outcomes, some of the defect and complication groupings could be argued since they are subjective categorizations.

The rareness of events contributed to the wide confidence intervals seen in most of our results. Although our study is one of the largest to date on this subject, we are still restricted by the small numbers of events; therefore, some findings that don't appear

statistically significant in this study may actually be realized as significant differences in a study with larger numbers.

This study also has several strengths. First, this is the largest and most comprehensive look at abnormal triple test results and adverse pregnancy outcomes to date. As described previously, many studies thus far have been case histories that could not provide meaningful comparative statistics and could not draw firm conclusions. By examining adverse pregnancy outcomes for the three combinations of abnormal test results separately, we were able to appreciate the risks associated with the different combinations of abnormal marker levels.

The use of the other markers to control for gestational age reliability in the groups is also a strength of this study. By making cut-offs close to the median for uE3 and the median for which ever of the other two markers was not under investigation, we attempted to reduce the number of women in whom the gestational age was either over or under estimated. Gestational age is extremely important since it determines the normal level for the analytes , therefore this additional check of accuracy lends considerable validity to the classification of the women in the various groups.

Future Directions

This relatively large retrospective cohort study strongly indicates that women with abnormal triple test results need to have their pregnancies monitored more closely due the increased risk of an adverse pregnancy outcome. However, more research separating the various combinations of abnormal test results is needed to confirm these results. Future

studies will need a large number of women in order to compensate for the infrequent occurrences of various individual outcomes of interest.

Additionally, the shortfalls in data collection in this study should be addressed. Future studies should gather more demographic and exposure information from the women through personal interviews rather than relying simply on the medical record information that the patient provided her doctor. Also, uniform abstraction of the relevant medical information from the woman's record by a member of the investigation team would help reduce the variability in collection we experienced by having the physician's office fill out the follow-up forms. In addition, autopsy of SAB infants could provide more accurate information on the association between elevated levels of the analytes and fetal defects. To appreciate the biologic mechanisms underlying the associations between markers and adverse pregnancy outcomes, more research also needs to be done on the causes of analyte variability during pregnancy.

Potentially preventable medical complications of pregnancy account for approximately 15 to 20 percent of preterm deliveries (72), and preterm delivery accounts for 80 percent of perinatal mortality not attributed to congenital malformations (38). Because of this, long term management of these at-risk pregnancies needs to be better defined. No studies, however, have specifically investigated management protocols. Development of an appropriate clinical approach to women with high values is complicated by the fact that most of the pregnancies will have normal outcomes. Suggestions for further management have included increased obstetric surveillance such as repeat ultrasounds to document fetal growth and amniotic fluid volume, and antenatal testing such as fetal movement counts, nonstress tests, and biophysical profiles. Until

prospective studies are performed evaluating optimal management, however, no specific protocols for evaluating these pregnancies can be accepted as the standard of care. For now, treatment of women with abnormal triple test results should be individualized according to the woman's risk factors, situation, and needs.

APPENDICES

Appendix A

Follow-up information form, Michigan State University Prenatal Screening Program.

Triple Test Follow-up Form

MSU Prenatal Screening Program
B245 Life Sciences Building
East Lansing, MI 48824-1317

PTINFO

Patient's Name	LMP	EDC	Patient's Date of Birth
Address	TT Code	Genetics Chart #	Weight (1st Draw)
	G P Ab Sb		SS #
County Ethnic Group	Ref. Physician		Phone
Is there a family history of birth defects, mental retardation, or recurrent pregnancy losses? ☐ No ☐ Yes Explain			
Has this patient had any previous premature deliveries (36 weeks or less)? ☐ No ☐ Yes How many			

PROCS

1ST DRAW	2ND DRAW	3RD DRAW
Gest. Age DS Risk	Gest. Age DS Risk	Gest. Age DS Risk
AFP uE3 BhCG	AFP uE3 BhCG	AFP uE3 BhCG
Ultrasound ☐ No ☐ Yes	Ultrasound Findings	Amniocentesis ☐ No ☐ Yes
Karyotype Results	Hemoglobin ☐ No ☐ Fetal ☐ Maternal	AChE-Positive ☐ No ☐ Yes

PROBLEMS

WAS THE PATIENT'S PREGNANCY COMPLICATED BY . . .			
Health problems?	☐ No ☐ Yes	Explain	
Diabetes?	☐ No ☐ Yes	☐ Gestational ☐ Insulin dependent ☐ Diet controlled	Date of onset
Hypertension?	☐ No ☐ Yes	Date of onset	
Vaginal bleeding?	☐ No ☐ Yes	Which trimester ☐ 1st ☐ 2nd ☐ 3rd	
Alcohol or drug use?	☐ No ☐ Yes	Explain (How much? How often?)	
Medications?	☐ No ☐ Yes	Explain	
Smoking?	☐ No ☐ Yes	How much	

OUTCOME

Date of delivery	Maternal weight at delivery	Gestational age	
Delivery Complications? ☐ No ☐ Yes Explain			
Outcome ☐ Live born ☐ Stillborn ☐ Spontaneous Abortion ☐ Elective Abortion		Infants sex ☐ Male ☐ Female	
Mode of delivery ☐ Vaginal ☐ Cesarean Section ☐ Forceps	Length	Birth weight	
Head circumference	Apgar score at 1 minute	Apgar score at 5 minutes	
Abnormalities observed & Additional comments			
Completed by	Date		

Appendix B

Follow-up information form, Henry Ford Hospital Prenatal Screening Program.

December 5, 1995

ATTN: NURSES

At your earliest convenience could you look up this information?

Name_____ EDC / /

Delivery date_____

Baby's Sex_____

How Delivered_____

Weight_____

Baby OK?_____

If not, what problems_____

_____

_____

Thanks so much. You can just fax this back to 876-2076.

Suzanne

Appendix C

Infant abnormality categories.

Major defects detectable pre-natally	**Major defects detectable post-natally**	**Minor defects detectable post-natally**
Prune Belly	Coaretation of the Aorta	Cryptorchidism
Cystic Adenomatoid Malformation	Ventricular/Atrial Septal Defect	No Helix Fold (Ear)
Anencephaly	Imperforate Anus	Single Umbilical Artery
Encephaloceile	Closed Sagital Sutures	Hypospadias
Gastroschisis	Osteogenesis Inperfecta	Hydronephrosis
Nermis Hypoplasia	Soto's Syndrome	Undescended Testes
Dextrocardia		Eartag
Arthrogyposis		Very Short Cord

Appendix D

Categories of maternal complications prior to pregnancy.

Autoimmune	**Endocrine**	**Renal**	**Asthma**
Type 1 Diabetes	Hyperthyroidism	Renal Failure	Asthma
Lupus erythematosis	Type 1 Diabetes	IgA Nephropathy	
Polymyositis	Synthroid requirement		
Crohn's Disease			
Ulcerative Colitis			
Rheumatoid arthritis			
IgA Nephropathy			

LIST OF REFERENCES

LIST OF REFERENCES

1. Beekhuis JR, Van Lith JMM, De Wolf BTHM, Mantingh A. Increased maternal serum alpha-fetoprotein and human chorionic gonadotropin in compromised pregnancies other than for neural tube defects or Down syndrome. *Prenat Diagn* 1992;12:643-647.

2. Bergstrand CG, Czar B. Demonstration of a new protein fraction in serum from the human fetus. *Scand J Clin Lab Invest* 1956;8:174.

3. Bogart MH, Jones OW, Felder RA, et al. Prospective evaluation of maternal serum human chorionic gonadotropin levels in 3428 pregnancies. *Am J Obstet Gynecol* 1991;165:663-7.

4. Bogart MH, Pandian MR, Jones OW. Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities. *Prenat Diagn* 1987;7:623-30.

5. Boyd PA, Keeling JW. Raised maternal serum alpha-fetoprotein in the absence of fetal abnormality-placental findings. A quantitative morphometric study. *Prenat Diagn* 1986;6:369-373.

6. Brock DJH, Barron L, Duncan P. Significance of elevated midtimester plasma alpha-fetoprotein values. *Lancet* 1979;1:1281-1282.

7. Brock DJH, Barron L, Jelen P, et al. Maternal serum-alpha-fetoprotein measurements as an early indicator of low birth-weight. *Lancet* 1977;2:267.

8. Brock DJH, Barron L, Raab GM. The potential of mid-trimester maternal plasma alpha-fetoprotein measurement in predicting infants of low birth weight. *Br J Obstet Gynaecol* 1980;87:582-585.

9. Brock DJH, Barron L, Watt M, et al. Maternal plasma alpha-fetoprotein and low birthweight: A prospective study throughout pregnancy. *Br J Obstet Gynaecol* 1982;89:348.

10. Brock DJH, Bolton AE, Monaghan JM. Prenatal diagnosis of anencephaly through maternal serum-alpha-fetoprotein measurement. *Lancet* 1973;2:923.

11. Brock DJH, Sutcliffe RG. Alpha-fetoprotein in the antenatal diagnosis of anencephaly and spina bifida. *Lancet* 1972;2:197-199.

12. Burton BK, Prins GA, Verp MS. A prospective trial of prenatal screening for Down syndrome by means of maternal serum α-fetoprotein, human chorionic gonadotropin, and unconjugated estriol. *Am J Obstet Gynecol* 1993;169:526-30.

13. Burton BK. Outcome of pregnancy in patients with unexplained elevated or low levels of maternal serum alpha-fetoprotein. *Obstet Gynecol* 1988;72:709-713.

14. Burton BK. Unexplained elevated maternal serum alpha-fetoprotein and adverse perinatal outcome. In Elias S, Simpson JL (Eds.). *Maternal Serum Screening for Fetal Genetic Disorders*. Churchill Livingstone Inc., 1992.

15. Buster JE. Gestational changes in steroid hormone biosynthesis, secretion, metabolism, and action. *Clin Perinatol* 1983;10:527-552.

16. Canez MS, Lee KJ, Olive DL. Progesterones and estrogens. *Infertil Reproduct Med Clin North Amer* 1992;3:59-78.

17. Canick JA, Knight GJ, Palomaki GE, et al. Low second trimester maternal serum unconjugated oestriol in pregnancies with Down's syndrome. *Br J Obstet Gynaecol* 1988;297:883-87.

18. Capeless EL, Kelleher PC, Walters CP. Elevated maternal serum αfetoprotein levels and maternal risk factors: their association with pregnancy complications. *J Reprod Med* 1992;37(3):257-260.

19. Carter CO, Evans K. Spina bifida and anencephaly in Greater London. *J of Med Gen* 1973;10:209-234.

20. Chitty LS, Bobrow M. Techniques in current use in prenatal diagnosis. *Intl J of Technology Assessment in Health Care* 1994;10(4):604-627.

21. Clark F, Dickinson JE, Walters BNJ, et al. Elevated mid-trimester hCG and maternal lupus anticoagulant. *Prenat Diagn* 1995;15:1035-1040.

22. Crandall BF, Robertson RD, Lebherz, et al. Maternal serum alpha-fetoprotein screening for the detection of neural tube defects. *West J Med* 1983;138:524-530.

23. Crandall BF, Robinson L, Grau P. Risks associated with an elevated maternal serum alpha-fetoprotein level. *Am J Obstet Gynecol* 1991;165:581-586.

24. Crossley JA, Aitken DA, Connor JM. Second trimester unconjugated oestriol levels in maternal serum from chromosomally abnormal pregnancies using an optimized assay. *Prenat Diagn* 1993;13:271.

25. Cuckle HS, Wald NJ, Lindenbaum RH. Maternal serum alpha-fetoprotein measurement: A screening test for Down syndrome. *Lancet* 1984;1:926.

26. Diagnostic Systems Laboratories Inc. *Ultra-sensitive Unconjugated estriol: Radioimmunoassay Kit for the Quantitative Measurement of Unconjugated Estriol in Serum* (insert). 1994.

27. DiMaio MS, Baumgarten A, Greenstein RM, et al. Screening for fetal Down syndrome in pregnancy by measuring alpha fetoprotein levels. *N Engl J Med* 1987; 317:342.

28. Doran TA, Montgomery RJ, Valentine GH, et al. Maternal serum alpha-fetoprotein screening: report of a Canadian pilot project. *Can Med Assoc J* 1987;137:285-293.

29. Evans J, Stokes IM. Outcome of pregnancies associated with raised serum and normal amniotic fluid alpha-fetoprotein concentrations. *BMJ* 1984;288:1494.

30. Fox H. The effect of hypoxia on trophoblast in organ culture: a morphologic and autoradiologic study. *Am J Obstet Gynecol* 1970;107:1058-64.

31. Frigoletto FD, Little GA, eds. *Guidelines for Perinatal Care*. Washington DC American Academy of Pediatrics and the American College of Obstetricians and Gynecologists, 1988.

32. Fuhrmann W, Altland K, Kohler A, et al. Feto-maternal transfusion after chorionic villus sampling: evaluation by maternal serum alpha-fetoprotein measurement. *Hum Genet* 1988;78:83.

33. Fuhrmann W, Weitzel HK. Maternal serum alpha-fetoprotein screening for neural tube defects. *Hum Genet* 1985;69:47.

34. Ghosh A, Tang MHY, Tai D, et al. Justification of maternal serum alpha-fetoprotein screening in a population with low incidence of neural tube defects. *Prenat Diagn* 1986;6:83.

35. Gitlin D, Boesman M. Serum alpha-fetoprotein, albumin, and gamma-globulin in the human conceptus. *J Clin Investigation* 1966;45(11):1826.

36. Gonen R, Perez R, David M, et al. The association between unexplained second-trimester maternal serum hCG elevation and pregnancy complications. *Obstet Gynecol* 1992;80(1):83-85.

37. Gordon YB, Grudzinskas JG, Kitau MJ, et al. Fetal wastage as a result of an alpha-fetoprotein screening programme. *Lancet* 1978;1:677.

38. Gravett CP, Buckmaster JG, Watson PT, Gravett MG. Elevated second trimester maternal serum β-hCG concentrations and subsequent adverse pregnancy outcome. *Am J Med Gen* 1992;44:485-486.

39. Green MF, Haddow JE, Palomaki GE, et al. Maternal serum alpha-fetoprotein levels in diabetic pregnancies. *Lancet* 1988;2:345.

40. Gross SJ, Phillips OP, Shulman LP, et al. Adverse prenatal outcome in patients screen-positive for neural tube defects and fetal Down syndrome. *Prenat Diagn* 1994;14:609-613.

41. Haddow JE, Kloza EM, Smith DE, Knight GJ. Data from an alpha-fetoprotein pilot screening program in Maine. *Obstet Gynecol* 1983;62(5):556.

42. Haddow JE, Knight GJ, Kloza EM, et al. Relation between maternal weight and serum alpha-fetoprotein concentration during the second trimester. *Clin Chem* 1981;27:133-134.

43. Haddow JE, Knight GJ, Kloza EM, Palomaki GE. Alpha-fetoprotein, vaginal bleeding and pregnancy risk. *Br J Obstet Gynaecol* 1986;93:589.

44. Haddow JE, Macri JN, Munson M. The amnion regulates movement of fetally derived alpha-fetoprotein into maternal blood. *J Lab Clin Med* 1979;94:344-347.

45. Haddow JE, Palomaki G, Knight GJ, Kloza, EM, Hitchcock J. *Prenatal Screening for Major Fetal Disorders: The Foundation for Blood Research Handbook.* Foundation for Blood Research, 1990.

46. Haddow JE, Palomaki GE, Knight GJ, et al. Prenatal screening for Down's syndrome with use of maternal serum markers. *N Engl J Med* 1992;327:588-93.

47. Haddow JE, Palomaki GE, Knight GJ. Can low birth weight after elevated maternal serum alpha-fetoprotein levels be explained by maternal weight? *Obstet Gynecol* 1987;70:26-28.

48. Haddow JE. Prenatal screening for open neural tube defects, Down's syndrome, and other major fetal disorders. *Seminars in Perinatology* 1990;14(6):488-503.

49. Hamilton MP, Abdalla HI, Whitfield CR. Significance of raised maternal serum alpha-fetoprotein in singleton pregnancies with normally formed fetuses. *Obstet Gynecol* 1985;65:465-470.

50. Hay DL, Barrie JU, Davison GB, et al. The relation between maternal serum alpha-fetoprotein levels and feto-maternal haemorrhage. *Br J Obstet Gynaecol* 1979;86:516.

51. Hay DL. Placental histology and the production of human choriogonadotropin and its subunits in pregnancy. *Br J Obstet Gynaecol* 1988;95:1268-1275.

52. Johnson AM. Racial differences in maternal serum alpha-fetoprotein screening. In Mizejewski GH, Porter IH (Eds.). *Alpha-fetoprotein and Congenital Disorders*. New York, NY, Academic Press, 1985.

53. Johnson MA, Palomaki GE, Haddow JE. The effect of adjusting maternal serum alpha-fetoprotein levels for maternal weight in pregnancies with fetal open spina bifida: A United States Collaborative Study. *Am J Obstet Gynecol* 1990;163:9-11.

54. Katz VL, Chescheir NC, Cefalo RC. Unexplained elevations of maternal serum alpha-fetoprotein. *Obstet Gynecol Survey* 1990;45(11):719-726.

55. Koontz WL, Seeds JW, Adams NJ, et al. Elevated maternal serum alpha-fetoprotein, second-trimester oligohydramnios, and pregnancy outcome. *Obstet Gynecol* 1983;62(3):301.

56. Lachman E, Hingley SM, Bates G, et al. Detection and measurement of fetomaternal haemorrhage: Serum alpha-fetoprotein and the Kleuhauer technique. *BMJ* 1977;1:1377.

57. Lidbjork G, Kjessler B, Johansson SGO. Alpha-fetoprotein (AFP) in maternal serum and human chorionic gonadatropin (hCG) in urine in 77 patients with vaginal bleeding in early pregnancy. *Acta Obstet Gynecol Scand* 1977;(Suppl.)69:54.

58. Lieppman RE, Williams MA, Cheng EY, et al. An association between elevated levels of human chorionic gonadotropin in the midtrimester and adverse pregnancy outcome. *Am J Obstet Gynecol* 1993;168(6):1852-57.

59. Lindsay P. Making sense of maternal serum screening. *Nursing Times* 1994;90(49):31-32.

60. Loncar J, Barnabei VM, Larson JW. Advent of maternal serum markers for Down syndrome screening. *Obstet Gyn Survey* 1995;50(4):316-320.

61. Los FJ, De Wolf BTHM, Huisjes HJ. Raised maternal serum-alpha-fetoprotein levels and spontaneous fetomaternal transfusion. *Lancet* 1979;2:1210.

62. Los FJ, Debruijn HWA, Van Beek CCT, et al. AFP transport across the fetal membranes in the human. *Prenat Diagn* 1985;139:518-523.

63. Los FJ, Hagenaars AM, Cohen-Overbeek TE, et al. Maternal serum markers in second-trimester oligohydramnios. *Prenat Diagn* 1994;14:565-568.

64. MacDonald ML, Wagner RM, Slotnick RN. Sensitivity and specificity of screening for Down syndrome with AFP, hCG, unconjugated estriol, and maternal age. *Obstet Gynecol* 1991;77:63.

65. Macri JN Krantz DA, Kasturi RV, et al. Maternal serum Down syndrome screening: Free-beta protein is a more effective marker than human chorionic gonadotropin. *Am J Obstet Gynecol* 1990;163:1248.

66. Macri JN, Kasturi RV, Krantz DA, et al. Maternal serum Down screening: Unconjugated estriol is not useful. *Am J Obstet Gynecol* 1990b;162:672.

67. Macri JN, Krantz DA, Kasturi RV, et al. Measurement of unconjugated estriol by enzyme-linked immunosorbant assay fails to show an association with Down syndrome. *Am J Obstet Gynecol* 1990c;162:1634.

68. Macri JN, Weiss RR, Libster B, Cagan MA. Maternal serum-alpha-fetoprotein and low birth-weight. *Lancet* 1978;1:660.

69. Macri JN, Weiss RR, Tillit R, et al. Prenatal diagnosis of neural tube defects. *JAMA* 1976;236(11):1251.

70. Macri JN, Weiss RR. Prenatal serum alpha-fetoprotein screening for neural tube defects. *Obstet Gynecol* 1982;59:633-639.

71. Main DM, Mennuti MT. Neural tube defects: Issues in prenatal diagnosis and counseling. *Obstet Gynecol* 1986;67(1):1.

72. Meis PJ, Ernest JM, Moore ML. Causes of low birth weight in public and private patients. *Am J Obstet Gynecol* 1987;156:1165-1168.

73. Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum α-fetoprotein and fetal chromosomal abnormalities. *Am J Obstet Gynecol* 1984;148:886-94.

74. Milunsky A, Alpert E. Results and benefits of a maternal serum alpha-fetoprotein screening program. *JAMA* 1984;252(11):1438.

75. Milunsky A, Jick SS, Bruell CL, et al. Predictive values, relative risks, and overall benefits of high and low maternal serum alpha-fetoprotein screening in singleton pregnancies: new epidemiologic data. *Am J Obstet Gynecol* 1989;161:291-297.

76. Moore MP, Redman CWG. Case-control study of severe pre-eclampsia of early onset. *BMJ* 1983;287:580.

77. Morssink LP, Kornman LH, Beekhuis JR, et al. Abnormal levels of maternal serum human chorionic gonadotropin and alpha-fetoprotein in the second trimester: relation to fetal weight and preterm delivery. *Prenat Diagn* 1995;15:1041-1046.

78. Muecke LN. Prenatal screening for neural tube defects: a choice for all. *Perspect Biol Med* 1992;36(1):87-96.

79. Muller F, Aegerter P, Boue A. Prospective maternal serum human chorionic gonadotropin screening for the risk of fetal chromosome anomalies and of subsequent fetal and neonatal deaths. *Prenat Diagn* 1993;13:29-43.

80. Mutton DE, Ide R, Alberman E, Bobrow M. Results of first year (1989) of a national register of Down's syndrome in England and Wales. *BMJ* 1993;306:431-32.

81. Nelson LH, Bensen J, Burton BK. Outcomes in patients with unusually high maternal serum alpha-fetoprotein levels. *Am J Obstet Gynecol* 1987;157:572-576.

82. New England Regional Genetics Group Prenatal Collaborative Study of Down Syndrome Screening. Combining maternal serum α-fetoprotein measurements and age to screen for fetal Down syndrome in pregnant women under age 35. *Am J Obstet Gynecol* 1989;160:175-79.

83. Persson PH, Kullander S, Gennser G, et al. Screening for fetal malformations using ultrasound and measurements of alpha-fetoprotein in maternal serum. *BMJ* 1983;286:747.

84. Phillips OP, Elias S, Shulman LP, et al. Maternal serum screening for fetal Down syndrome in women less than 35 years of age using α-fetoprotein, hCG, and unconjugated estriol: a prospective 2-year study. *Obstet Gynecol* 1992;80:353-58.

85. Prenatal detection of neural tube defects. *ACOG Technical Bulletin* December 1986; Number 99.

86. Purdie DW, Young JL, Guthrie KA, Picton CE. Fetal growth achievement and elevated maternal serum alpha-fetoprotein. *Br J Obstet Gynaecol* 1983;90:433.

87. Read AP, Donnai D, Harris R. Comparison of pregnancy outcome after amniocentesis for previous neural tube defect or raised maternal serum alpha-fetoprotein. *Br J Obstet Gynaecol* 1980;87:372-376.

88. Roberts JM, Taylor RN, Friedman SA, Goldfein A. New developments in pre-eclampsia. *Fetal Med Rev* 1990;2:125-141.

89. Robinson L, Grau P, Crandall B. Pregnancy outcomes after increasing maternal serum alpha-fetoprotein levels. *Obstet Gynecol* 1989;74:17-20.

90. Rushton DI. Pathology of the placenta. In Wigglesworth JS, Singer DB (Eds.). *Textbook of Fetal and Perinatal Pathology*, Cambiridge: Blackwell Scientific Publications, 1991.

91. Salafia CM, Silberman C, Herrerra NE, Mahoney MJ. Placental pathology at term associated with elevated midtrimester maternal serum AFP concentration. *Am J Obstet Gynecol* 1988;158:1064.

92. Sanofi Diagnostics Pasteur Inc. *Kallestad AFP/OB Kit* (insert). 1995.

93. SAS Institute Inc. *The SAS System for Windows Version 6.10.* Computer Software. 1994.

94. Schnittger A, Kjessler B. Alpha-fetoprotein screening in obstetric practice and the use of differential action limits. *Acta Obstet Gynecol Scand Suppl* 1984;119:33.

95. Secher NJ, Ericksen PS, Hansen PK, et al. High maternal serum alpha-fetoprotein and low birthweight: A study concerning possible correlation between maternal weight, smoking, and serum alpha-fetoprotein and subsequent birthweight and gestational age. *Am J Perinat* 1985;2(2):78.

96. Seller MJ, Singer JD, Coltart TM, Campbell S. Maternal serum-alpha-fetoprotein levels and prenatal diagnosis of neural tube defects. *Lancet* 1974;1:428.

97. Seppala M, Ruoslahti E. Alpha-fetoprotein in maternal serum: a new marker for detection of fetal distress and intrauterine death. *Am J Obstet Gynecol* 1973;115(1):48.

98. Serono Diagnostics. *hCG Maiaclone Kit* (insert). 1993.

99. Silver RM, Draper ML, Byrne JLB, et al. Unexplained elevations of maternal serum alpha-fetoprotein in women with antiphospholipid antibodies: A harbinger of fetal death. *Obstet Gynecol* 1994;83:150-155.

100. Smith ML. Raised maternal serum alpha-fetoprotein levels and low birth weight babies. *Br J Obstet Gynaecol* 1980;87:1099-1102.

101. Sorenson TK, Williams MA, Zingheim RW, et al. Elevated second-trimester human chorionic gonadotropin and subsequent pregnancy-induced hypertension. *Am J Obstet Gynecol* 1993;169:834-8.

102. Spencer K, Coobes EJ, Mallard AS, et al. Free beta hCG in Down syndrome screening: A multicentre study of its role compared with other biochemical markers. *Ann Clin Biochem* 1992;29:506.

103. Spencer K. Evaluation of an assay of the free beta subunit of choriogonadotropin and its potential value in screening for Down syndrome. *Clin Chem* 1991;37:809.

104. Stiller RJ, Lockwood CJ, Belanger K, et al. Amniotic fluid alpha-fetoprotein concentrations in twin gestations: dependence on placental membrane anatomy. *Am J Obstet Gynecol* 1981;88:231.

105. Stirrat GM, Gough JD, Bullock S, et al. Raised maternal serum AFP, oligohydramnios and poor fetal outcome. *Br J Obstet Gynaecol* 1984;91:296.

106.Sundaram SG, Goldstein PJ, Manimekalai S, Wenk RE. Alpha-fetoprotein and screening markers of congenital disease. *Clin Lab Med* 1992;12(3):481-92.

107.Tanaka M, Natori M, Kohono H, et al. Fetal growth in patients with elevated maternal serum hCG levels. *Obstet Gynecol* 1993;81(3):341-343.

108.United Kingdom collaborative study on alpha-fetoprotein in relation to neural tube defects, second report. Amniotic fluid alpha-fetoprotein measurement in antenatal diagnosis of anencephaly and open spina bifida in early pregnancy. *Lancet* 1979;2:651.

109.Wald NJ, Brock DJH, Bonnar J. Prenatal diagnosis of spina bifida and anencephaly by maternal serum-alpha-fetoprotein measurement. *Lancet* 1974;1:765.

110.Wald NJ, Cuckle HS, Borcham J, et al. The effect of maternal weight on maternal serum alpha-fetoprotein levels. *Br J Obstet Gynaecol* 1981;88:1094-1096.

111.Wald NJ, Cuckle HS, Boreham J, et al. Maternal serum alpha-fetoprotein and diabetes mellitus. *Br J Obstet Gynaecol* 1979;86:101-105.

112.Wald NJ, Cuckle HS, Boreham J, Turnbull AC. Maternal serum alpha-fetoprotein and birth weight. *Br J Obstet Gynaecol* 1980;87:860-863.

113.Wald NJ, Cuckle HS, Densem JW, et al. Maternal serum screening for Down's syndrome in early pregnancy. *BMJ* 1988;297:883-87.

114.Wald NJ, Cuckle HS, Stirrat GM, et al. Maternal serum-alpha-fetoprotein and low birth-weight. *Lancet* 1977;2:268.

115.Wald NJ, Cuckle HS. Biochemical Screening. In: Brock DJ, Rodeck CH, Ferguson-Smithe MA (Eds.). *Prenatal Diagnosis and Screening*. London: Churchill Livingston, 1992.

116.Wald NJ, Cuckle HS. Maternal serum-alpha-fetoprotein in antenatal screening for anencephaly and spina bifida in early pregnancy, report of the U.K. Collaborative study on alpha-fetoprotein in relation to neural-tube defects. *Lancet* 1977;1:1323-1332.

117.Wald NJ, Cuckle HS. Recent advances in screening for neural tube defects and Down's syndrome. *Bailliere Clin Obstet Gynaecol* 1987;1:649.

118.Wald NJ, Densem JW, Smith D, Klee GG. Four-marker serum screening for Down's syndrome. *Prenat Diagn* 1994;14:707-716.

119.Wald NJ, Kennard A, Densem JW, et al. Antenatal maternal serum screening for Down's syndrome: results of a demonstration project. *BMJ* 1992;305:391-94.

120.Waller DK, Lustig LS, Cunningham GC, et al. Second trimester serum alpha-fetoprotein levels and the risk of subsequent fetal death. *N Engl J Med* 1991;325:6-10.

121.Waller DK, Lustig LS, Smith AH, Hook EB. Alpha-fetoprotein: a biomarker for pregnancy outcome. *Epidemiology* 1993;4:471-476.

122.Walters BNJ, Lao T, Smith V, De Swiet M. Alpha-fetoprotein elevation and proteinuric pre-eclampsia. *Br J Obstet Gynaecol* 1985;92:341.

123.Walters CP. Poor pregnancy outcomes associated with elevated maternal serum alpha-fetoprotein in combination with increased risk for Down syndrome. *Prenat Diagn* 1993;13:221-222.

124.Wenstrom KD, Owen J, Boots LR, DuBard MB. Elevated second-trimester human chorionic gonadatropin levels in association with poor pregnancy outcome. *Am J Obstet Gynecol* 1994;171:1038-41.

125.Williams RL, Creasy RK, Cunningham GC, et al. Fetal growth and perinatal viability in California. *Obstet Gynecol.* 1982;59(5):624-632.

126.Youings S, Gregson N, Jacobs P. The efficacy of maternal age screening for Down's syndrome in Wessex. *Prenat Diagn* 1991;11:419-25.