AN INVESTIGATION OF MATERNAL SERUM PREGNANCY PROTEIN MARKERS IN THE FIRST TRIMESTER

Submitted by

Gary McCaw

in

July 1993

to

The University of Glasgow

for the degree of

Master of Science

Research undertaken at the Duncan Guthrie Institute of Medical Genetics at Yorkhill Hospital, Glasgow

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

"Old houses were scaffolding once

and workmen whistling"

T. E. Hulme

"Parturient montes, nascetur ridiculus mus"

Horace

(Ars Poetica, 139)

CONTENTS

(over)

Contents
========

Acknowledgements

My thanks go to Prof. J Michael Connor for the opportunity to undertake this research project.

Also to Dr. David A Aitken for his supervision and advice.

To Jenny A Crossley for generous (unsolicited!) help and suggestions.

To Elspeth Gracey, Jenny and Esther Berry for covering my routine work during writing up.

To David Farquharson for putting up with my moods and "petulent frenzies" and for making me smile.

To Glasgow University for relieving me of the burden of renewing my passport for the past three years.

To William Coyle for my weekly "therapy" sessions without which none of this would have been probable!

And lastly to Caroline for.... everything else!!

Abbreviations

AFP	- Alphafoetoprotein
EPPS	- Hydroxyethyl Piperazine Propane Sulphonic acid
EQAS	- External Quality Assurance Scheme
FBHCG	- Free-B Human Chorionic Gonadotrophin
esp.	- Especially
g/L	- Grams per Litre
HCG	- Human Chorionic Gonadotrophin
hFSH	- Human Follicle Stimulation Hormone
hLH	- Human Luteinising Hormone
hTSH	- Human Thyroid Stimulation Hormone
IQ	- Intelligence Quotient
IU/ml	- International Units per Millilitre
IRMA	- Immuno-Radiometric Assay
KU/L	- Kilo Units per Litre
L	- Litre
LMP	- Last Menstrual Period
min.	- Minutes
mIU/ml	- Milli-International Units per millilitre
ml	- Millilitres
MoM	- Multiples of the Median
msAFP	- Maternal serum AFP
msHCG	- Maternal serum HCG
msUE3	- Maternal serum UE3
NaCl	- Sodium Chloride
NaN_3	- Sodium Azide
nmol	- Nanomoles
nmol/L	- Nanomoles per Litre
NTD	- Neural Tube Defect
RIA	- Radio-Immuno Assay
rpm	- Revolutions per minute
UE3	- Unconjugated Oestriol
ul	- Microlitres

Summary

It is well established that abnormally high levels of maternal serum alpha-foeto protein (msAFP) are associated, in the second trimester, with pregnancies affected by open neural tube defects (NTDs). More recently, low levels of msAFP, low levels of unconjugated oestriol (UE3), and high levels of human chorionic gonadotrophin (HCG) have been associated with trisomy 21 pregnancies and this, in conjunction with the known correlation between trisomy 21 and maternal age risk, has been used as the basis for population screening in the second trimester.

However, screening at this stage of pregnancy carries the inherent disadvantages of late diagnosis and thus late termination of affected pregnancies. This study set out to investigate the levels of the above three analytes (AFP, HCG, UE3), in maternal serum sampled from pregnancies in the first trimester, to determine their potential as detectors of trisomy 21 and NTDs at this stage of foetal development.

Between January 1987 and April 1990 a series of 14,000 serum samples were prospectively collected from women between 6 and 15 weeks pregnant who were routinely attending three local hospitals for ante-natal care. Upon receiving the samples, the clotted red blood cells were spun down and the serum separated into two aliquots, one for immediate use and one to be kept in frozen storage. Each sample was

allocated a unique number and this was entered into a computer database system along with the patient's details. The computer inputs these patient numbers into a daily worksheet and they were then assayed for AFP along with the routine second trimester samples. The HCG and UE3 assays were carried out separately and at a later date on the stored frozen samples. Within this series 16 trisomy 21, 5 trisomy 18, 1 trisomy 13 and 14 open NTD pregnancies were identified and the serum samples isolated for later use. In addition to these there was a twin pregnancy, in which one foetus was affected with spina bifida and one unaffected, and another pregnancy in which the foetus was affected by both spina bifida and gastroschisis. From the total series of samples a set of 632 controls was selected in order to determine median values for each gestational age. The sample size for each gestation was as follows: 14 at 6 weeks, 45 at 7 weeks, 69 at 8 weeks, 71 at 9 weeks, 68 at 10 weeks, 69 at 11 weeks, 69 at 12 weeks, 68 at 13 weeks, 68 at 14 weeks, and 69 at 15 weeks.

The respective levels of the three analytes studied were determined by Immuno-Radiometric assays (IRMA), in the cases of AFP and HCG, and by Radio-Immuno assay (RIA) for UE3. AFP levels were assayed using an in-house method which employs a two-site IRMA with Iodine-125 labelled monoclonal anti-AFP and polyclonal anti-AFP Sepharose solid phase. Separation is achieved by bead settlement in a sucrose density gradient.

The HCG levels were assayed by use of a commercial kit, supplied by Serono. This uses three monoclonal antibodies to HCG, two of which are labelled with Iodine-125. The third antibody is labelled with fluoroscein and binds to the B-subunit. Separation is achieved by use of a sheep antiserum to fluoroscein coupled to a magnetic solid phase which binds to the HCG-monoclonal antibody complex. Due to the sensitivity of this assay it was necessary to dilute the sera 1:500 with horse serum.

The UE3 RIA was also a commercial kit, supplied by Amersham. Because this kit was designed for the second trimester it was necessary to adapt this assay to increase its sensitivity and thus render it useful as a first trimester test. After several trials optimum results were obtained by using the supplied standards at 20% with the sample sera and all other reagents at 100% volume. The RIA depends on competition between oestriol in the serum and Iodine-125 labelled oestriol for a limited number of binding sites on oestriol specific rabbit antibody. Separation is again by magnetic polymer particles.

For each of the three analytes, the concentration varies with gestation. As the gestational age increases the AFP level rises steadily from about 1.4 KU/L at 6 weeks to 5.84 KU/L at 10 weeks and to 23.67 KU/L at 15 weeks. HCG levels rise rapidly to a peak of 98 IU/ml at 8 weeks and fall again, levelling off to around 20 IU/ml at about 20 weeks. UE3 levels increase slowly at first to about 0.46 nmol/L

then, at around 11 weeks, rise more steeply as gestation progresses.

The sera from the 36 abnormal pregnancies were then assayed and the results converted into multiples of the median (MoM) for each analyte at the stated gestation for that pregnancy. In the group of 16 trisomy 21 pregnancies the median MoM values for each analyte were as follows:

AFP 0.65 ($p<0.02$), HCG 0.97 ($p<0.52$), UE3 0.67 ($p<0.01$)

In the group of 5 trisomy 18 pregnancies median MoMs for each analyte were: AFP 0.71, HCG 0.27, UE3 0.34

The median maternal age for these autosomal trisomies was 29 years, ranging from 21 to 43 years of age.

None of the 14 pregnancies with open NTDs had msAFP levels which were considered to be elevated in the first trimester sampling, that is > 2.0 MoMs for the stated gestation. However when compared to their matched samples all 14 cases had significantly raised msAFP levels in the second trimester. From these results it is clear that biochemical screening for NTDs in the first trimester is not reliable using these analytes and a separate program to screen for NTDs in the second trimester will continue to be necessary.

With regard to Down's syndrome pregnancies, in this study, intact HCG varied little from the median value in trisomy 21 pregnancies in the first trimester suggesting that it may not be useful in a screening program. It can also be seen that the median MoMs for AFP and UE3 in this group are virtually identical and significantly reduced. However,

there is some correlation between these markers (Correlation Coefficient r=0.22) indicating that they cannot be used as independent measures of risk in the first trimester. However, more recent studies indicate that the use of a free β-HCG assay, rather than intact HCG, greatly improves first trimester screening efficiency having a median MoM of 1.96 in trisomy 21 pregnancies. Thus, using a combination of AFP, or UE3, free β-HCG and maternal age, screening for chromosomal abnormalities at any gestation from 7 to 20 weeks may be possible.

1. Introduction

1.1

Since modern medical and nursing care have greatly reduced perinatal mortality and morbidity, genetically determined diseases and congenital malformations have become increasingly important factors of ill health in the community. It is therefore natural that those diseases with the highest birth incidences and severest pathologies have been the ones studied with the greatest interest. Amongst these, and of specific interest here, are the neural tube defects (NTDs) and chromosome abnormalities, particularly trisomy 21 (Down's syndrome).

1.2 Neural Tube Defects

Neural tube defects have a relatively high birth incidence albeit with wide geographical variations. Typically quoted as 1-3/1000 (Connor and Ferguson-Smith, 1992a), NTDs principally comprise anencephaly (failure of the anterior neuropore to close) and spina bifida (failure of neural tube closure further down). The two conditions are equally frequent and coexist in approximately 20% of cases. Other malformations, especially exomphalos, may also coexist. Several sub-divisions of spina bifida are made but of particular interest here is the distinction between "open"

and "closed" lesions. In 15-20% of cases an intact layer of opaque tissue covers the lesion and are thus termed closed lesions. These tend to cause less neurological disability than open lesions where there is little or no covering tissue. The majority of cases, however, are open NTDs where the lesion is exposed and foetal metabolites can pass directly into the amniotic fluid from exposed capillaries and may even enter the maternal circulation. Thus only the open lesions are detectable biochemically.

Work done in the early 1970s (Brock & Sutcliffe, 1972; Leek et al, 1973; Brock et al, 1973; Seller et al, 1974; Harris et al, 1974; Wald et al, 1974; Brock et al, 1974) indicated an association between, initially, high levels of amniotic fluid alphafetoprotein (AFP) and NTDs and, subsequently, high levels of maternal serum AFP (msAFP) and NTDs. However, the relatively small sample sizes of these studies led to the setting up of the first UK Collaborative Study in January 1975, reporting in 1977 (Report of UK Collaborative Study on Alphafetoprotein in Relation to Neural Tube Defects, 1977). This large study, of some 18,684 unaffected pregnancies and 301 NTD pregnancies, confirmed the association between high msAFP and NTD pregnancies, further concluding that the optimum serum sampling time was between 16-18 weeks gestation. The study also recommended the use of multiples of the normal median (MoMs), as opposed to percentiles, and suggested a cut-off level of > 2.5 MoM.

It had already been clear by the early 1970s that, due to the overlapping distributions of msAFP from affected and unaffected pregnancies (Fig. 1), msAFP measurement would not be a useful diagnostic test but that it could form the basis of an effective screening program. This has proved to be the case, the first ante-natal serum screening programs run for NTDs were established in the mid-1970s and such screening is now widely available throughout the UK. A blood sample is taken from the pregnant woman, usually at 16 or 17 weeks gestation, and assayed for AFP. Should the AFP be above a certain cut-off level (greater than 2.0 MoM in the west of Scotland) a second sample is requested and if this, also, indicates a high risk of an NTD then a detailed ultrasound scan and amniocentesis may be recommended. The amniotic fluid sample is assayed for AFP by immunoelectrophoresis (Brock & Sutcliffe, 1972) and, following other findings in relation to NTDs, also assayed for acetylcholinesterase to obtain a diagnostic result (Smith et al, 1979). These screening programs typically identify 100% of cases of anencephaly and 88% of open spina bifida. (cf. amniocentesis which identifies 100% of anencephalics and more than 98% of open spina bifidas, Ferguson-Smith, 1983). In the west of Scotland, screening 75-80% of the pregnant population, this program has resulted in a 74% reduction of NTD births in the region (Ferguson-Smith, 1983).

Further studies of analytes other than AFP, such as human

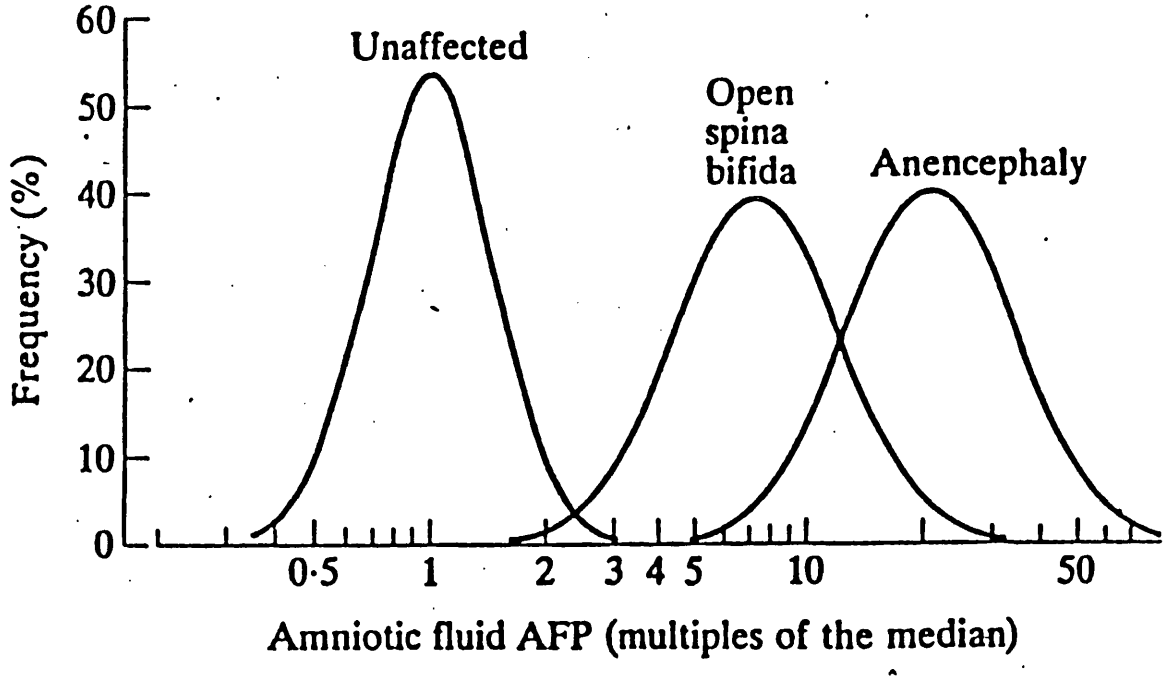

Figure 1. Overlap in the Distributions of AFP in Normal Pregnancies and those affected by NTDs.

chorionic gonadotrophin (HCG) and unconjugated oestriol (UE3), have been carried out in an attempt to improve screening efficiency (Canick et al, 1990). These indicated no significant changes in spina bifida pregnancies for either HCG or UE3 though both analytes were below the median level in anencephalic pregnancies, this being especially low for UE3 with a median MoM of 0.17.

1.3 Down's Syndrome

Trisomy 21, or Down's syndrome, was the first chromosome abnormality identified in humans (Lejeune et al, 1959) and was also the first to be prenatally diagnosed, by amniocentesis, in 1969 (Connor and Ferguson-Smith, 1992b). Although over 600 chromosomal diseases are now known, trisomy 21 is still the most common with a birth incidence of 1.3/1000 (Valenti et al, 1968). It is the single commonest cause of mental retardation in newborns and is responsible for about 25% of all moderate to severe mental handicap in children of school age. The IQ is usually less than 50 and other complications may include congenital heart malformations, epilepsy, hypothyroidism, cataracts and leukaemia. Puberty is often delayed and incomplete and presenile dementia common after 40 years of age.

An association between an increased risk of a Down's syndrome pregnancy and increasing maternal age has been known since the early 1930's (Penrose, 1933; Jenkins,

1933). This increases from a birth risk of 1:1500 at 20 years of age to 1:1000 at 29, 1:380 at 35, 1:112 at 40 and about 1:30 at 45. However, although women 35 years and over have a significantly greater risk they only account for about 30% of Down's births because of the vast bulk of the pregnant population (over 90%) are under this age. Therefore offering amniocentesis to all women over 35 years of age could detect a maximum of 30% of Down's pregnancies. Clearly, due to the slight risk of foetal mortality associated with amniocentesis, (0.5-1.0%), this could not be routinely offered to all women under 35. Thus some method of screening the pregnant population to identify women at an increased risk of a Down's pregnancy was desirable.

Due to the serendipitous persistence of a woman pregnant with what turned out to be a trisomy 18 (Edward's syndrome) infant, Merkatz and his colleagues (in 1984) conducted a retrospective study of amniotic fluid and msAFP levels in pregnancies associated with prenatally diagnosed chromosome abnormalities. This data was first presented in 1983 and published in 1984 (Merkatz et al, 1984) concluding that, while no special pattern emerged for trisomic pregnancies when looking at amniotic fluid, there was a significant association between such pregnancies and low msAFP. Many subsequent studies (Cuckle and Wald, 1984; Fuhrmann et al, 1984; Tabor et al, 1984; Cowchock and Ruch, 1984; Seller, 1984; Spencer and Carpenter, 1985; Murday and Slack, 1985; Hershey et al, 1985; Doran et al, 1986; Brambati et al,

1986; Bogart et al, 1987; Canick et al, 1988) confirmed this association indicating a typical value of 0.72 multiples of the normal median (MoM) for Down's syndrome pregnancies. This and other work (esp. Cuckle et al, 1987) suggested that the current AFP screening programs for NTDs could, in conjunction with known maternal age specific risk data (Ferguson-Smith & Yates, 1984), be adapted to screen for aneuploidies. This was first done in the UK in the 1980s and was introduced in the West of Scotland in 1987. These screening programs typically identify up to 40% of Down's syndrome pregnancies, with a 6-7% false positive rate, compared with up to 30% detection and a 6.7% false positive rate by maternal age alone.

With a view to increasing both sensitivity and specificity a whole range of other maternal serum pregnancy protein markers have been studied in recent years. Chief amongst these analytes have been HCG, (Bogart et al, 1987), and UE3, (Canick et al, 1988).

It has been known since the mid-1960's that low levels of HCG in early pregnancies is predictive of an abnormal outcome, commonly a spontaneous abortion (Brody and Carlstrom, 1965). It was thus a prime candidate for investigation and indeed the value of HCG in improving prenatal screening has been confirmed by several studies (Wald et al,1988a; Bogart et al,1989; Crossley et al, 1991a). Wald et al (1988a) in a study of serum samples from 77 Down's syndrome pregnancies with 385 normal controls,

indicated that high levels of maternal serum HCG (msHCG) were associated with trisomy 21 pregnancies. The level for the median HCG concentration in such pregnancies was 2.04 MoM. This and another large study, of 7815 unaffected pregnancies and 15 Down's syndrome pregnancies (Crossley et al, 1991), indicated that the integration of HCG into a screening program using AFP and maternal age risk could provide a detection rate of over 60% with a false positive rate of 5.6%.

UE3 has also been shown to be useful (Canick et al, 1988; Wald et al, 1988b) having a median value of 0.73 MoM in Down's syndrome pregnancies and in combination with maternal age data and AFP yields a detection rate of 45% with a false positive rate of 5.2%. These analytes have now been integrated into many screening programs, either HCG singly or HCG and UE3 together, and in conjunction with AFP and maternal age data can provide a Down's detection rate of upwards of 60% with a 5% false positive rate, though the value of including UE3 in such programs has been questioned (Macri et al, 1990a).

As stated above, several other analytes have been examined such as pregnancy-specific B1 glycoprotein (SP-1) (Bogart, 1987,1989; Bartles and Lindemann, 1988; Knight et al, 1989; Graham et al, 1992), pregnancy associated plasma protein-A (Stabile et al, 1988), human placental lactogen (hPL) and progesterone (Knight et al, 1989), and free-β-HCG (FBHCG) (Macri et al, 1990b). These have met with varying degrees

of success and work is ongoing. It remains the case, however, that AFP, HCG and UE3 are the main focus of interest and as they are the three analytes which were examined in this present study they will be considered in more detail.

1.4 Alphafetoprotein

First identified by Bergstrand and Czar in 1956, human AFP is a glycoprotein with a molecular weight of about 65,000 and a single polypeptide chain of 590 amino acids. Though no conclusive evidence has yet been produced, since it is closely linked to albumen, it has been speculated that it functions as a transport protein (Ruoslahti and Hirai, 1978). While the early source of AFP is almost certainly in the yolk sac, production later shifts to the foetal liver though some evidence exists of synthesis in the gastro-intestinal tract (Gitlin, 1975) . Production of AFP in the foetus begins with the development of the yolk sac at around 4-6 weeks and the foetal serum concentration peaks at about 13 weeks gestation (Gitlin, 1975). In normal, non-pregnant adults the serum concentration is between 2-20 ng/ml, corresponding to about 1.6-16 KU/L (Gitlin and Boesmann, 1966; Talermann et al, 1978). Maternal serum AFP rises from normal adult levels at about 4-6 weeks gestation, peaking at between 200-400 KU/L around 25 weeks (Gitlin, 1975).

1.5 Human Chorionic Gonadotrophin

HCG is a sialoglycoprotein with a molecular weight of about 40,000. It is composed of two sub-units, termed α and β, the larger α sub-unit being virtually identical to that of the pituitary hormones human luteinising hormone (hLH), human follicle stimulating hormone (hFSH), and human thyroid stimulating hormone (hTSH). Variation in biological activity is determined by differences in the β sub-unit and it is these which allow the molecules to be immunologically distinguished (Rothfield et al, 1974). The site of HCG production is the placental syncytio-trophoblast where it is produced in large quantities and functions in early pregnancy to sustain the corpus luteum beyond its normal lifetime, ensuring as a result the continued production of oestrogen and progesterone which help to maintain the endometrium, thus allowing pregnancy to proceed (Lunenfeld and Insber, 1978). In later pregnancy the placenta can produce adequate amounts of the steroid hormones itself and HCG production is reduced. HCG also serves to stimulate the production of testosterone by the testes of the foetal male necessary for normal masculinisation. It has further been proposed that placental secretion of HCG helps to protect the foetus from the maternal immune system (Adock et al, 1973).

Detectable amounts of HCG may be found in the maternal serum and urine very soon after conception with levels

rising to a peak during the third month of gestation. Levels then fall steadily until about 20 weeks and then remain fairly constant until term.

The actual values range from about 50 mIU/ml after the first week's gestation to a peak of between 30 and 200 IU/ml at 12 weeks. This falls to around 15 IU/ml at 20 weeks and persists at this value for the duration of pregnancy (Vaitukaitus et al, 1977).

1.6 Unconjugated Oestriol

Oestriol belongs to the oestrogens, which are one of the five major classes of steroid hormones derived from cholesterol, and is produced predominantly in the foeto-placental unit (Diczfalusy and Mancuso, 1969). Hydroxylated steroid intermediates, formed mainly by the foetal adrenals (Frandsen and Stakemann, 1964; Siiteri and MacDonald, 1966; Adlercreutz and Luukkainen, 1970), are metabolised to oestriol by the syncytio-trophoblast cells of the placenta and are conjugated in the maternal liver to form glucuronides and sulphates (Lauritzen, 1971). Thus oestriol exists in the blood as a mixture of the unconjugated form and several conjugates. The level of UE3 in the maternal serum increases slowly through the first trimester, then rapidly after that. Oestriol in the maternal blood has a half-life of only 20-30 minutes, thus variations in foetal-placental production should be rapidly reflected

by changes in maternal serum levels.

1.7 First Trimester Screening

All the above work on screening for foetal neural tube defects and chromosome abnormalities has focussed on analytes in the second trimester, especially from 16-20 weeks gestation following the recommendation of 16-18 weeks as optimum for NTD detection in the 1977 UK Collaborative Study. However, any screening at this stage of pregnancy carries the intrinsic disadvantages of late diagnosis and consequent late termination of affected pregnancies . This has led to considerable interest in the levels of marker analytes in the first trimester. Several studies as early as the mid-1980's (Brambati et al, 1986; Barkai et al, 1987) reported low msAFP in first trimester Down's pregnancies. These had a low sample size, however, and a larger study, looking at AFP, HCG and $UE3_f$ was carried out by Cuckle et al, 1988. This took samples from 22 affected Down's pregnancies, at gestations from 7-12 weeks, each being matched with 5 samples from unaffected pregnancies at the same gestation. The study showed lowered median values of 0.35 MoM for UE3 and 0.72 MoM for AFP. No significant change in HCG levels was reported. This study also recommended that further investigations on a larger scale be undertaken.

A significant problem with much of the above work, valuable though it was, is, as was suggested by Cuckle et al

in their 1988 study, the relatively small sample sizes. In addition, the majority of the samples had been obtained from patients prior to first trimester chorionic villus sampling procedures where the indication for this sampling was advanced maternal age and they are thus not truly representative of the screened population. Further, all looked at Down's syndrome exclusively, none including any NTDs. It was with a view to contributing to the data available that this study was undertaken.

1.8 Aims

Four principle objectives were identified:

(i) To optimise the available AFP, HCG and UE3 assays for first trimester maternal serum samples.

(ii) To establish normal ranges for AFP, HCG and UE3 at each week of gestation to be studied.

(iii) To investigate the levels of AFP, HCG and UE3 in maternal serum from known trisomy 21 pregnancies from an unselected pregnant population for the detection of Down's syndrome in the first trimester.

(iv) To investigate the levels of maternal serum AFP, HCG and UE3 in pregnancies affected by neural tube defects from an unselected pregnant population in the first trimester.

2. Materials and Methods.

2.1 Maternal Serum Samples

Between January 1987 and April 1990 maternal serum samples were prospectively collected from 14,000 pregnant women at between 6-15 weeks gestation who were routinely attending ante-natal clinics in the West of Scotland. From each sample an aliquot was used for routine maternal serum alphafoetoprotein (msAFP) screening, the remainder being stored at $-20^{\circ}C$ for later use. Between July 1989 and April 1990 first trimester serum samples from three of these local clinics were also routinely assayed for human chorionic gonadotrophin (HCG).

Three methods of assessing gestational age are routinely used:

1. From the date of last menstrual period (LMP).
2. Estimation by clinical examination.
3. From the bi-parietal diameter assessed by ultrasonic scan.

Where estimates of gestation by the different methods varied, the following hierarchy was used: if the date of the last menstrual period was certain then gestation was calculated on that basis; if uncertain, or unknown, then estimation by ultrascan was used; if an ultrascan had not been carried out then gestation estimation by uncertain dates was used and failing that, by clinical examination.

Where assessment by ultrascan differed by two or more weeks from estimation by dates, even if certain, then the ultrascan estimate was used. For the majority of patient samples used as controls,the different methods of measuring gestational age concurred. Within this series of 14,000 samples, 16 trisomy 21, 5 trisomy 18, 1 trisomy 13 and 14 open NTD pregnancies were identified and the sera isolated and frozen at -20 C for later use. From the total series a set of 632 controls were selected in order to determine median values for each gestational age. The sample size for each gestation was as follows:
14 at 6 weeks, 45 at 7 weeks, 69 at 8 weeks, 71 at 9 weeks, 68 at 10 weeks, 69 at 11 weeks, 69 at 12 weeks, 68 at 13 weeks, 68 at 14 weeks, 69 at 15 weeks.

Patient details and assay results were stored in a computer database though no data analysis was undertaken at this stage. Most patients with continuing, viable pregnancies returned for routine serum screening in their second trimester and this further data was entered into the database. The outcomes of the above pregnancies were then awaited and the clinical results of any abnormal births recorded, these again being entered into the database.

2.2 Immunoassay Methodology

Two assay types were used in the determination of the

levels of the three analytes that were studied: a Radio-Immuno assay (RIA) in the case of Unconjugated Oestriol (UE3), and Immuno-Radiometric assays (IRMA) in the cases of AFP and HCG.

RIAs have rapidly proliferated in range and type since the first publication by Yalow and Berson in 1960, offering as they do the advantages of sensitivity, specificity and simplicity. RIAs and IRMAs utilise antibody adsorbed onto a solid-phase. However, the ways in which this reagent is used is quite different for the two assay types.

In RIAs a limited and constant concentration of antibody is reacted with a much larger, varying amount of antigen in the presence of a fixed and constant amount of radio-labelled antigen which competes with the native antigen for binding sites on the antibody. Thus the amount of radio-labelled antigen which is bound to the antibody / solid-phase complex varies inversely to the original total concentration of antigen. That is, the more radioactivity measured at the end-point, the less antigen there was present in the original sample.

With IRMAs, however, the varying concentrations of antigen are reacted with a constant but, this time, 'excess' amount of radio-labelled antibody. Therefore, after equilibrium is reached, the amount of bound antigen is directly proportional to the original concentration of the antigen. That is, the more radioactivity measured at the end-point, the more antigen there was present initially.

Both RIAs and IRMAs discriminate between the bound and unbound fractions by use of an appropriate antibody which is adsorbed onto a solid-phase. This is used to produce a precipitate, containing the bound fraction, and a supernatant containing the unbound fraction which is discarded.

2.3.1 Alphafetoprotein Assay

The assay used for both this first trimester study and the routine second trimester samples was an in-house IRMA method (Stevenson et al, 1987). This assay has been in constant use for routine ante-natal screening in the west of Scotland area since 1985 and the laboratory participates in the UK External Quality Assurance Scheme (EQAS).

Two antibodies to AFP are employed in this assay, one monoclonal and one polyclonal. The monoclonal anti-AFP is radio-labelled with Iodine-125 while the polyclonal antibody is attached to a Sepharose solid-phase. Separation of the bound fraction is achieved by bead settlement, under gravity, in a sucrose density gradient.

2.3.2 Alphafoetoprotein Assay Methodology

25 ul aliquots of serum are diluted with 200 ul of assay buffer (EPPS, Tween 20, Na N_3). This is carried out in duplicate for each sample, standard and quality control. In

each assay there are 10 standards, 0-9, 5 quality controls at the begining and end of the assay, and a drift control every 15 samples (see Table 1 for values and assay layout). To this is added 200 ul of tracer mix, (270ul radio-labelled anti-AFP, 14 ml anti-AFP solid-phase, 40ml assay buffer, and 1.2 ml sheep serum per 240 tube assay). The assay tubes are incubated at room temperature for 2 hours on an orbital shaker at about 300rpm. 1 ml of isotonic saline wash, (0.9% NaCl, Tween 20), is then added to each tube. A peristaltic pump and inspiration manifold are then used to add 3 ml of concentrated sucrose solution (100g/L, Tween 20) to each tube FROM THE BOTTOM UP. In this way the bound fraction is floated to the top of the tube whence the Sepharose beads are left to settle for one hour.

At the end of this time an aspiration manifold, attached to an electric vacuum pump, is used to suck off the upper layers containing the unbound fraction. (Both the inspiration and aspiration manifolds deal with 40 tubes at a time).The bound fraction is then resuspended with another 1ml of saline wash, to reduce non-specific binding, and again floated on 3 ml of the sucrose solution. After a further one hour settlement any remaining unbound fraction is removed as before.

Having been previously mounted in the appropriate racks, the assay tubes are transferred to a Packard Cobra gamma multi-counter which counts 10 tubes simultaneously. The raw counts (1 min./tube) are stored to floppy-disk and the

Assay Tube Nos.	Tube Contents	Values (KU/L)
1-2	Standard 0	0.00
3-4	" 1	0.94
5-6	" 2	1.76
7-8	" 3	5.29
9-10	" 4	10.50
11-12	" 5	29.70
13-14	" 6	61.60
15-16	" 7	126.00
17-18	" 8	245.00
19-20	" 9	511.00
21-22	Qual. Cont. 1	5.30
23-24	" " 2	16.80
25-26	" " 3	79.40
27-28	" " 4	275.00
29-30	" " 5	BLANK
31-32	Drift control	115.00
33-34	Sample	
"	"	
"	"	
61-62	Drift Control	
	(And again after every 14 samples)	
"		
231-232	Qual. Cont. 1	
233-234	" " 2	
235-236	" " 3	
237-238	" " 4	
239-240	" " 5	

Table 1: AFP Assay Layout and Standard Values.

data then processed with the SASPRO calculation package which uses the 4-parameter mass action model (Edwards and Ekins, 1983). These results are then transmitted downline to a DEC PDP 11/84 mainframe computer and entered into the database.

2.4.1 Human Chorionic Gonadotrophin Assay

The assay type used, as for AFP, was an IRMA. A proprietary kit was used, that being the HCG MAIA Clone kit, supplied by SERONO Diagnostics. This measures intact HCG, not the dissociated subunits. It employs THREE high affinity monoclonal antibodies, two of which are radio-labelled with Iodine-125 and the third labelled with fluoroscein. These bind to unique sites on both subunits of the intact molecule. After a short incubation, separation is achieved by addition of a sheep antisera to fluoroscein which is coupled to a magnetic solid-phase thus enabling a rapid and consistant sedimentation of the bound fraction.

2.4.2 Human Chorionic Gonadotrophin Assay Methodology

Using solely the reagents supplied with this kit the highest concentration of HCG measurable without dilution is 500 mIU/ml. Thus, after preliminary investigation, a 1:500 dilution was carried out on all patient samples and internal quality controls. This was done using horse serum with 0.2%

Sodium Azide as an antibacterial agent. The dilutions were carried out in two steps: 10 ul of sample is added to 190 ul of horse serum (1:20); after vortex mixing, 10 ul of this dilution is added to 240 ul of horse serum (1:25) and again vortex mixed, giving a total dilution of 1:500. The kit standards, were used at the concentrations supplied and the sample results derived were scaled up accordingly. Quality controls, in addition to the basic positive and negative controls supplied with the kit, were made up using pooled maternal sera of known gestation which were then aliquoted and frozen for storage at -20°C. (see Table 2 for assay values and layout)

Next, 25 ul of diluted samples, controls and standards are dispensed, in duplicate, into reaction tubes. To all of these is added 500 ul of the radio-labelled anti-HCG. The tubes are vortex mixed then incubated in a water bath at 37°C for 15 min. After this time 200 ul of magnetic anti--fluoroscein solid-phase is added, the tubes vortexed and incubated at room temperature for a further 5 min. They are then transferred to magnetised racks whence the solid-phase/ HCG/ antibody complex precipitates out after a minute or so. To reduce non-specific binding the supernatant is drained off and 500 ul of wash buffer (TRIS) is added, the tubes again vortexed and the separation step repeated. After again discarding the supernatant the tubes are left to drain for about 10 mins.

The tubes are then removed to a gamma multi-

Assay Tube Nos.	Tube Contents	Values (IU/ml)
1-2	Standard 0	0
3-4	" 1	5
5-6	" 2	10
7-8	" 3	25
9-10	" 4	100
11-12	" 5	250
13-14	" 6	500
15-16	Serono +ve.	26
17-18	Qual. Cont. 1	75*
19-20	" " 2	12*
21-22	Drift Control	295
23-24	Sample	
"	"	
"	"	
53-54	Drift Control	295
"	Sample	
"	"	
85-86	Drift Control	295
"	Sample	
"	"	
117-118	Qual. Cont. 1	75
119-120	" " 2	12

* Qual. Cont. 1 + 2: These two quality controls were made up from pooled 9 week and 17 week maternal serum respectively. The serum was first pooled, then aliquoted and frozen. The values given are thus mean values.

Table 2: HCG Assay Layout with Standard Values.

-counter which counts 10 tubes simultaneously, each one for two mins. The raw counts, standard curve and results were recorded onto the computer print-out and later typed into a database/ statistics package (SPSS/PC+) for subsequent analysis.

2.5.1 Unconjugated Oestriol Assay

Unlike the AFP and HCG IRMAs, an RIA method was employed here. Again this was a commercial kit, the "Amerlex-M 2nd. Trimester Unconjugated Estriol RIA Kit" manufactured by Amerlite Diagnotics Ltd. Being specifically a second trimester kit it was necessary to carry out an extensive preliminary investigation into increasing the sensitivity of the kit to suit first trimester levels.

This RIA method depends on competition between UE3 in the serum and Iodine-125 labelled oestriol for a limited number of binding sites on a rabbit anti-oestriol. The proportion of the radio-labelled bound fraction is inversely related to the concentration of oestriol in the serum sample. Separation of this bound fraction is achieved by use of a magnetic solid-phase to which a further antibody is attached.

2.5.2 Adaptation of the Unconjugated Oestriol Assay Kit

Several experiments were carried out to try and improve

the assay sensitivity. These trials were all done using the same set of early gestation samples, randomly selected from the pool and covered most of the range of gestations studied (Samples at 6 and 7 weeks gestation could not be used due to shortage of numbers).

2.5.3

The first assay variation was simply to use the standards and reagents at 1/2 normal volumes while retaining full volumes for the sample sera. This proved very unsatisfactory, however, due to the volume specific reaction dynamics of RIAs.

2.5.4

The next trial used the assay standards at 20% volume with all assay reagents at full volume. Sample sera were used at both 20% and 50% of normal in an attempt to conserve the limited supplies of sera. This again gave inconsistant results.

2.5.5

The third trial again used standards at 20% but made up to 50% of normal volume with pooled male human serum. Again, sample sera were tried at 20% and 50% of normal. This method yielded improved results though again with insufficient consistancy.

2.5.6

The next trial again ran standards 20% but this time made up to 100% volume with pooled male human serum. All reagents and sample sera were used at full volumes. This proved the most satisfactory and consistant variant. Final sample values were obtained by using this method and multiplying all results by 0.2. Unfortunately, having to use full volume aliquots (100ul) of sera resulted in being unable to obtain results for some of the abnormal samples due to there being insufficient sera available.

2.5.7 Unconjugated Oestriol Assay Methodology

20ul aliquots of the standards and 100 ul aliquots of the sera, both in duplicate, were dispensed into the reaction tubes (Table 3). Then, 100ul aliquots of Iodine-125 labelled oestriol were added to each tube. A further 1.0 ml of anti-oestriol solid-phase was added to all the tubes and these were then vortex mixed and incubated in a water bath at 37 $^{\circ}$C. for 1 hour. Upon removal the reaction tubes are transferred to magnetised racks and left for 15 mins. for the bound fraction to precipitate. The supernatant is then discarded and the tubes allowed to drain on blotting paper for 20 mins. Following this the tubes are transferred to a gamma multi-counter, each one for 2 mins., the raw counts printed out and these were later entered into a database / statistical package (SPSS/PS+).

Assay Tube Nos.	Tube Contents	Values (nmol/L)
1-2	Standard 0	0.00
3-4	" 1	1.10
5-6	" 2	2.20
7-8	" 3	4.90
9-10	" 4	9.20
11-12	" 5	19.50
13-14	" 6	48.00
15-16	Qual. Cont. 1	27.00*
17-18	" " 2	1.50*
19-20	Drift Control	30.00**
21-22	Sample	
"	"	
"	"	
51-52	Drift Control	
"	Sample	
"	"	
83-84	Drift Control	
"	Sample	
"	"	
117-118	Qual. Cont. 1	
119-120	" " 2	

* Qual. Cont. 1 + 2: These quality controls were made up from pooled maternal serum from 16 weeks and 8 weeks respectively. The serum was first pooled, aliquoted and then frozen. The values given are mean values.

** Drift Controls: These too were made up from pooled maternal serum, again at 16 weeks though in this case from a different batch to that of Quality Control 1. The values given are mean values.

Table 3: UE3 Assay Layout with Standard Values.

2.6 Statistical Methods Employed in Data Analysis

Having obtained the normal control values for all three analytes, a median value for each analyte at each week of gestation was derived by weighted linear regression. This was done using the statistics software package SPSS/PC+ which also served as the database to which all the analyte assay data was stored.

Upon obtaining these normal medians the results from the abnormal pregnancies were converted to multiples of these medians (MoMs) by simply dividing the value for the abnormal sample at a given gestation by the normal median value at that gestation.

Any deviations from normal values in the abnormal populations were tested for significance using the Mann-Whitney test. This test, also known as the Wilcoxon test, does not require any assumptions about the shape of the underlying distributions. It tests the hypothesis that two independent samples come from populations having the same distribution. The form of the distribution need not be specified. The test does not require that the variable be measured on an interval scale; an ordinal scale is sufficient.

The p-values derived using this test were again obtained using SPSS/PC+.

3. Results Section

3.1.1 Assay Optimisation

The in-house AFP assay employed was of sufficient sensitivity (0.6 KU/L) and precision (10.8% at 6.3KU/L) as to require no modification for first trimester samples. (Sensitivity being defined as the mean of the zero standard plus two standard deviations).

The HCG assay, as with sample sera from second trimester pregnancies, was modified by a 1:500 dilution of maternal sera using horse serum. With this modification the assay sensitivity was 0.8 IU/ml and the assay precision was 5.7% at 10 IU/ml.

The UE3 assay required several modifications for first trimester work before a satisfactory method was found, the original modification described by Canick et al (1988) and Wald et al (1988b) being inappropriate due to differences in the Amersham UE3 assay kits used (the assay used here was the Amersham Amerlex 2nd. Trimester kit). Two criteria were considered in modifiying this kit: the desirability of conserving sera from abnormal pregnancies and the need to increase the assay sensitivity to deal with first trimester sera.

3.1.2 Unconjugated Oestriol Assay Optimisation

The first variation, using the standards and reagents at

1/2 volumes with the sample sera at full volume, proved very unsatisfactory resulting in a poor curve fit and an inability to fit some results to the curve.

3.1.3

The next trial used the assay standards at 20% volume with all other reagents at full volume. Sample sera were used at both 20% and 50% of normal volume in attempt to conserve sera. This gave poor sensitivity (0.22 nmol/L) and many sample results were too high to fit onto the the standard curve.

3.1.4

The third trial again used the standards at 20% but made up to 50% of normal volume using pooled male human sera. Again sample sera were tried at both 20% and 50% of normal volumes. This gave poorer sensitivity (0.28 nmol/L) though the sample sera results did map better to the curve at 20% of normal volume.

3.1.5

The next trial again ran the standards at 20% of normal volume but made up to 100% using pooled male human serum. All reagents and sample sera were used at full volumes. This proved the most satisfactory and consistant variant and yielded a sensitivity of 0.12 nmol/L with a precision of 21.3% at 0.3 nmol/L.

Final sample values were obtained using this method and multiplying all results by a factor of 0.2. Unfortunately, having to use full volume aliquots (100ul) of sera resulted in being unable to obtain results for some of the abnormal samples due to a shortage of available sera.

3.2 Normal Controls

Having established the criteria for assessing gestation, a total pool of 632 samples with a range of gestations from 6 to 15 weeks were selected and assayed for each of the three analytes. Stable median values were obtained at, typically, around 50 samples. This sample size was not possible, however, for the gestational periods of 6 and 7 weeks due to a combination of insufficient sample numbers in the total series at these gestations or samples failing to meet the gestation assessment criteria (Table 4). In addition, the full set of matched controls were not available for each analyte, again due to a shortfall in sera volume. The results from these assays are summarised as median values in Table 5 and are presented in full in Appendix I. They are also presented as graphs of concentration against gestation in Figures 2, 3 and 4 showing the spread of the data around the medians.

Sample List No.	Gestation (Weeks)	Number of Samples per Analyte		
		AFP	HCG	UE3
1-69	15	69	63	66
70-140	14	68	67	63
141-208	13	68	65	61
209-278	12	69	61	57
279-347	11	69	62	62
348-421	10	64	68	66
422-492	9	71	66	66
493-561	8	69	66	66
562-618	7	37	45	45
619-632	6	13	12	14
Total No. Samples Assayed		597	575	566
Missing Cases (*)		35	57	66
Total Sample Number		632	632	632

(*) = Due to insufficient volumes of sera

Table 4. Breakdown of Sample Numbers Assayed to Establish Median Values for Normal Controls.

	Analytes		
Gestation (in Weeks)	AFP (KU/L)	HCG (IU/ml)	UE (nmol/L)
6	1.41	71.0	0.32
7	3.21	81.0	0.34
8	3.99	98.0	0.37
9	4.49	84.0	0.40
10	5.84	75.0	0.46
11	10.12	72.0	0.70
12	13.51	64.0	0.82
13	17.08	49.0	1.45
14	22.08	39.5	2.02
15	23.69	34.0	2.67

Table 5: Median Analyte values in Normal Pregnancies.

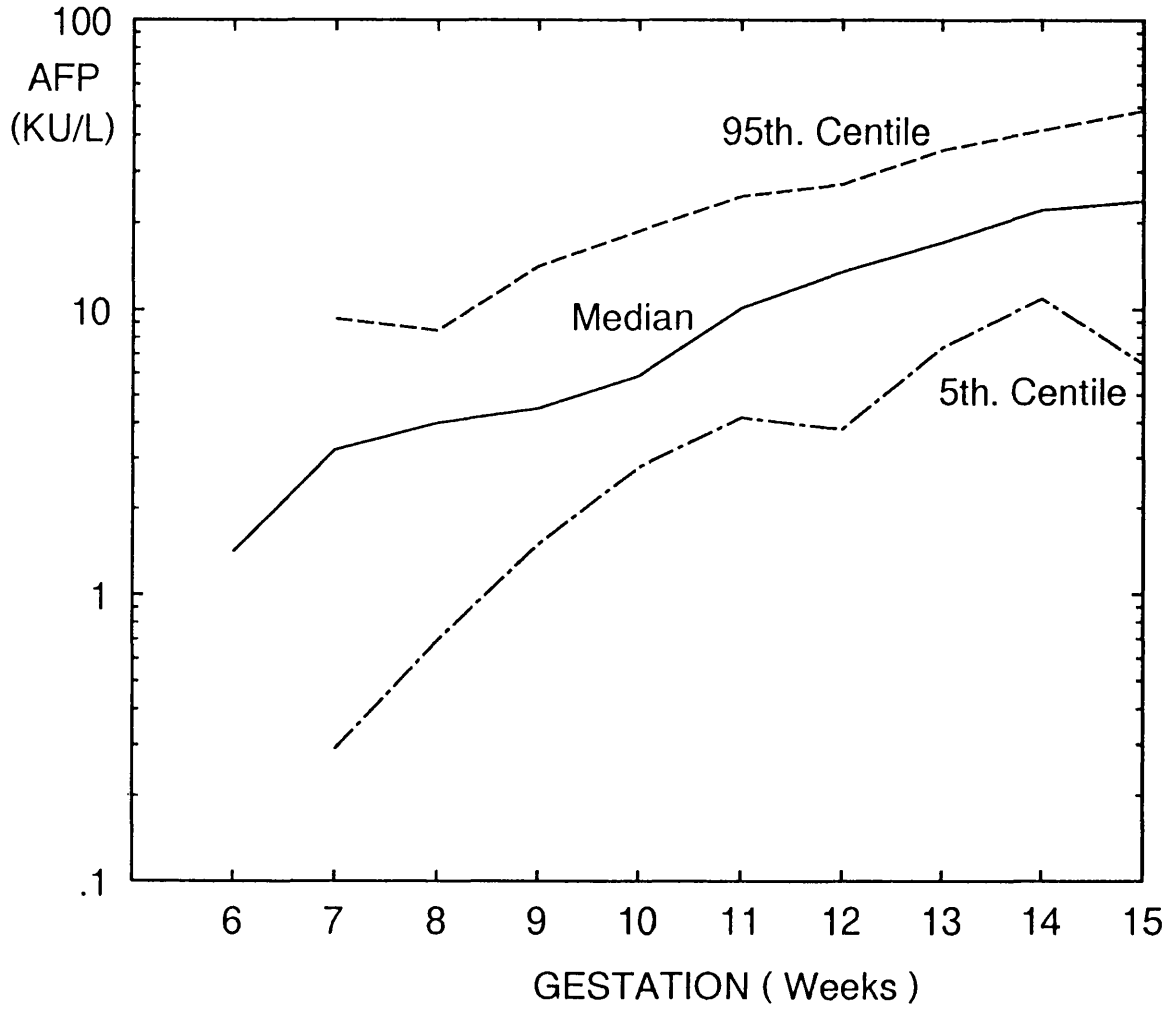

Figure 2. AFP versus Gestation.

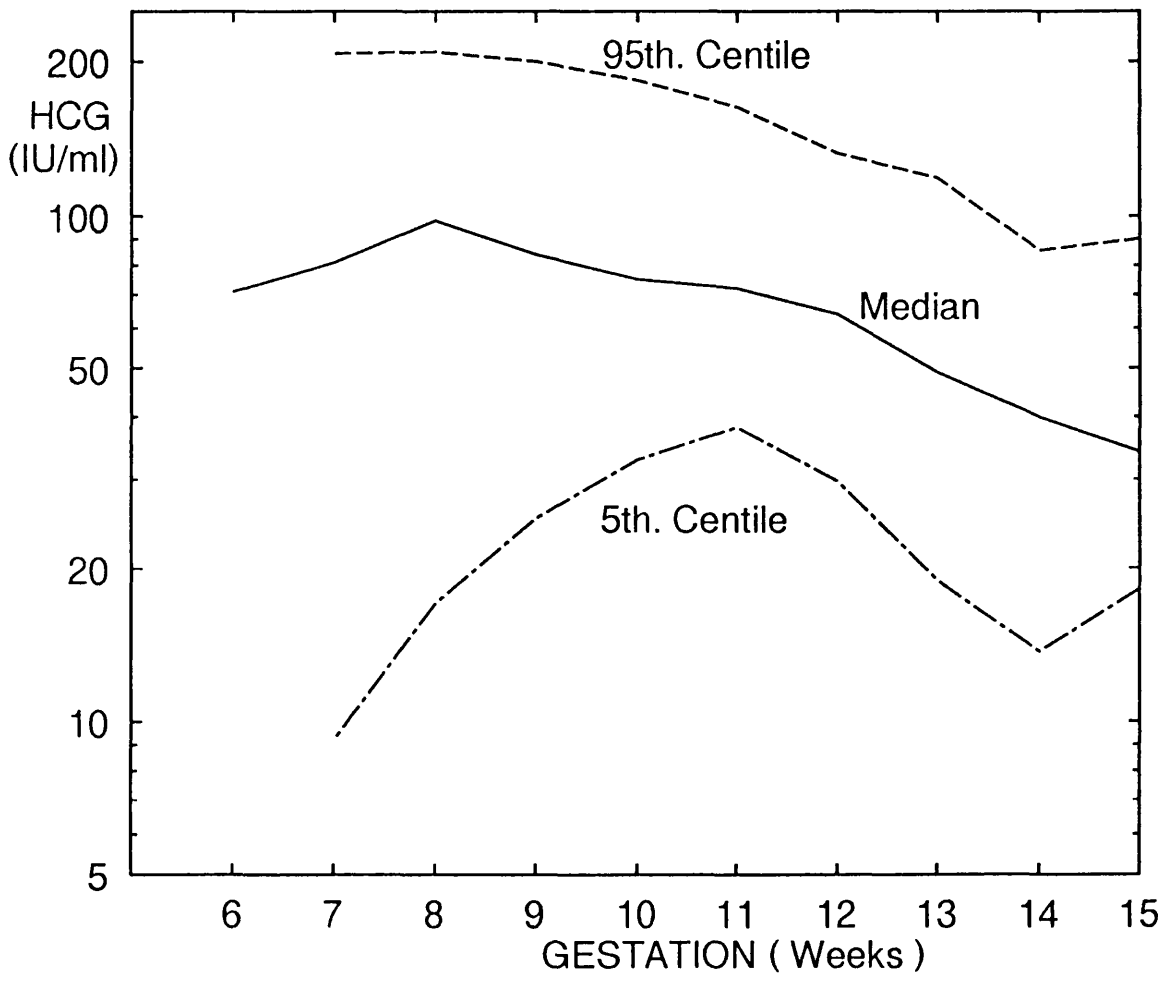

Figure 3. HCG versus Gestation.

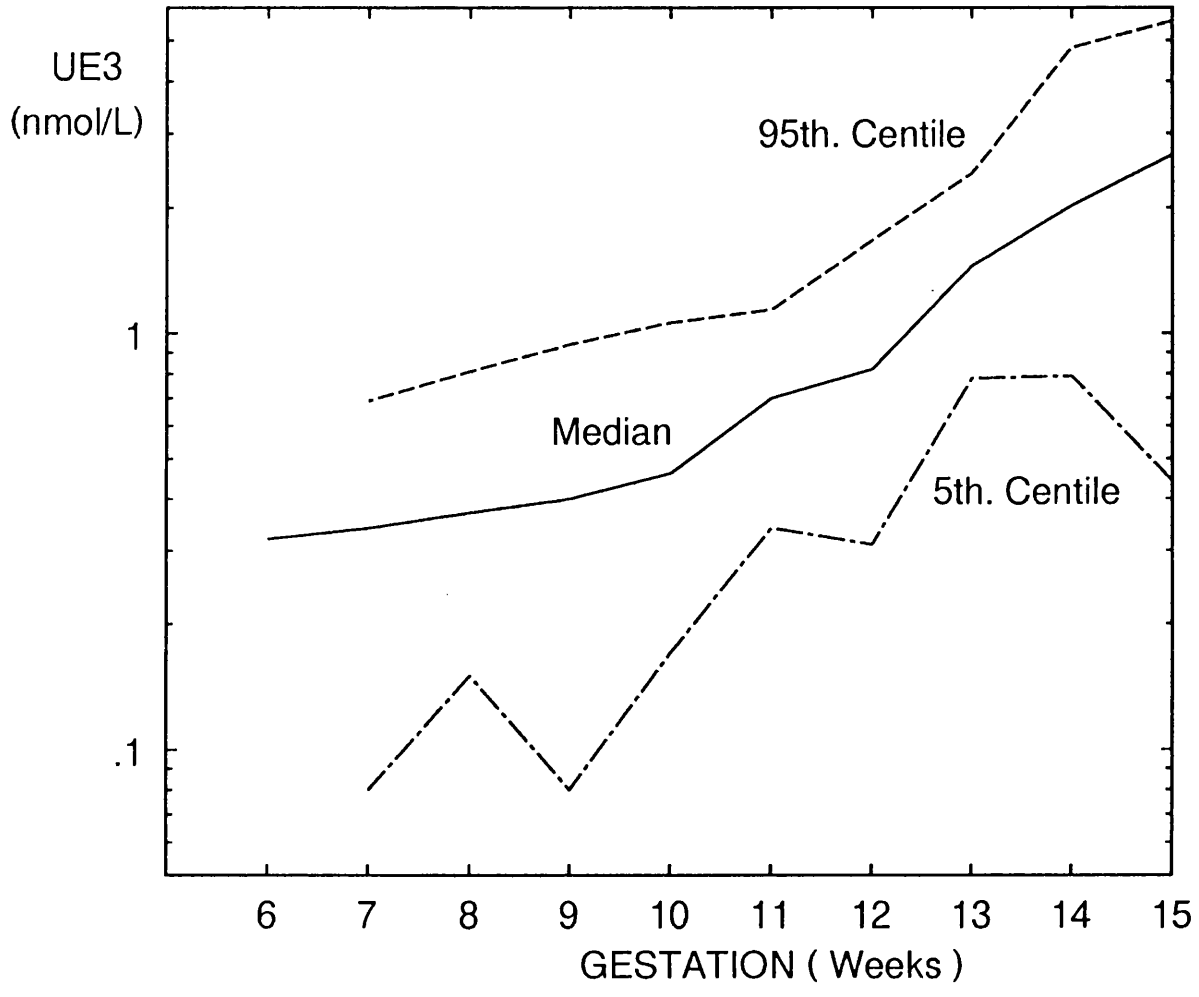

Figure 4. UE3 versus Gestation.

3.3 Abnormal Samples

Using the above normal control median data the results from pregnancies with known abnormal outcomes were studied. The serum samples from these pregnancies had been randomly inserted into the assays used to determine normal controls at which times nothing was known about them other than their laboratory identification numbers. Multiples of the median (MoMs) were then calculated for each abnormal sample by the simple formula:

$$\frac{\text{Sample analyte value at gestation X}}{\text{Median analyte value at gestation X}} = \text{MoM for Sample}$$

From the prospective series of 14,000 pregnancies a total of 16 trisomy 21 and 14 open NTDS were identified. In addition, 5 trisomy 18, 1 trisomy 13, 1 exomphalos, 1 anencephaly with spina bifida and gastroschisis and a twin pregnancy, where one foetus had spina bifida and one was unaffected, were also identified. However, given the small numbers of these latter cases, discussion will be largely confined to the trisomy 21 and NTD cases.

Having been converted to MoMs, the results from these abnormal pregnancies are presented in Tables 6, 7 and 8. These results are also presented as scatter plots (Figs. 5 and 6) and the raw data is presented in full in

Appendix-II. Significance of any deviations from the normal median values was assessed by the Mann-Whitney test, the results of which are presented in Table 9.

3.3.1 Alphafetoprotein

As may be seen from Fig. 5, AFP levels are significantly lower (p = 0.02) than the median normal in Down's pregnancies, having a median MoM of 0.65. With regard to open NTDs, no significant difference from normal values was detected (p = 0.75), as may be seen from Fig. 6.

3.3.2 Human Chorionic Gonadotrophin

Intact HCG levels in first trimester Down's pregnancies show no significant deviation from normal median values, having a median MoM of 0.97 (p = 0.52). However, HCG levels show some reduction in trisomy 18 pregnancies, having a median MoM of 0.27 (Fig. 5).

Again, no significant differences were observed in open spina bifida (p = 0.61), though all three anencephalics had values > 2.0 MoM (Fig. 6).

Trisomy 21 Cases	Gestn. (Weeks)	Analytes AFP (MoM)	HCG (MoM)	UE3 (MoM)
1	13	0.88	2.88	0.90
2	8	0.53	0.87	0
3	9	0.87	1.45	0.50
4	13	0.47	1.96	1.09
5	13	0.99	1.41	0.84
6	9	0.22	1.62	1.25
7	9	0.89	1.10	0
8	7	0.31	0.96	0.82
9	14	0.50	6.08	0.68
10	11	0.10	0.63	0.26
11	13	0.70	0.98	NA *
12	10	2.05	0.93	0.65
13	14	0.60	0.96	0.37
14	10	1.20	0.77	0.46
15	13	0.59	0.96	0.89
16	10	1.13	0.43	0

* NA = Not Available

Table 6: Analyte Results as Multiples of the normal Median (MoM) in Trisomy 21 pregnancies.

		Analytes		
	Gestn. (Weeks)	AFP (MoM)	HCG (MoM)	UE3 (MoM)
Trisomy 18	13	0.70	0.27	0.41
	11	0.59	0.58	NA *
	11	0.59	0.14	0.34
	8	1.00	0.13	0.62
	12	0.79	0.48	0.28
Trisomy 13	15	0.35	1.03	1.90

* NA = Not Available

Table 7: Analyte Results in Trisomy 18 Pregnancies as Multiples of the normal Median (MoM) plus values for the Trisomy 13 Pregnancy.

ABNORMALITY	Gestn. (weeks)	AFP (MoM)	HCG (MoM)	UE3 (MoM)
Spina bifida	9	0.67	0.69	1.38
	10	0.86	1.80	1.39
	10	0.69	0.83	1.61
	11	0.59	0.56	0.77
	11	1.09	0.88	0.96
	12	1.41	1.20	1.60
	12	1.04	0.25	1.30
	12	1.41	1.28	0.85
	12	1.18	0.61	NA*
	13	1.05	0.80	0.63
	14	2.90	1.11	1.01
Anencephaly	8	0.75	2.32	2.54
	8	0.50	2.09	2.00
	10	0.86	2.20	0.74
Exomphalos	12	2.15	3.61	1.27
Anencephaly with spina bifida and gastroschisis	12	1.78	1.47	0.98
Twin pregnancy: 1 spina bifida, 1 unaffected	11	2.96	0.68	1.00

*NA = Not Available

Table 8: Analyte Results from Pregnancies with Non--Chromosomal Defects Expressed as Multiples of the Normal Median (MoMs).

Mann-Whitney Tests

	Analyte	p-value
Trisomy 21	AFP	0.02
	HCG	0.52
	UE3	0.0005
Open NTDs	AFP	0.75
	HCG	0.61
	UE3	0.18

Table 9: Outcome of Mann-Whitney tests on the Results from the Abnormal Pregnancies.

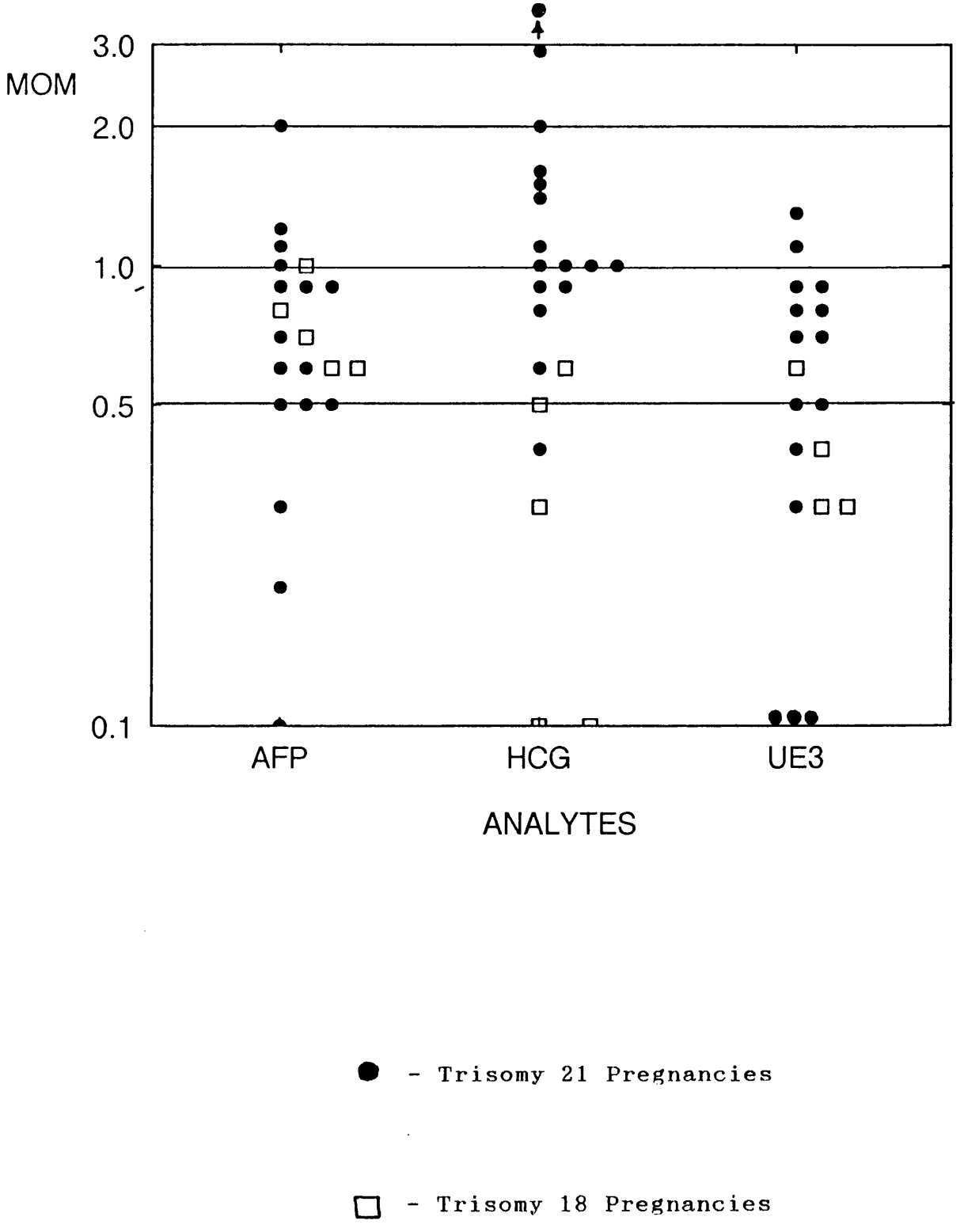

Figure 5. Scatter Plot of Trisomy 21 and Trisomy 18 Results.

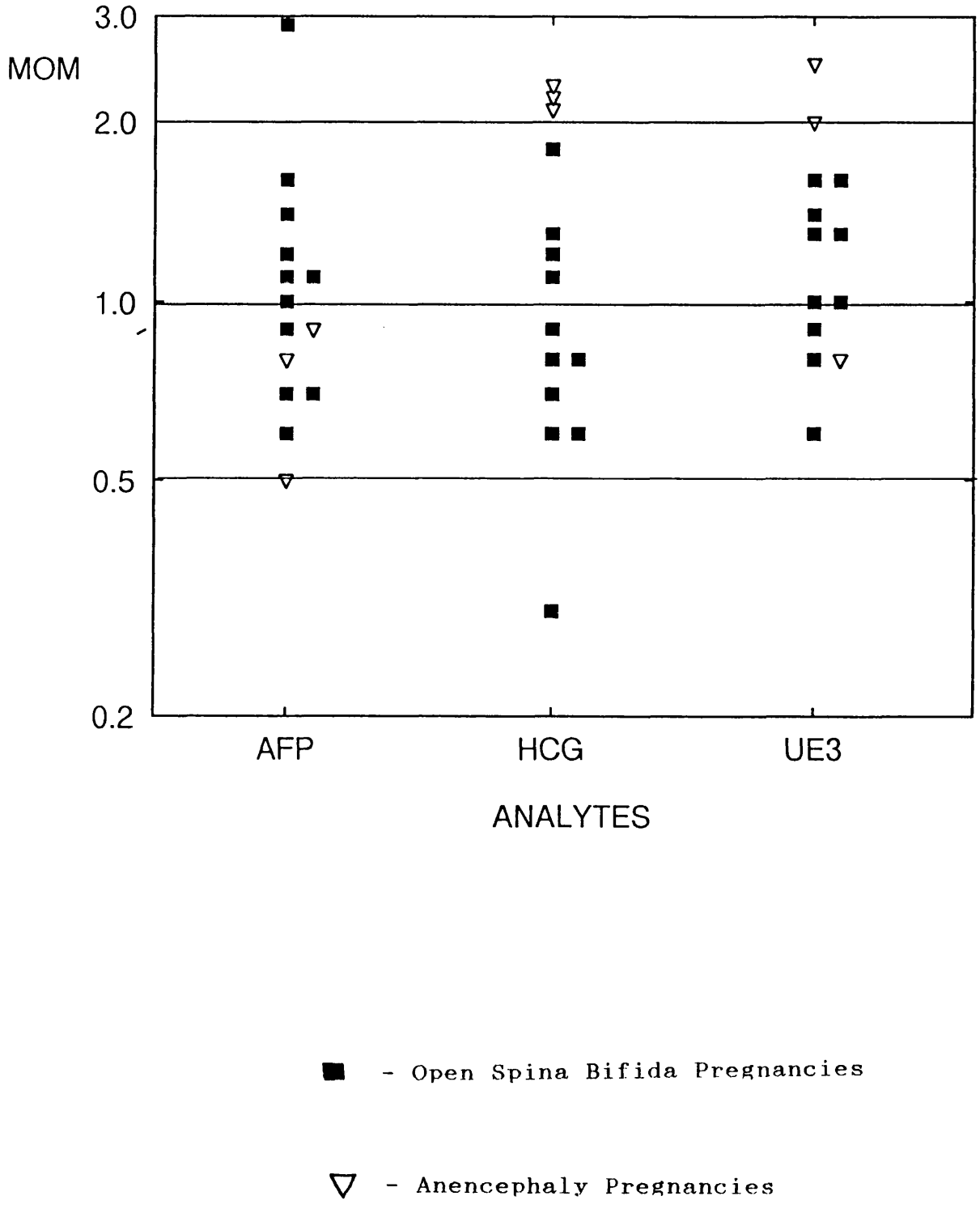

Figure 6. Scatter Plot of Spina Bifida and Anencephalic Results.

3.3.3 Unconjugated Oestriol

UE3 levels in trisomy 21 affected pregnancies show a highly significant (p = 0.0005) drop below normal values, with a median MoM of 0.67. Levels in trisomy 18 pregnancies are even more depressed with a median MoM of 0.34 (Fig.5).

Open spina bifida again shows no significantly abnormal pattern (p = 0.18), though anencephaly levels do appear elevated with two of the three having values > 2.0 MoM (Fig. 6).

3.3.4 Other Abnormalities

The results from these pregnancies are also presented in Tables 7 and 8. The values for AFP, especially, in pregnancies with non-chromosomal defects does appear elevated from the normal, but no reliable conclusions may be drawn given the sample sizes.

4. Discussion

4.1 Chromosome Abnormalities

The results presented here for Down's syndrome pregnancies, identified from a true screening population, show that AFP and UE3 levels are significantly reduced in affected pregnancies but that intact HCG, the most powerful marker in the second trimester, is virtually unaltered. These results are again presented, in Table 10, along with the data from previous significant studies for purposes of comparison. As may be seen from this table, the data from this study accords well with the findings of many other studies (Brambati et al, 1986; Barkai et al, 1987; Cuckle et al, 1988; Milunsky et al, 1988; Mantigh et al, 1988; Bogart et al, 1989; Brock et al, 1990; Nebiolo et al,1990; Kratzer et al, 1991; Crandall et al, 1991; Johnson et al, 1991; Check et al, 1990; van Lith et al, 1991; Shalev et al, 1992; van Lith reporting, 1992; Wald et al, 1992).

The data presented for trisomy 18 pregnancies in the first trimester is, at the time of writing, unique in both case numbers and in the range of analytes examined. While AFP levels show some decrease, both UE3 and HCG levels are considerably depressed which mirrors the pattern in the second trimester.

With regard to intact HCG, although no significant deviation from normal median values has been demonstrated in

Reference	Normal Controls	Trisomy 21 Case Numbers	Trisomy 21 AFP MoM	Trisomy 21 HCG MoM	Trisomy 21 UE3 MoM	Trisomy 18 Case Numbers	Trisomy 18 AFP MoM	Trisomy 18 HCG MoM	Trisomy 18 UE3 MoM
Brambati et al. (1986)	446	8	0.75	NA	NA	2	0.50	NA	NA
Barkai et al. (1987)	99	2	0.65	NA	NA	NA	NA	NA	NA
Cuckle et al. (1988)	110	22	0.72	NQ	0.35	NA	NA	NA	NA
Brock et al. (1990)	63	21	0.71	1.43	0.67	NA	NA	NA	NA
This Study. (1993)	632	16	0.65	0.97	0.67	5	0.71	0.27	0.34

NA: Data Not Available
NQ: Data Not Quoted

Table 10: Comparison of median MoMs from the current study with those from previous studies of trisomy 21 and 18 in the first trimester.

the present study, a modest increase (1.43 MoM) was noted by Brock et al (1990) in a series of 21 Down's syndrome pregnancies. More recent studies which looked at free-B HCG (FBHCG) have found the pattern of high maternal serum levels associated with second trimester Down's pregnancies reflected in the first trimester also. (Macri et al, 1990b; Spencer et al, 1992)

The similarity of the above relationships, that is, low AFP and UE3 and high FBHCG, might lead one to hope that the detection rates for Down's syndrome achieved in the second trimester may also be attainable in the first trimester. Clearly, though, further prospective studies will be necessary to determine whether this is indeed viable.

As for the one case of trisomy 13 found in the series, while there is a marked drop in the AFP level (see Table 7) the most striking feature is the very high HCG level (3.61 MoM). However, this is only one case and further data are required before any conclusions can be drawn.

4.2 Neural Tube Defects

The inclusion of msAFP estimation in routine second trimester prenatal screening programs not only contributes to the detection of Down's syndrome pregnancies but has a key role in the detection of open neural tube defects at this stage of pregnancy, to which end it was originally introduced. However, this study, in common with previous ones (UK Collaborative study, 1977; Shalev et al, 1992), has been unable to demonstrate any significant deviation

from normal median values for the analytes AFP, intact HCG and UE3 in first trimester pregnancies with open spina bifida.

HCG and UE3 levels in the few cases of anencephaly do show a marked increase and this is of some interest as it is exactly the opposite of the trends identified by Canick et al in 1990. Their study gave a median MoM of 0.75 for HCG compared with >2.0 MoM here and, perhaps more strikingly, a very low median MoM of 0.17 for UE3 compared with a marked increase to over 2.0 MoMs in two out of the three in this study. The Canick study, though, was much larger with some 19 anencephalics and further data are required before concluding that the current results are of significance.

Overall, however, there is no significant pattern thus moving screening for chromosome abnormalities to the first trimester would have the disadvantage of loss of detection of NTDs. Although ultrasound scanning is an effective diagnostic measure for NTDs in the second trimester in a high risk group defined by maternal serum screening, its sensitivity as a primary screening measure is open to question (Whittle, 1992). This is illustrated by the 14 cases of open NTDs reported in this study where all except one case was not diagnosed until after investigation of a routine second trimester elevated msAFP result. It is therefore concluded that, for the near future, first trimester biochemical screening for NTDs will continue to be impracticable and that second trimester screening will

retain its central role in such ante-natal programs.

4.3 Estimating Gestation

Another factor of central importance in all areas of ante-natal screening is the accurate estimation of gestational age. It is well recognised that accurate ascertainment of this is critical in the calculation of MoMs and hence the derivation of any maternal risk. The current method usually employed is to round gestation down to the nearest whole week. That is, if accurate dates for the last menstrual period indicate a gestation of 13 weeks and 5 days then gestation is taken as 13 weeks. Clearly, there is some significant margin for error here and there may be a case for examining the possibility of reckoning gestation in days and therefore determining maternal risk values in a more continuous manner. This may be of even greater value given that the rate of change of maternal serum levels of some analytes varies at different ranges of gestational age. For example, from 6 to 12 weeks UE3 levels increase only moderately and thus an 'error' of a few days in estimating gestation would have little effect. However, from 12 weeks onwards the rise in levels increases substantially and those same few days 'error' may effect a significant alteration of maternal risk (see Fig. 4). From 6 to 12 weeks there is a mean rise of 0.08 nmol/L/week in the median value of UE3, while from 12 weeks to 15 weeks there is a mean rise in UE3

levels of 0.61 nmol/L/week: almost an eight-fold rate increase. However, refining the use of gestation to single days will require the estimation of median values in the control population for each individual day of gestation to allow accurate conversion of analyte values to MoMs.

4.4 Alternative Analytes

With regard to both Down's syndrome and NTDs, continued examination of maternal serum pregnancy markers, other than those discussed above, will certainly be an area of intense activity. Already many of the alternative analytes which have been investigated in the second trimester have also been investigated in the first trimester. Mentioned previously, free-β HCG (FBHCG), (Macri et al, 1990b; Spencer, 1991), is of considerable interest showing a marked elevation in both the second and first trimesters unlike intact HCG which, as stated, shows no significant deviation from normal levels in the first trimester. Others include pregnancy-specific B1-glycoprotein (SP-1) and placental alkaline phosphatase (PALP), (Brock et al, 1990); progesterone (Kratzer et al, 1991); cancer antigen 125 (CA125), (Check et al, 1990; van Lith et al, 1991); placental protein 14 (PP-14), (Wald et al, 1992).

Apart from FBHCG, few of these studies, all of which looked only at Down's pregnancies, indicated that any of the analytes mentioned would be particularly useful. At the time

of writing only the work on pregnancy associated plasma protein-A, (PAPP-A), (Wald et al, 1992; Brambati et al, 1993) appears to offer a significantly large deviation from normal median values (0.27 MoM in Down's pregnancies) to warrant consideration for inclusion in first trimester screening. However, PAPP-A shows significant correlation with FBHCG in the first trimester (Aitken et al, 1993) and the values are unaltered in Down's syndrome pregnancies in the second trimester (Knight et al, 1993). More data are required to assess the rate of change in PAPP-A MoMs across gestation. This whole area is still evolving and no doubt will be aided by an understanding of the basic patho-physiology.

4.5 Conclusions

From the results of the present study, and others previously mentioned, several analytes in maternal serum such as AFP, UE3, FBHCG and PAPP-A can be identified as possible candidates for a first trimester screening program for foetal chromosome abnormalities. However, in the first trimester AFP is no longer the first choice marker, as it is in the second trimester screening programs, as it does not predict NTDs at this stage although it still a useful predictor of Down's syndrome. The most powerful marker in the first trimester is free-β HCG (FBHCG) which, in combination with AFP is predicted to give a 54% detection

rate at a 5% false positive rate (Aitken et al, 1993). UE3 and PAPP-A each show significant median shifts in Down's syndrome pregnancies in the first trimester, but are technically difficult to measure at the low concentrations prevalent in very early pregnancy. The advantage of using AFP/FBHCG in combination with maternal age would be consistency with current second trimester screening programs, as this marker combination could be used at any gestation from 7-20 weeks.

If pre-natal screening for chromosome abnormalities became routine before 15 weeks gestation, detection of NTDs would have to rely on the use of detailed ultrasonography. While anencephaly is readily detected by ultrasound in the first trimester, spina bifida is difficult to detect before the second trimester, and there may be a case for continuing to offer AFP screening at 16-18 weeks gestation as a primary screening test for NTDs in combination with ultrasound.

Little is understood about the mechanisms which cause variations in the levels of certain maternal serum markers in chromosomally abnormal pregnancies. The different patterns of variations seen between markers and between first and second trimesters (Table 11) is difficult to rationalise with simple explanations of foetal/placental immaturity as originally proposed by Wald et al (1988a). Further studies on the control of production and excretion of these markers in appropriate foetal and placental tissues are necessary to elicit these mechanisms in order to refine

the selection of the optimal markers for the various stages of the screening process.

However, given the public debate engendered by the anxiety created in expectant mothers by current second trimester screening, any future multi-stage schemes would be heavily dependent on the careful provision of trained, skilled counselling to ensure that pregnant women fully understand the complexities of the screening process and are thus not rendered unduly anxious.

Analyte	First Trimester	Second Trimester
AFP	Low	Low
UE3	Low	Low
HCG (intact)	Normal	High
FBHCG (free-β)	High	High
PAPP-A	Low	Normal
SP-1	Low	High

Low - Below Normal Median Values.
Normal - At the Normal Median Value.
High - Above the Normal Median Value.

Table 11: Comparison of Marker Analyte Levels in First and Second Trimester Down's Syndrome Pregnancies.

Appendix I : Normal Control Raw Data

Lab No	Sort Code	Gestn	Maternal Age	AFP (KU/L)	HCG (IU/ml)	UE3 (nmol/L)
150003	1	15	25	24.79	68	1.94
150615	1	15	28	23.04	49	3.08
150117	1	15	26	11.84	84	2.18
150640	1	15	24	24.24	32	3.27
150287	1	15	27	34.03	34	3.44
150106	1	15	41	47.35	19	3.64
150378	1	15	35	16.26	38	2.48
150008	1	15	23	25.94	26	2.64
150543	1	15	25	30.43	N/A	3.70
150306	1	15	28	33.62	20	6.26
150574	1	15	31	19.25	37	2.72
150582	1	15	31	59.95	23	3.52
150531	1	15	29	30.76	87	3.54
150585	1	15	28	17.17	20	3.34
150533	1	15	30	16.31	65	0.80
150607	1	15	22	26.44	21	5.96
150456	1	15	22	12.38	23	1.70
150282	1	15	29	21.67	23	1.99
150223	1	15	25	20.72	20	1.46
150222	1	15	21	27.18	19	1.82
150313	1	15	20	35.04	20	3.98
150220	1	15	29	23.75	33	2.42
150229	1	15	22	26.78	37	2.46
151847	1	15	23	29.68	40	1.88
151965	1	15	31	12.01	70	1.81
151090	1	15	19	16.55	20	2.52
151344	1	15	16	34.85	26	5.57
151803	1	15	26	25.58	25	3.32
151352	1	15	17	20.87	50	2.76
151959	1	15	21	10.63	99	0.40
151823	1	15	32	49.98	43	3.57
151676	1	15	28	28.73	35	3.64
151134	1	15	31	22.22	N/A	N/A
151071	1	15	21	45.47	50	4.58
151176	1	15	26	17.38	N/A	0.70
150891	1	15	25	23.28	30	1.77
150647	1	15	26	23.62	19	4.24
150814	1	15	23	34.98	23	4.08
150866	1	15	27	63.19	51	3.04
150649	1	15	30	25.88	37	2.46
152043	1	15	19	25.10	33	0.95
151207	1	15	27	8.00	N/A	0.73
150644	1	15	21	15.48	49	1.98
151741	1	15	30	4.70	36	0.70
151672	1	15	17	22.60	35	3.77
150711	1	15	22	22.80	20	2.06
150868	1	15	29	27.55	33	3.24
810127	1	15	30	26.00	N/A	N/A

Lab No	Sort Code	Gestn	Maternal Age	AFP (KU/L)	HCG (IU/ml)	UE3 (nmol/L)
150706	1	15	26	46.00	35	5.56
151590	1	15	31	15.00	18	2.98
150709	1	15	22	21.80	37	2.44
150690	1	15	25	4.87	91	0.52
150655	1	15	29	15.85	16	2.30
151294	1	15	26	33.17	63	2.74
150897	1	15	28	16.05	47	2.66
150892	1	15	24	21.39	34	3.96
151667	1	15	30	22.24	19	2.67
150707	1	15	25	23.80	55	2.20
151434	1	15	31	24.69	20	4.34
151936	1	15	24	8.67	87	0.82
151937	1	15	32	36.69	19	2.82
151557	1	15	34	24.00	22	3.10
151929	1	15	24	33.44	N/A	N/A
151911	1	15	22	2.31	20	0.14
151595	1	15	29	10.00	95	0.38
151181	1	15	24	23.24	37	3.86
152017	1	15	18	39.38	19	2.56
151400	1	15	28	19.01	13	2.26
151045	1	15	22	12.00	54	1.44
150215	1	14	26	20.44	44	2.98
150213	1	14	20	10.98	55	0.84
150628	1	14	22	21.83	25	3.82
150166	1	14	26	16.92	52	1.92
150244	1	14	28	27.45	44	1.46
150137	1	14	34	16.10	30	1.04
150291	1	14	31	14.64	40	2.22
150195	1	14	35	30.95	35	N/A
150498	1	14	29	38.82	86	2.40
150377	1	14	37	15.92	12	N/A
150167	1	14	38	23.77	30	N/A
150492	1	14	32	24.83	15	3.40
150511	1	14	31	17.59	21	2.00
150259	1	14	21	18.33	0	5.28
150047	1	14	24	20.85	32	1.84
150147	1	14	27	20.86	41	2.52
150234	1	14	27	36.55	25	4.82
150273	1	14	26	13.13	28	3.30
150013	1	14	27	26.17	23	2.02
150077	1	14	36	20.73	85	1.78
150062	1	14	24	25.89	18	0.20
150061	1	14	33	38.71	24	1.84
150494	1	14	22	22.87	65	2.14
150466	1	14	27	6.92	67	0.48
150243	1	14	24	35.08	33	1.60
150240	1	14	27	24.70	54	1.36
150451	1	14	29	17.43	35	1.98
150349	1	14	24	20.15	23	2.28
150103	1	14	27	15.88	72	1.06

Lab No	Sort Code	Gestn	Maternal Age	AFP (KU/L)	HCG (IU/ml)	UE3 (nmol/L)
150221	1	14	36	35.39	46	15.60
150563	1	14	14	12.58	69	2.34
150407	1	14	26	12.12	55	1.30
150430	1	14	33	23.44	17	2.70
150100	1	14	22	10.87	47	1.52
150802	1	14	30	4.58	125	2.40
151907	1	14	22	27.18	32	1.94
152007	1	14	31	27.26	51	1.34
151986	1	14	22	44.73	58	2.22
151685	1	14	27	36.09	67	3.17
151347	1	14	22	25.25	29	1.21
150793	1	14	27	22.94	45	2.80
151221	1	14	24	17.21	46	1.50
150876	1	14	25	26.14	39	2.00
150834	1	14	17	33.61	52	3.80
151880	1	14	29	11.71	58	2.50
150862	1	14	26	51.50	28	2.94
151327	1	14	27	23.76	31	3.12
150779	1	14	27	19.23	30	1.82
150943	1	14	28	20.40	49	N/A
151925	1	14	38	23.24	48	1.76
151292	1	14	24	25.78	32	2.40
151868	1	14	23	21.16	20	1.65
150683	1	14	27	26.40	43	2.40
151380	1	14	28	22.07	37	1.46
151160	1	14	28	14.31	N/A	N/A
150745	1	14	25	22.33	17	0.86
151705	1	14	29	30.58	71	2.66
151566	1	14	16	44.00	27	2.85
151227	1	14	28	26.42	55	0.78
151317	1	14	23	30.40	45	1.57
150656	1	14	34	19.94	20	2.32
150700	1	14	31	19.90	51	3.34
151508	1	14	20	19.10	86	1.14
151397	1	14	20	14.42	58	2.75
151040	1	14	26	11.00	N/A	N/A
151950	1	14	29	19.86	N/A	2.24
153329	1	14	28	21.43	N/A	N/A
152708	1	14	25	12.00	N/A	N/A
152342	1	14	22	N/A	20	4.77
153288	1	14	30	N/A	13	2.25
153226	1	14	27	N/A	18	2.16
150082	1	13	33	45.56	29	1.92
150331	1	13	27	25.56	27	1.68
150334	1	13	24	7.31	56	0.78
150613	1	13	36	17.58	274	1.80
150145	1	13	23	13.22	115	2.18
150288	1	13	27	18.66	49	2.22
150444	1	13	31	14.08	23	1.30
150411	1	13	34	19.72	25	1.78

Lab No	Sort Code	Gestn	Maternal Age	AFP (KU/L)	HCG (IU/ml)	UE3 (nmol/L)
150261	1	13	23	11.69	21	1.62
150488	1	13	22	30.82	31	1.14
150113	1	13	29	37.45	N/A	1.78
150022	1	13	25	9.96	50	N/A
150366	1	13	24	8.07	43	1.30
150012	1	13	30	15.76	75	N/A
150108	1	13	34	26.61	48	1.44
150257	1	13	26	19.13	52	1.50
150151	1	13	32	18.84	59	1.54
150048	1	13	25	24.27	31	1.14
150408	1	13	28	14.20	35	0.90
150263	1	13	30	14.91	58	1.66
150540	1	13	29	10.19	57	2.10
150004	1	13	29	21.59	27	1.50
150448	1	13	35	14.75	55	1.16
150118	1	13	29	33.29	67	2.42
150389	1	13	20	12.87	71	1.86
150547	1	13	23	24.00	62	2.06
150445	1	13	25	20.77	62	0.82
150038	1	13	22	13.31	18	0.52
150401	1	13	27	21.28	29	1.26
150075	1	13	29	7.39	120	1.10
150612	1	13	32	18.54	55	0.88
150414	1	13	29	25.57	31	2.36
150115	1	13	30	15.95	36	1.58
150410	1	13	27	11.64	31	1.08
150399	1	13	28	12.28	37	0.76
150162	1	13	22	9.23	38	1.02
150016	1	13	35	15.34	47	1.88
150242	1	13	27	25.88	30	1.18
150454	1	13	28	16.98	43	1.74
150609	1	13	25	15.67	55	1.32
150386	1	13	27	19.12	91	1.54
150230	1	13	33	16.63	82	1.34
150552	1	13	27	21.55	32	0.90
150560	1	13	25	11.08	67	1.80
150632	1	13	17	17.51	115	0.82
150203	1	13	25	31.33	54	N/A
151063	1	13	20	5.96	103	N/A
151649	1	13	19	17.21	55	0.92
151120	1	13	24	6.88	N/A	N/A
150998	1	13	20	11.55	24	N/A
151303	1	13	23	19.56	36	1.06
151173	1	13	29	13.48	33	1.24
151682	1	13	21	16.83	17	1.55
151093	1	13	26	25.62	N/A	N/A
151799	1	13	23	23.19	42	2.58
151838	1	13	36	18.67	75	1.18
151260	1	13	19	20.00	64	1.18
150757	1	13	32	37.57	147	1.45

Lab No	Sort Code	Gestn	Maternal Age	AFP (KU/L)	HCG (IU/ml)	UE3 (nmol/L)
151118	1	13	29	9.12	34	1.56
151898	1	13	25	13.83	58	1.64
151430	1	13	42	28.04	35	1.34
151533	1	13	30	20.16	30	0.98
151910	1	13	27	19.53	78	1.04
150922	1	13	30	22.17	75	2.01
151798	1	13	40	14.05	15	1.20
151127	1	13	24	20.88	54	1.31
151555	1	13	21	12.00	49	0.99
150665	1	13	35	9.01	73	1.28
150000	1	12	29	11.00	N/A	N/A
150001	1	12	40	9.00	N/A	N/A
150480	1	12	27	9.51	70	0.56
150056	1	12	31	27.04	54	1.28
150092	1	12	25	23.49	39	0.74
150098	1	12	27	19.37	N/A	0.80
150095	1	12	36	23.80	72	1.66
150091	1	12	30	10.78	47	N/A
150508	1	12	30	17.58	55	0.94
150590	1	12	22	12.08	55	0.96
150141	1	12	32	10.52	45	0.84
150007	1	12	32	7.39	N/A	N/A
150021	1	12	37	19.59	65	N/A
150315	1	12	26	11.40	62	0.66
150372	1	12	32	12.85	20	N/A
150032	1	12	31	32.09	71	1.12
150193	1	12	33	14.46	55	0.70
150390	1	12	36	N/A	N/A	N/A
150119	1	12	24	5.03	69	1.66
150256	1	12	27	10.49	67	1.96
150442	1	12	30	12.46	67	N/A
150130	1	12	22	10.33	36	0.80
150398	1	12	35	10.32	57	1.18
150002	1	12	34	24.56	49	0.82
150114	1	12	30	13.88	57	0.42
150184	1	12	24	10.17	80	N/A
150583	1	12	30	5.82	26	1.20
150473	1	12	28	16.71	48	0.64
150606	1	12	29	19.60	50	0.50
150159	1	12	22	6.20	52	0.48
150403	1	12	29	1.13	98	0.22
150409	1	12	38	5.00	40	0.72
150253	1	12	18	6.93	107	0.62
150153	1	12	29	15.70	79	1.30
150633	1	12	18	5.21	135	0.82
151339	1	12	30	8.13	83	0.98
150737	1	12	20	12.60	75	0.57
151966	1	12	37	12.34	64	0.36
151442	1	12	20	3.36	75	N/A
151964	1	12	17	15.24	79	0.50

Lab No	Sort Code	Gestn	Maternal Age	AFP (KU/L)	HCG (IU/ml)	UE3 (nmol/L)
151386	1	12	25	30.78	N/A	1.42
151859	1	12	26	25.71	71	0.88
150754	1	12	16	17.16	62	1.16
151707	1	12	27	9.18	72	0.56
151805	1	12	37	25.21	39	0.54
151300	1	12	23	5.06	45	0.42
150750	1	12	24	7.88	N/A	0.48
151515	1	12	21	8.17	44	0.92
151113	1	12	20	17.47	109	N/A
151021	1	12	21	15.82	N/A	N/A
150838	1	12	27	12.62	56	0.62
151254	1	12	26	6.00	N/A	N/A
150835	1	12	23	13.63	56	1.08
151980	1	12	18	9.63	29	1.26
152010	1	12	32	16.47	69	0.88
150971	1	12	27	11.90	107	0.85
150920	1	12	31	8.74	95	0.76
151095	1	12	26	10.33	63	0.86
150953	1	12	27	27.10	186	1.50
152061	1	12	28	8.86	150	0.46
151425	1	12	19	3.05	49	0.24
150923	1	12	29	13.49	109	0.90
150968	1	12	22	7.28	92	0.32
150914	1	12	28	15.29	79	0.68
151546	1	12	21	13.70	55	0.90
151329	1	12	17	13.20	68	0.63
150935	1	12	21	4.21	52	0.34
151131	1	12	30	7.84	39	0.66
151147	1	12	30	17.51	74	1.12
151137	1	12	28	21.12	76	0.44
150040	1	11	24	22.81	78	0.62
150096	1	11	29	4.32	117	0.50
150078	1	11	32	8.75	71	0.44
150019	1	11	28	10.53	228	1.28
150622	1	11	25	9.26	165	0.48
150139	1	11	21	21.95	61	N/A
151161	1	11	26	29.01	N/A	0.84
150489	1	11	35	11.90	109	0.66
150014	1	11	29	9.38	137	0.34
150393	1	11	33	17.25	98	0.58
150510	1	11	29	9.83	62	N/A
150034	1	11	28	11.02	44	0.72
150392	1	11	25	20.02	N/A	0.84
150049	1	11	32	14.72	103	0.98
150144	1	11	19	9.89	83	0.88
150639	1	11	33	15.14	78	1.18
150146	1	11	29	22.90	86	0.56
150026	1	11	25	16.08	44	0.76
150491	1	11	28	15.11	151	0.70
150148	1	11	35	13.66	51	0.62

Lab No	Sort Code	Gestn	Maternal Age	AFP (KU/L)	HCG (IU/ml)	UE3 (nmol/L)
150194	1	11	29	15.41	92	0.62
150371	1	11	25	22.52	47	N/A
150504	1	11	26	14.34	33	0.42
150635	1	11	28	7.71	53	0.62
151232	1	11	30	17.57	79	0.70
150294	1	11	30	5.36	48	0.82
150541	1	11	26	17.04	N/A	N/A
150235	1	11	31	6.65	62	0.72
150260	1	11	30	5.52	69	0.82
150524	1	11	26	22.77	84	0.80
150079	1	11	27	4.81	77	0.32
150415	1	11	33	17.17	74	0.66
151181	1	11	27	11.22	N/A	0.66
150530	1	11	35	10.46	63	0.88
150519	1	11	28	16.84	100	N/A
150065	1	11	39	15.69	82	0.78
150782	1	11	20	2.77	70	0.44
151648	1	11	24	2.39	121	0.76
151065	1	11	20	15.98	N/A	N/A
150762	1	11	26	25.18	25	0.74
150718	1	11	26	8.00	71	0.46
150843	1	11	31	8.27	60	1.16
151216	1	11	20	4.56	48	0.88
151722	1	11	28	5.10	37	0.80
151717	1	11	23	6.51	59	0.53
150955	1	11	18	31.30	93	0.72
151461	1	11	22	6.80	70	0.52
151725	1	11	22	11.49	112	0.81
150719	1	11	28	9.17	99	0.52
150764	1	11	22	11.66	N/A	0.52
151445	1	11	30	7.53	46	0.35
151019	1	11	22	5.27	N/A	N/A
150908	1	11	27	9.51	52	0.64
150860	1	11	31	10.40	45	0.46
150751	1	11	23	7.23	68	0.84
152060	1	11	26	6.68	73	0.44
151423	1	11	29	16.56	74	0.70
151307	1	11	34	10.98	61	0.60
151258	1	11	28	4.00	59	0.66
151257	1	11	20	17.00	141	0.72
151797	1	11	20	5.76	68	0.68
151518	1	11	25	8.17	134	0.98
152006	1	11	32	13.28	57	0.78
151067	1	11	29	24.06	114	1.00
151457	1	11	26	5.99	59	0.42
151677	1	11	33	5.66	365	0.34
150660	1	11	32	8.37	75	0.70
151233	1	11	28	12.35	95	0.72
151715	1	11	30	7.69	54	0.74
150094	1	10	27	6.34	224	0.32

Lab No	Sort Code	Gestn	Maternal Age	AFP (KU/L)	HCG (IU/ml)	UE3 (nmol/L)
150341	1	10	23	5.41	232	1.16
150055	1	10	17	5.84	68	1.14
150168	1	10	29	14.51	171	1.08
150093	1	10	21	6.48	139	0.16
150172	1	10	31	11.73	N/A	0.78
150340	1	10	25	5.51	95	0.34
150475	1	10	22	2.77	63	0.36
150478	1	10	28	9.23	N/A	N/A
150169	1	10	29	20.22	43	N/A
150088	1	10	21	4.23	119	0.26
150427	1	10	20	8.41	63	1.02
150631	1	10	23	4.28	65	0.26
150335	1	10	21	8.98	186	0.34
150514	1	10	23	6.07	74	0.08
150290	1	10	29	5.24	77	0.42
150611	1	10	30	10.91	60	0.56
150589	1	10	27	5.34	118	0.58
150150	1	10	24	7.16	39	0.54
150076	1	10	25	5.30	90	0.52
150602	1	10	20	2.67	67	0.48
150520	1	10	34	4.44	39	0.30
150156	1	10	24	4.07	181	0.68
150562	1	10	38	4.26	82	N/A
150071	1	10	26	6.06	30	N/A
150739	1	10	20	5.00	86	0.94
150675	1	10	28	3.87	118	N/A
151477	1	10	15	5.94	76	0.61
151479	1	10	38	8.92	36	0.49
152036	1	10	22	3.18	71	0.88
151478	1	10	27	11.09	151	0.59
151656	1	10	35	6.84	58	0.89
150734	1	10	23	3.02	54	0.46
150977	1	10	28	6.22	41	0.44
150678	1	10	17	3.85	79	0.36
151197	1	10	22	3.45	69	0.00
151522	1	10	25	24.24	107	0.79
151808	1	10	23	19.24	57	0.41
151716	1	10	28	7.77	153	0.38
150939	1	10	20	8.57	92	0.54
151018	1	10	27	8.61	61	0.36
150873	1	10	20	4.58	90	0.52
151835	1	10	29	4.54	N/A	N/A
151581	1	10	27	6.00	112	0.50
151214	1	10	22	5.96	93	0.47
151804	1	10	28	3.16	80	0.20
151981	1	10	29	4.03	73	0.38
151899	1	10	29	2.82	44	0.26
151529	1	10	24	3.66	144	0.44
151310	1	10	31	8.48	57	0.22
150919	1	10	24	2.35	31	0.32

Lab No	Sort Code	Gestn	Maternal Age	AFP (KU/L)	HCG (IU/ml)	UE3 (nmol/L)
150859	1	10	26	5.13	156	1.02
151215	1	10	24	3.97	77	0.72
150773	1	10	36	9.41	87	0.73
151308	1	10	31	3.52	42	0.28
151459	1	10	25	4.61	63	0.58
150942	1	10	24	6.74	126	0.46
150671	1	10	26	12.91	65	0.52
151145	1	10	27	16.27	35	0.40
150941	1	10	30	6.86	55	0.40
151146	1	10	26	5.40	57	0.50
151735	1	10	33	4.01	61	0.59
151733	1	10	21	16.90	25	0.54
151671	1	10	20	8.11	87	0.47
153568	1	10	36	N/A	N/A	N/A
152921	1	10	30	N/A	101	0.24
152275	1	10	26	N/A	70	0.34
153571	1	10	34	N/A	N/A	N/A
152424	1	10	23	N/A	54	0.90
152102	1	10	35	N/A	97	0.60
152927	1	10	28	N/A	115	0.36
153210	1	10	26	N/A	N/A	N/A
152506	1	10	26	N/A	134	0.46
152129	1	10	26	N/A	46	0.46
150218	1	9	29	9.33	56	0.32
150474	1	9	33	4.32	101	0.38
150479	1	9	34	5.33	112	0.40
150553	1	9	33	3.22	91	0.24
150337	1	9	28	1.68	36	0.50
150505	1	9	32	2.44	99	0.40
150446	1	9	30	4.22	168	0.46
150402	1	9	31	33.82	64	0.34
150165	1	9	35	6.41	64	0.44
150413	1	9	23	5.98	104	0.40
150507	1	9	30	4.83	71	0.32
150140	1	9	25	8.54	158	0.86
150465	1	9	22	3.20	N/A	N/A
150603	1	9	26	2.85	113	0.50
150312	1	9	21	10.61	99	0.38
150623	1	9	27	4.47	126	0.38
150518	1	9	23	8.47	108	0.28
150187	1	9	17	1.56	20	0.32
150101	1	9	30	3.23	117	0.48
150228	1	9	27	3.47	107	0.18
150559	1	9	26	2.54	131	0.32
150068	1	9	23	9.24	178	0.88
150129	1	9	27	4.85	68	0.80
150252	1	9	31	4.49	71	0.32
150158	1	9	29	7.08	95	0.26
150102	1	9	34	4.51	57	0.30
150517	1	9	24	1.39	86	0.56

Lab No	Sort Code	Gestn	Maternal Age	AFP (KU/L)	HCG (IU/ml)	UE3 (nmol/L)
150280	1	9	34	9.12	76	0.58
150154	1	9	20	1.64	31	0.38
150251	1	9	32	7.50	198	0.46
152052	1	9	19	4.65	201	0.34
151654	1	9	17	13.95	N/A	N/A
150740	1	9	23	7.33	40	0.40
152035	1	9	36	3.18	111	0.42
151066	1	9	18	5.28	N/A	0.68
151288	1	9	28	1.94	209	0.50
151483	1	9	22	3.14	58	0.51
151967	1	9	24	3.14	76	0.54
151062	1	9	21	0.56	N/A	N/A
152032	1	9	29	3.74	145	0.56
152031	1	9	31	0.99	31	1.10
151305	1	9	20	7.64	108	0.97
151084	1	9	26	5.07	N/A	N/A
151800	1	9	27	4.10	75	0.65
151879	1	9	25	14.43	75	0.48
152064	1	9	25	4.04	N/A	N/A
151903	1	9	26	2.55	106	0.00
150959	1	9	28	9.42	47	0.46
151218	1	9	27	3.57	101	0.76
152009	1	9	32	7.16	140	0.14
151130	1	9	22	9.80	24	0.22
151112	1	9	19	7.94	84	0.30
151869	1	9	26	2.46	55	0.26
150836	1	9	26	4.99	72	0.34
150858	1	9	26	15.76	80	0.52
151874	1	9	25	4.37	75	0.52
151727	1	9	24	4.12	69	0.28
151168	1	9	27	4.42	39	0.50
150954	1	9	26	11.40	29	0.58
150854	1	9	26	4.35	59	0.30
151140	1	9	27	2.97	27	0.04
150667	1	9	31	3.47	154	0.56
150863	1	9	32	8.21	174	0.34
150369	1	9	27	5.38	66	0.32
151792	1	9	37	7.21	118	0.42
151739	1	9	24	3.42	102	0.59
151436	1	9	35	3.99	63	0.58
151209	1	9	20	6.73	41	0.22
151796	1	9	20	4.78	56	0.46
151983	1	9	27	3.34	93	0.00
151972	1	9	28	2.16	311	N/A
150339	1	8	30	2.64	40	0.30
150301	1	8	30	4.12	126	0.32
150211	1	8	18	3.20	200	0.52
150214	1	8	24	5.07	157	0.50
150292	1	8	31	3.25	41	0.28
150116	1	8	27	7.27	113	0.80

Lab No	Sort Code	Gestn	Maternal Age	AFP (KU/L)	HCG (IU/ml)	UE3 (nmol/L)
150006	1	8	26	4.39	125	0.42
150024	1	8	28	6.67	110	0.42
150262	1	8	28	4.05	233	0.52
150616	1	8	21	4.15	228	0.24
150111	1	8	32	3.99	159	N/A
150059	1	8	30	5.28	86	0.24
150417	1	8	26	3.00	N/A	N/A
150126	1	8	31	5.65	127	N/A
150310	1	8	22	3.03	59	0.52
150385	1	8	19	2.48	59	0.58
150278	1	8	36	3.52	38	0.22
150436	1	8	23	1.00	27	0.10
150805	1	8	27	4.37	123	0.42
150735	1	8	25	1.71	69	0.42
151652	1	8	23	3.41	123	0.28
150980	1	8	29	3.28	16	0.32
152034	1	8	23	1.35	13	0.30
151979	1	8	26	1.72	147	0.56
150855	1	8	30	9.63	64	0.36
151577	1	8	26	3.00	83	0.51
151523	1	8	29	1.89	93	0.49
150915	1	8	23	4.33	125	0.42
151349	1	8	21	5.48	41	0.73
151897	1	8	30	4.51	71	0.54
151345	1	8	37	0.89	95	1.21
151877	1	8	32	1.83	161	0.44
151578	1	8	34	5.00	112	0.25
151960	1	8	22	2.78	109	0.46
151616	1	8	31	3.00	186	0.53
151217	1	8	27	0.49	43	0.26
151128	1	8	16	12.72	101	0.36
151424	1	8	26	4.42	116	0.58
151302	1	8	31	1.29	72	0.26
151524	1	8	29	1.92	140	0.32
151428	1	8	23	3.20	167	0.36
151987	1	8	24	2.53	95	0.34
151958	1	8	33	2.52	95	0.26
150774	1	8	23	13.93	14	0.20
151516	1	8	24	4.91	213	0.42
151466	1	8	28	3.31	85	0.50
151343	1	8	28	0.00	124	0.38
151309	1	8	31	1.14	89	0.56
151219	1	8	33	6.81	90	0.23
151213	1	8	35	2.25	146	0.47
151408	1	8	24	2.96	176	0.87
151990	1	8	26	3.98	73	0.22
151332	1	8	23	0.00	61	0.24
151892	1	8	30	4.45	138	0.18
152041	1	8	41	3.18	66	0.00
151969	1	8	29	4.04	N/A	0.22

Lab No	Sort Code	Gestn	Maternal Age	AFP (KU/L)	HCG (IU/ml)	UE3 (nmol/L)
151997	1	8	31	2.79	62	0.26
150681	1	8	29	3.06	19	0.14
151993	1	8	27	4.37	61	0.38
151996	1	8	24	2.00	64	0.22
151520	1	8	21	2.28	193	0.40
151314	1	8	26	2.18	73	0.40
150827	1	8	38	1.15	63	0.30
151881	1	8	29	2.42	111	0.36
151690	1	8	23	4.59	119	0.63
150952	1	8	19	4.60	123	0.82
151885	1	8	28	3.45	117	0.16
151815	1	8	28	3.61	112	0.54
151086	1	8	34	5.52	N/A	0.42
150626	1	7	27	1.71	218	0.38
150424	1	7	28	2.00	92	0.44
150054	1	7	35	3.38	N/A	0.62
150342	1	7	30	4.69	137	0.50
150043	1	7	39	3.95	N/A	N/A
150293	1	7	35	2.71	123	0.32
150621	1	7	26	1.79	69	0.22
150600	1	7	21	1.39	37	N/A
150104	1	7	27	4.41	96	0.24
150625	1	7	25	0.98	48	0.38
150248	1	7	22	3.93	103	N/A
151855	1	7	31	0.31	81	0.46
151285	1	7	19	2.14	90	0.39
152011	1	7	32	0.14	4	0.28
151688	1	7	38	9.21	73	0.38
151985	1	7	37	9.88	133	0.26
151870	1	7	26	2.18	81	0.72
151619	1	7	32	1.00	59	0.52
150913	1	7	34	4.32	64	0.06
150878	1	7	36	3.21	65	0.34
151526	1	7	23	2.03	98	0.40
151125	1	7	31	1.68	233	0.26
152008	1	7	29	7.32	54	0.28
151617	1	7	32	2.00	95	0.50
151514	1	7	21	0.64	61	0.28
151464	1	7	29	1.80	N/A	N/A
151878	1	7	21	N/A	131	0.00
150724	1	7	27	1.94	167	0.38
150733	1	7	30	8.10	56	0.56
152046	1	7	34	4.15	N/A	N/A
150657	1	7	39	3.41	112	0.28
151563	1	7	41	6.00	91	0.30
151268	1	7	39	7.00	179	0.35
151273	1	7	26	3.00	182	1.03
151274	1	7	26	1.00	8	0.32
152026	1	7	21	3.27	50	0.30
152344	1	7	22	1.10	52	0.28

Lab No	Sort Code	Gestn	Maternal Age	AFP (KU/L)	HCG (IU/ml)	UE3 (nmol/L)
152707	1	7	27	1.00	50	0.30
153405	1	7	29	N/A	N/A	N/A
152855	1	7	17	N/A	43	0.42
152368	1	7	29	N/A	60	0.48
153520	1	7	22	N/A	N/A	N/A
152920	1	7	29	N/A	50	0.46
152669	1	7	31	N/A	N/A	0.48
153496	1	7	23	N/A	N/A	N/A
152925	1	7	21	N/A	83	0.14
152089	1	7	31	N/A	12	0.42
152926	1	7	29	N/A	N/A	N/A
152183	1	7	25	N/A	109	0.32
153146	1	7	27	N/A	106	0.54
153437	1	7	31	N/A	N/A	N/A
153020	1	7	23	N/A	N/A	N/A
152392	1	7	20	N/A	65	0.12
152871	1	7	30	N/A	63	0.30
152445	1	7	23	N/A	93	0.32
152194	1	7	19	N/A	56	0.26
153552	1	7	24	N/A	N/A	N/A
150423	1	6	36	2.00	16	0.48
151000	1	6	28	1.39	N/A	0.24
151426	1	6	20	2.26	N/A	1.76
150918	1	6	21	1.11	34	0.52
151080	1	5	30	2.38	11	0.32
150905	1	6	29	1.42	73	0.22
150768	1	6	26	0.86	31	0.28
151817	1	5	19	2.00	N/A	N/A
151622	1	6	33	N/A	71	0.28
151639	1	6	20	0.63	39	0.62
151640	1	6	24	0.94	108	0.62
152218	1	6	28	1.15	71	0.24
152283	1	6	35	2.00	104	0.76
153104	1	6	21	4.00	113	0.26

Appendix II : Abnormal Pregnancy Raw Data

Lab. No.	Type Abnorm.	Maternal Age	Gestn. (Weeks)	AFP KU/L	HCG IU/ml	UE3 nmol/L
151531	Tri-21	27	13	15.1	141	1.3
152056	Tri-21	24	8	2.1	85	0
157126	Tri-21	29	9	3.9	122	0.2
161233	Tri-21	25	13	8.0	96	1.58
162617	Tri-21	23	13	17.0	69	1.21
161566	Tri-21	29	9	1.0	136	0.50
162804	Tri-21	29	9	4.0	92	0
163154	Tri-21	36	7	1.0	78	0.28
162632	Tri-21	21	14	11.0	240	1.38
165443	Tri-21	31	11	1.0	45	0.18
165913	Tri-21	24	13	12.0	48	NA
163610	Tri-21	30	10	12.0	70	0.30
152190	Tri-21	35	14	13.2	38	0.75
923164	Tri-21	39	10	7.0	58	0.21
923428	Tri-21	28	13	10.0	47	1.29
968731	Tri-21	25	10	6.6	32	0
163898	Tri-18	33	13	12.0	13	0.60
911399	Tri-18	37	11	6.0	42	0
907057	Tri-18	35	11	6.0	10	0.24
911439	Tri-18	43	8	4.0	13	0.23
957231	Tri-18	39	12	10.7	31	0.23
151211	Tri-13	28	15	8.2	35	5.06
152390	Anenc.		8	3.0	227	0.94
154175	Anenc.		8	2.0	205	0.74
160034	Anenc.		10	5.0	165	0.34
156492	Sp.Bif.		9	3.0	58	0.55
160086	Sp.Bif.		10	5.0	135	0.64
152514	Sp.Bif.		10	4.0	62	0.74
161106	Sp.Bif.		11	6.0	40	0.54
165794	Sp.Bif.		11	11.0	63	0.67
152762	Sp.Bif.		12	19.0	77	1.31
160374	Sp.Bif.		12	14.0	16	1.07
160582	Sp.Bif.		12	19.0	82	0.70
164348	Sp.Bif.		12	16.0	39	NA
153931	Sp.Bif.		13	18.0	39	0.91
152199	Sp.Bif.		14	64.0	44	2.04

Bibliography

Adlercreutz H and Luukkainen T (1970).
Identification and determination of oestrogen in various biological materials in pregnancy.
Ann. of Clin. Research, 2:365-380.

Adock E W et al (1973).
HCG: Its possible role in maternal lymphocyte suppression.
Science, p.118-845.

Aitken D A, McCaw G, Crossley J A, Berry E, Connor J M, Spencer K and Macri J N (1993).
Biochemical screening for chromosome abnormalities and neural tube defects in the first trimester.
J. Med. Genet., Vol. 30, No. 4:336.

Barkai G, Shaki R, Pariente C and Goldman B (1987).
First trimester alphafetoprotein levels in normal and chromosomally abnormal pregnancies.
The Lancet, ii:p.389.

Bartles I and Lindemann A (1988).
Maternal levels of pregnancy-specific B1-glycoprotein (SP1) are elevated in pregnancies affected by Down's syndrome.
Hum. Genet., 80:46-48.

Bergstrand C D and Czar B (1956)
Demonstration of a new protein fraction in serum from the human foetus.
Scandanavian J. of Clin. Investig., 8:174.

Bogart M H, Pandian M R, Jones O W (1987).
Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities.
Prenatal Diagnosis, Vol. 7:623-630.

Bogart M H, Golbus M S, Sorg N D, Jones O W (1989).
Human chorionic gonadotropin levels in pregnancies with aneuploid fetuses.
Prenatal Diagnosis, Vol. 9:379-384.

Brambati B, Simoni G, Bonacchi I and Piceni L (1986).
Fetal chromosomal aneuploidies and maternal serum alphafetoprotein levels in first trimester.
The Lancet, ii:165.

Brambati B, MacIntosh M C M, Teisner B, Maguiness S, Shrimanker K, Lanzani A, Bonacchi I, Tului L, Chard T and Grudzinskas J G (1993).
Low maternal serum levels of pregnancy associated plasma protein-A (PAPP-A) in the first trimester in association with abnormal fetal karyotype.
Br. J. Obstet. Gynaecol., Vol. 100:324-326.

Brock D J H and Sutcliffe R G (1972).
Alphafoetoprotein in the antenatal diagnosis of anencephaly and spina bifida.
The Lancet, ii:197-199.

Brock D J H and Sutcliffe R G (1973).
Prenatal diagnosis of anencephaly.
Biochem. Soc. Transactions, i:149-152.

Brock D J H, Bolton A E, Scrimgeour J B (1974).
Prenatal diagnosis of spina bifida and anencephaly through maternal plasma alphafoetoprotein measurement.
The Lancet, i:767.

Brock D J H, Barron L, Holloway S, Liston W A, Hillier S G, Seppala M (1990).
First trimester maternal serum biochemical indicators in Down's syndrome.
Prenatal Diagnosis, Vol. 10:245-251.

Brody S and Carlstrom G (1965).
Human chorionic gonadotropin in abnormal pregnancy. Serum and urinary findings using various immunoassay techniques.
Acta Obstet. Gynaec., Scandinav., 44:32-44.

Canick J A, Knight G J, Palomaki G E, Haddow J E, Cuckle H S and Wald N J (1988).
Low second trimester serum unconjugated oestriol in pregnancies with Down's syndrome.
Brit. J. Obstet. and Gynaecol., 95:330-333.

Canick J A, Knight G J, Palomaki G E, Haddow J E (1990).
Second trimester levels of maternal serum unconjugated oestriol and human chorionic gonadotropin in pregnancies affected by fetal anencephaly and open spina bifida.
Prenatal Diagnosis, Vol. 10:733-737.

Check J H, Nowroozi K, Vaze M, Wapner R, Seefried S (1990).
Very high CA 125 levels during early first trimester in three cases of spontaneous abortion with chromosomal abnormalities.
Am. J. Obstet. Gynecol., 162:674-5

Connor J M and Ferguson-Smith M A (1992a).
Essential Medical Genetics, 3rd. edition, Blackwell Scientific Publications, Oxford:p. 195.

Connor J M and Ferguson-Smith M A (1992b).
Ibid.:p. 8.

Connor J M and Ferguson-Smith M A (1992c).
Ibid.:p. 132.

Cowchock F S and Ruch D A (1985).
Low maternal serum AFP and Down's syndrome.
The Lancet, ii:161-162.

Crossley J A, Aitken D A, Connor J M (1991a).
Prenatal screening for chromosome abnormalities
using maternal serum Chorionic Gonadotrophin,
Alphafetoprotein and age.
Prenatal Diagnosis, 11:83-101.

Crandall B F, Golbus M S, Goldberg J D, Matsumoto M (1991).
First trimester maternal serum unconjugated oestriol and alphafoetoprotein in foetal Down's protein.
Prenatal Diagnosis, Vol. 11:377-380.

← 1991a
Crossley J A, McCaw G, Aitken D A, Cameron A, Pont J M, Whittle M J, Connor J M (1991b).
A prospective trial of prenatal screening for chromosome abnormalities using maternal serum hCG and AFP levels.
Jour. Med. Genet., 28:565-566.

Cuckle H S, Wald N J, Lindenbaum R H (1984).
Maternal serum alphafoetoprotein measurement: a screening test for Down's syndrome.
The Lancet, April 28:926-929.

Cuckle H S, Wald N J, Thompson S G (1987).
Estimating a woman's risk of having a pregnancy with Down's syndrome using her age and serum alphafoetoprotein level.
Brit. J. of Obstet. and Gynaecol., Vol. 94:387-402.

Cuckle H S, Wald N J, Barkai G, Fuhrmann W, Altland K, Brambati B, Knight G, Palomaki G, Haddow J E, Canick J (1988).
First trimester biochemical screening for Down syndrome.
The Lancet, Oct. 8, (1988): 851-852.

Diczfalusy E and Mancuso S (1969).
Oestrogen metabolism in pregnancy.
From Foetus and Placenta, eds. Klopper A and Diczfalusy E, Blackwell Scientific publications, Oxford: 191-248.

Doran T, Cadesky K, Wong P, Mastrogiacomo C, Capello T (1986).
Maternal serum alphafetoprotein and foetal autosomal trisomies.
Am. J. Ob. Gyn., 154(2):277-281.

Edwards P R and Ekins R P (1983).
Mass action model based microprocessor program for RIA data processing.
In: Hunter W M and Corrie J E T eds.: "Immunoassays for Clinical Chemistry", 2nd. edn., Churchill-Livingstone, Edinburgh: 640-652.

Ferguson-Smith M A (1983).
The reduction of anencephalic spina bifida births by maternal serum alphafoetoprotein screening.
Brit. Med. Bulletin, 39:365-372.

Ferguson-Smith M A and Yates J R W (1984).
Maternal age specific rates for chromosome aberrations and factors influencing them: Report of a collaborative European study on 52,965 amniocentesis.
Prenatal Diagnosis, 4:5-44.

Frandsen V A and Stakemann G (1964).
The site of production of oestrogenic hormones in human pregnancy.
Acta Endocrinologica, 47:265-276.

Fuhrmann W, Wendt P, Weitzel H K (1984).
Maternal serum AFP as a screening test for Down's syndrome.
The Lancet, ii:413.

Gitlin D and Boesman M (1966).
Serum alphafoetoprotein, albumen and gamma globulin in the human conceptus.
Journ. Clin. Investig., 45:1826-1838.

Gitlin D (1975).
Normal biology of alphfoetoprotein.
Annals of the New York Academy of Science, 259:7-16.

Graham G W, Crossley J A, Aitken D A et al (1992).
Variation in the levels of pregnancy-specific B1-glycoprotein in maternal serum from chromosomally abnormal pregnancies.
Prenatal Diagnosis, 12:505-512.

Harris R, Jennison R F, Barson A J, Laurence K, Ruoslahti E and Seppala M (1974).
The Lancet, i:429.

Hershey D W, Crandall B F, Schroth P S (1985).
Maternal serum alphafoetoprotein screening of foetal trisomies.
Am. J. Obstet. Gynecol., 153:224-225.

Jenkins R L (1933).
Aetiology of mongolism.
Am. J. Dis. Child., 45:506.

Johnson A, Cowchock F S, Darby M, Wapner R, Jackson L G (1991).
First trimester maternal serum alphafoetoprotein and chorionic gonadotropin in aneuploid pregnancies.
Prenatal Diagnosis, Vol. 11:443-450.

Knight G J, Palomaki G E, Haddow J E (1988).
Use of maternal serum alphafoetoprotein measurements to screen for Down's syndrome.
Clin. Obstet. Gynecol., 31:306-327.

Knight G J, Palomaki G E, Haddow J E et al (1989).
Maternal serum levels of the placental products hCG, hPL, SP1 and progesterone are all elevated in cases of foetal Down's syndrome.
Am. J. Hum. Genet., 45:261.

Knight G J, Palomaki G E, Haddow J E, Miller W, Bersinger N and Schneider H (1993).
Pregnancy associated plasma protein-A as a marker for Down's syndrome in the second trimester of pregnancy.
Prenatal Diagnosis, Vol. 13, No. 3:222-223.

Kratzer P G, Golbus M S, Monroe S E, Finkelstein D E, Taylor R N (1991).
First trimester aneuploidy screening using serum human chorionic gonadotropin (hCG), free alpha-hCG, and progesterone. Prenatal Diagnosis, Vol. 11:751-765.

Lauritzen C (1971).
Placental steroidogenesis.
Excerpta Medica: 137-157.

Leek A E, Ruoss C F, Kitau C F, Chard T (1973).
Raised alphafetoprotein in maternal serum with anencephalic pregnancy.
The Lancet, ii:385.

Lunenfeld B and Insber V (1978).
Gonadotropins In: Diagnosis and treatment of functional infertility,
Grosse Velag Berlin, pp 66-68.

Macri J N, Kasturi R V, Krantz D A et al (1990 a).
Maternal serum Down's syndrome screening: Unconjugated oestriol is not useful.
AM. J. Obstet. Gynecol., 162:672-673.

Macri J N, Kasturi R V, Krantz D A et al (1990 b).
Maternal serum Down's syndrome screening: Free-B protein is a more effective marker than human chorionic gonadotropin.
Am. J. Obstet. Gynecol., 163:4,1.1248-1253.

Mantingh A, Marrink J, de Wolf B, Breed A S P M, Beekhuis J R (1988).
Low maternal serum alpfafoetoprotein at 10 weeks geatation and fetal Down's syndrome.
Short Communications:499-500.

Merkatz I R, Nitowsky H M, Macri J N, Johnson W E (1984).
An association between low maternal serum alphafoetoprotein and fetal chromosomal abnormalities.
Am. J. Obstet. and Gynecol., April 1:886-894.

Milunski A, Wands J, Brambati B, Bonacchi I, Currie K (1988).
First trimester maternal serum alphafoetoprotein screening for chromosome defects.
Am. J. Obstet. Gynecol., Vol. 159, no.5:1209-1213

Murday V and Slack J (1985).
Screening for Down's syndrome in the North East Thames region.
Br. Med. J., 291:1315-1318.

Nebiolo L M, Ozturk M, Brambati B, Miller S, Wands J, Milunsky A (1990).
First trimester serum alphafoetoprotein and human chorionic gonadotropin screening for chromosome defects.
Prenatal Diagnosis, Vol. 10:575-581.

Penrose L S (1933).
The relative effects of paternal and maternal age in mongolism.
J. Genet., 27:219.

Report of UK Collaborative study on Alphafoeto-protein in relation to Neural-Tube Defects (1977).
Maternal serum alphafoetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy.
The Lancet, 25 June:1323-1332.

Rothfield B et al, in "Nuclear Medicine in Vitro",
J. P. Lippincott Co., Philadelphia, 1974.
pp. 220-230.

Ruoslahti E. and Hirai H. (1978).
Alphafoetoprotein (Collaborative review).
Scandanavian J. of Immunology, 8, Supp. 8:3-26.

Seller M D, Singer J D, Coltart T M, Campbell S (1974).
Maternal serum alphafoetoprotein levels and prenatal diagnosis of neural tube defects.
The Lancet, i:428.

Seller M J (1984).
Prenatal screening for Down's syndrome.
The Lancet, i:1359.

Shalev E, Zalel Y, Dan U, Sacran W, Rakover Y, Weiner E (1992).
Maternal serum alphafoetoprotein in the first trimester cannot predict neural tube defects.
Prenatal Diagnosis, Vol. 12:309-312.

Siiteri P K and MacDonald P C (1966).
Placental estrogen biosynthesis during human pregnancy.
J. Clin. Endocrin. and Metab., 26:751-761.

Smith A.D., Wald N.J., Cuckle H.S., Stirrat G.H., Bobrow M., Lagercrantz H. (1979).
Amniotic fluid acetylcholinesterase as a possible diagnostic test for neural tube defects in early pregnancy.
The Lancet, i:685-688.

Spencer K, Macri J N, Aitken D A, Connor J M (1992).
Free-B HCG as First Trimester Marker for Foetal Trisomy.
The Lancet, 339:1480.

Spencer K and Carpenter P (1985).
Screening for Down's syndrome using serum alphafoetoprotein:
A retrospective study indicating caution.
Br. Med. J., 290:1940-1943.

Stabile I, Grudzinskas J G, Chard T (1988).
Review: Clinical applications of pregnancy protein
estimations with particular reference to
pregnancy-associated plasma-protein-A (PAPP-A).
Obstet. Gynecol. survey, Vol. 43, No. 2:73-82.

Stevenson J D, Chapman R S, Perry B, Logue F C (1987).
Evaluation and clinical application of a two site
immunoradiometric assay for alpha-1- foetoprotein using
readily available reagents.
Ann. Clin. Biochem. 24:411-418.

Tabor A, Nordgaard-Pederson B and Jacobson J C (1984).
Low maternal serum AFP and Down's syndrome.
The Lancet, ii:161.

Talerman A., Haije W.G., Baggerman L. (1978).
Histological patterns in germ cell tumours associated with
raised serum AFP.
Scandanavian J. of Immunology, 8, 8:97-102.

Vaitukaitis J (1977).
J. Human Chorionic Gonadotropin in "Endocrinology of
Pregnancy", Fuchs and Klopper (eds.).
Harper and Row, New York:p.67

Van Lith J M M, Mantingh A, Beekhuis J R, de Bruijn H W A
(1991).
First trimester CA 125 and Down's syndrome.
Brit. J. Obst. and Gynaecol., Vol. 98:493-494.

Van Lith J M M reporting for the Dutch working party on
prenatal diagnosis (1992).
First trimester maternal serum human chorionic gonadotrophin
as a marker for fetal chromosomal disorsers.
Prenatal Diagnosis, Vol. 12:495-504.

Wald N J, Brock D J H, Bonnar J (1974).
Prenatal diagnosis of spina bifida and anencephaly by
maternal serum alphafoetoprotein measurement
The Lancet, i:765.

Wald N J, Cuckle H S , Densem J W, Nanchahal K, Royston P, Chard P, Haddow J E, Knight G J, Palomaki G E, Canick J A (1988a).
Maternal serum screening for Down's syndrome in early pregnancy.
Brit. Med. J., Vol. 297:883-887.

Wald N J, Cuckle H S, Densem J W, Nanchahal K, Canick J A, Haddow J E, Knight G J, Palomaki G E (1988b).
Maternal serum unconjugated oestriol as an antenatal test for Down's syndrome.
Brit. J. of Obstet. and Gynaecol., Vol. 95:334-341.

Wald N J, Stone R, Cuckle H S, Grudzinskas J G, Barkai G, Brambati B, Teisner B, Fuhrmann W (1992).
First trimester concentrations of pregnancy associated plasma protein-A and placental protein 14 in Down's syndrome.
Brit. Med. J., 305:391-394.

Wenger D, Miny P, Holzgreve W, Fuhrmann W, Altland K (1990).
First trimester maternal serum alpfafoetoprotein screening for Down's syndrome and other aneuploidies.
Am. J. Med. Gen. Supp., 7:89-90.

Whittle M J (1992).
Screening for foetal anomalies.
Curr, Obstet. Gynaecol., 2:72-76.

Yalow R S and Berson R P (1960).
Immunoassay of endogenous plasma insulin in man.
Journ. of Clin. Investig., 39:1157-1175.