BIOCHEMICAL MARKERS AND THE PATHOPHYSIOLOGY OF CHROMOSOMALLY ABNORMAL PREGNANCIES.

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ABBREVIATIONS.

AFP	alphafetoprotein
ALP	alkaline phosphatase
AMPD	2-amino-2-methyl-1,3-propandiol
BSA	bovine serum albumin
CA 125	cancer antigen 125
CdL	Cornelia de Lange syndrome
cm	centimetre
Con-A	concanavalin A
CV	co-efficient of variation
Da	dalton
DAB	diaminobenzidine
DNA	deoxyribonucleic acid
EECF	extra embryonic coelomic fluid
FαhCG	free alpha human chorionic gonadotropin
FβhCG	free beta human chorionic gonadotropin
FSH	follicle stimulating hormone
g	gramme
GGT	gamma glutamyl transferase
HBR	heterophillic blocking reagent
hCG	human chorionic gonadopin
hPL	human placental lactogen
hr	hour
HRP	horseradish peroxidase
IFNα	interferon α
IgG	immunoglobulin G
IU	International Units
kDa	kilodalton

kU	kilo-units
l	litre
LH	luteinising hormone
LMP	last menstrual period
M	molar
mUI	milli international units
ml	millilitre
MBP	eosinophil major basic protein
mg	milligramme
MOM	multiples of the median
mm	millimetre
mM	millimolar
MU	mega units
4-MU	4-methylumbliferone
4-MUP	4-methylumbelliferyl phosphate
NHS	normal human serum
OPD	ortho-phenylenediamine
PAPP-A	pregnancy associated plasma protein-A
PBS	phosphate buffered saline
PLD	phospholipase-D
proMBP	pro-form of eosinophil major basic protein
rpm	revolutions per minute
SP1	pregnancy specific beta 1 glycoprotein
TMB	3,3',5,5' tetramethylbenzidine
TSH	thyroid stimulating hormone
U	units
UE_3	unconjugated oestriol
US	ultrasound

URNAP	urea-resistant alkaline phosphatase
v/v	volume to volume
w/v	weight to volume
ηm	nanometre
ηmoles	nanomoles
ηM	nanomolar
μg	microgramme
μl	microlitre
μm	micrometre
μM	micromolar

SUMMARY.

The feto-placental unit synthesises a variety of proteins and hormones which are secreted into the maternal circulation and amniotic fluid from early pregnancy. In pregnancies where the fetus has an autosomal trisomy, the normal concentration profiles of these markers in maternal serum and amniotic fluid are disturbed. These marker changes can be used to estimate the risk that a pregnancy is affected by Down's syndrome (or Trisomy 18) and thus allow the parents to make an informed decision regarding prenatal diagnosis by invasive testing. However, the factors which give rise to the varying patterns of marker concentrations in chromosomally abnormal pregnancies are poorly understood. The aim of this project was to investigate the underlying causes of abnormal marker concentrations in Down's syndrome, Trisomy 13 and Trisomy 18 pregnancies. Endogenous levels of a series of products of the feto-placental unit were measured in fetal tissues and in corresponding maternal serum and amniotic fluid samples from affected pregnancies, and compared with the results obtained in the same tissues from unaffected pregnancies.

Samples of fetal liver, fetal ileum and placental tissue were collected from 71 chromosomally abnormal pregnancies (67 Down's syndrome, one Trisomy 13, three Trisomy 18) following prenatal diagnosis by chorionic villous sampling (CVS) or amniocentesis and termination of pregnancy at 10 to 24 weeks gestation. Corresponding tissues for use as controls were obtained from 75 putatively normal pregnancies terminated for psycho-social reasons at 12 to 22 weeks gestation.

Alphafetoprotein (AFP), intact human chorionic gonadotropin (hCG), free beta human chorionic gonadotropin (FβhCG), pregnancy associated plasma protein-A (PAPP-A), pregnancy specific beta-1 glycoprotein (SP1), total and placental alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) were measured in placental tissue, AFP and GGT in fetal liver and GGT in fetal ileum from affected and unaffected pregnancies. Specific immunoassay methods were used to measure AFP, intact hCG,

FβhCG, PAPP-A and SP1. Fluorometric and spectrophotometric kinetic assays were used to measure ALP isoenzymes and GGT respectively. Marker levels in the fetal tissues were expressed as units per mg of total protein and converted to multiples of the control median (MOM) at the appropriate week of gestation.

FβhCG, PAPP-A, SP1, total ALP and placental ALP levels were measured in maternal serum previously collected from 58 of the Down's syndrome pregnancies and two of the Trisomy 18 pregnancies. AFP and intact hCG concentrations (expressed as MOM) in maternal serum from 62 of the Down's syndrome pregnancies and two of the Trisomy 18 pregnancies were obtained from the West of Scotland routine screening database. Normal medians for SP1, total ALP and placental ALP were established by analysis of 114 randomly selected maternal serum samples at 15-20 weeks gestation. Weekly maternal serum FβhCG and PAPP-A medians were available from previous studies in this laboratory. Marker levels in maternal serum from affected and unaffected pregnancies were converted to MOM.

Thirty four of the Down's syndrome pregnancies and one of the Trisomy 18 pregnancies from which fetal tissues were collected were prenatally diagnosed following amniocentesis. Amniotic fluid AFP levels were measured prospectively in these cases as part of the routine analysis of AFP in all amniotic fluid specimens. Amniotic fluid samples from 33 Down's syndrome and one Trisomy 18 pregnancy were available from frozen storage for further marker analysis. Normal medians for intact hCG, FβhCG, PAPP-A, SP1, total ALP, placental ALP and GGT levels were established by analysis of 132 randomly selected amniotic fluid samples at 15-21 weeks gestation. Marker levels in amniotic fluid from affected and unaffected pregnancies were converted to MOM.

Placental localisation of AFP, hCG, PAPP-A, SP1 and placental ALP was investigated by immunohistochemistry in placental tissue from 16 Down's syndrome and 16 unaffected pregnancies, arranged into pairs matched for gestational age (14-20

weeks). Frozen tissue sections were prepared for light microscopy by a two-step indirect immunoperoxidase staining technique.

AFP levels in fetal liver from Down's syndrome pregnancies were not significantly different from the controls (Down's syndrome median MOM = 0.97; control median MOM = 1.01; p=0.259). AFP levels were significantly elevated in placental tissue from Down's syndrome pregnancies (2.43 MOM; p<0.001), while maternal serum and amniotic fluid AFP levels were reduced to 0.69 MOM and 0.87 MOM respectively. There was no correlation between tissue, maternal serum and amniotic fluid concentrations of AFP in Down's syndrome pregnancies. AFP was localised to the syncytiotrophoblast in placental tissue from both Down's syndrome and unaffected pregnancies. Synthesis of AFP by the fetal liver in Down's syndrome pregnancies does not appear to be altered in the first half of pregnancy. Elevated placental levels of AFP in Down's syndrome pregnancies suggest that AFP is accumulating in the syncytiotrophoblast and therefore reduced maternal serum levels of AFP may be caused by defective placental transport of AFP from the fetal circulation to the maternal circulation. Low amniotic fluid AFP levels in Down's syndrome pregnancies may result from impaired excretion of AFP by the fetal kidneys.

Intact hCG levels were elevated in placental tissue (4.06 MOM; p<0.001), maternal serum (2.72 MOM) and amniotic fluid (1.39 MOM; p=0.017) with significant correlation (p<0.02) between the three compartments in the Down's syndrome pregnancies. FβhCG levels were also elevated in placental tissue (3.40 MOM; p<0.001), maternal serum (3.06 MOM) and amniotic fluid (1.33 MOM; p=0.017) from Down's syndrome pregnancies. There was also a significant correlation (p<0.002) between placental and maternal serum levels of FβhCG, and between maternal serum and amniotic fluid FβhCG levels. HCG was localised to the syncytiotrophoblast in placental tissue from both Down's syndrome and unaffected pregnancies. The similarity in the change in levels of intact hCG and FβhCG between placenta, maternal serum and amniotic fluid suggests that in Down's syndrome pregnancies secretion of intact hCG and FβhCG

from the syncytiotrophoblast into the maternal circulation is not altered, and that higher maternal serum levels may be the result of increased placental synthesis of hCG subunits.

PAPP-A levels in placental tissue, maternal serum and amniotic fluid from Down's syndrome pregnancies were not significantly different from controls (placental median MOM = 0.96, p=0.304; maternal serum median MOM = 0.92; amniotic fluid median MOM = 0.95, p=0.126). Despite the similarity in the median MOM levels of PAPP-A in the different feto-maternal compartments, there was no significant correlation between placental, maternal serum and amniotic fluid PAPP-A levels. Placental synthesis and secretion of PAPP-A does not appear to be disturbed in Down's syndrome pregnancies in the second trimester.

SP1 levels in placental tissue (1.79 MOM) and maternal serum (1.33 MOM) from Down's syndrome pregnancies were significantly higher ($p<0.001$) than in unaffected pregnancies. However, amniotic fluid SP1 levels in Down's syndrome pregnancies (0.91 MOM) were not significantly different from normal (p=0.749). In contrast to intact hCG and FβhCG , there was no correlation between placental levels of SP1 and either maternal serum or amniotic fluid SP1 levels in Down's syndrome pregnancies. However, maternal serum and amniotic fluid SP1 levels in Down's syndrome pregnancies were significantly correlated ($p<0.001$). SP1 was localised to the syncytiotrophoblast in placental tissue from both Down's syndrome and unaffected pregnancies.

There was no difference in the activity of the placental isoenzyme of ALP in placental tissue from Down's syndrome pregnancies (0.91 MOM) compared to controls (p=0.318). In maternal serum from Down's syndrome pregnancies, total ALP activity was reduced to 0.83 MOM (p=0.002) of the unaffected pregnancies but placental ALP activity (1.04 MOM) was not significantly different from normal (p=0.505). In amniotic fluid from Down's syndrome pregnancies, placental ALP (0.75 MOM) was reduced but

to a much smaller extent than total ALP activity (0.39 MOM). There was some degree of correlation between placental ALP activity in placental tissue and in corresponding maternal serum and amniotic fluid samples from Down's syndrome pregnancies, however this was not statistically significant. Placental ALP was localised to the syncytiotrophoblast in placental tissue from both Down's syndrome and unaffected pregnancies. This suggests that placental synthesis and transport of placental ALP from the microvillar membrane of the syncytiotrophoblast is not disturbed in Down's syndrome pregnancies. However, low amniotic fluid placental ALP activity may indicate a reduction in the rate of transfer of the enzyme from the maternal circulation to the amniotic fluid or reduced synthesis of placental ALP from an alternative source. The substantial reduction in total amniotic fluid ALP activity in Down's syndrome pregnancies indicates a possible reduction in the tissue non-specific and/ or intestinal isoenzymes.

GGT activity was significantly elevated in extracts of fetal liver (2.01 MOM; $p<0.001$) and placental tissue (1.29 MOM; $p<0.01$), and significantly reduced in extracts of fetal ileum (0.43 MOM; $p<0.001$) from Down's syndrome pregnancies. In amniotic fluid from Down's syndrome pregnancies, GGT activity was significantly reduced to 0.46 MOM ($p<0.001$) of the unaffected pregnancies. There was no significant correlation between GGT activity in fetal tissues and amniotic fluid from the Down' syndrome pregnancies. However, the similarity between the median MOM levels of GGT activity in fetal ileum and amniotic fluid from the Down's syndrome pregnancies suggests that GGT activity in amniotic fluid is of fetal intestinal origin. There appears to be tissue-specific differences in the expression of GGT in Down's syndrome pregnancies.

Due to the small numbers of Trisomy 13 and Trisomy 18 pregnancies included in this study, it was not possible to identify likely causes of the marker variations associated with fetal Trisomy 13 and Trisomy 18. However, the variation in fetal tissue, maternal serum and amniotic fluid marker concentrations between Down's syndrome, Trisomy 13 and Trisomy 18 pregnancies suggests that the mechanisms giving rise to the

abnormal marker levels associated with the different autosomal trisomies are not the same.

The results of this investigation indicate that in Down's syndrome pregnancies, maternal serum levels of placental products reflect those found in the placenta: intact hCG, FβhCG and SP1 levels were elevated while PAPP-A and placental ALP levels were little changed. This suggests that transport of these proteins from the placenta into the maternal circulation is not affected but there is altered synthesis of hCG subunits and SP1. Hepatic synthesis of AFP does not appear to be altered in Down's syndrome pregnancies, but increased placental and reduced maternal serum levels of AFP point to a possible placental transport defect specific to AFP. Similarly reduced GGT levels in fetal intestine and in corresponding amniotic fluid from Down's syndrome pregnancies suggest that amniotic fluid GGT activity is of fetal intestinal origin since GGT activity was elevated in fetal liver and placental from the same series of Down's syndrome pregnancies.

CHAPTER 1.

INTRODUCTION.

1.1 CHROMOSOME ABNORMALITIES.

1.1.1 DOWN'S SYNDROME (TRISOMY 21).

Down's Syndrome is the most common autosomal chromosome abnormality with an overall birth incidence of 1/700 live births. The incidence of Down's syndrome is increased at advanced maternal age. The main complication associated with Down's syndrome is mental handicap. Presenile dementia is common in Down's syndrome individuals after 40 years of age, apparently caused by Alzheimers-like lesions in the brain. Other features of Down's syndrome include heart defects, increased susceptibility to infection, respiratory ailments and leukaemia, duodenal atresia and hypotonia. Individuals with Down's syndrome have characteristic physical features such as shorter than average height, flat facial profile, small low set ears, small nose, upwards slanting eyes, slightly protuberant tongue and single palmar creases (Cooper and Hall 1988, Serra and Neri 1990, Connor and Ferguson-Smith 1991).

In 95% of cases, trisomy 21 arises from meiotic non-disjunction mainly occurring at the first meiotic division but sometimes at the second. Mosaicism, the presence of normal and trisomic cell lines, occurs in approximately 1% of Down's syndrome individuals and in such cases the clinical features tend to be less severe. Approximately 4% of Down's syndrome cases are caused by a translocation involving chromosome 21, either inherited or de-novo.

It is not fully understood how the Down's syndrome phenotype arises from the presence of an extra copy of chromosome 21. The occurrence of Down's syndrome as a result of translocations involving chromosome 21 led to the realisation that an extra copy of the entire chromosome 21 is not required to cause Down's syndrome. Triplication of a region of the long arm of chromosome 21 (Down's syndrome 'critical region') at 21q22, is thought to be sufficient for the expression of the Down's syndrome phenotype. However, this does not exclude the possibility that regions of

chromosome 21 outside the critical region may be involved in the pathogenesis of Down's syndrome.

1.1.2 EDWARDS' SYNDROME (TRISOMY 18).

Edwards' syndrome is the second most common aneuploidy at term with an incidence of 1/3000 live births (Connor and Ferguson-Smith 1991). Clinical features associated with Trisomy 18 include low birth weight, heart defects, small chin, low set malformed ears, short sternum, clenched hands and single palmar creases. Only 10% of infants with Trisomy 18 survive beyond one year. The majority of trisomy 18 cases result from parental non-disjunction at meiosis either during the first or second meiotic divisions. Trisomy 18 rarely results from a translocation and mosaicism is occasionally observed. The incidence of trisomy 18 is increased at advanced maternal age.

1.1.3 PATAU'S SYNDROME (TRISOMY 13).

The incidence of Patau's syndrome (Trisomy 13) is 1/5000 live births (Connor and Ferguson-Smith 1991). In the majority of cases Trisomy 13 is caused by meiotic non-disjunction at either the first or second meiotic divisions. Around 20% of cases result from parental translocation and mosaicism is present in 5% of patients. The incidence of Trisomy 13 is increased at advanced maternal age. Clinical features associated with Trisomy 13 include heart defects, hypotelorism, cleft lip and palate, redundant skin around the nape of the neck, abnormal ears, clenched fists and single palmar creases. Only 10% of affected babies survive beyond one year.

1.2 PRENATAL DIAGNOSIS OF CHROMOSOME ABNORMALITIES.

Autosomal trisomies and other serious chromosome abnormalities can be diagnosed prenatally by fetal chromosome analysis of cultured amniotic fluid, chorionic villous or fetal blood cells. The diagnosis of such chromosome abnormalities in utero provides

the option of selective termination of affected pregnancies or can allow adequate preparation for the birth of a disabled child. However, amniocentesis, chorionic villous sampling and fetal blood sampling are invasive techniques and all carry defined risks for the pregnancy (MacLachlan 1992, Silverman and Wapner 1992, Nicolini and Rodeck 1992). These techniques are also time consuming and expensive, and for these reasons it is not desirable for all women to have such a diagnostic test. Prenatal screening for chromosome abnormalities, in particular Down's syndrome, provides a means of identification of women with a risk of carrying an affected fetus high enough to justify diagnostic testing.

1.3 PRENATAL SCREENING.

Various steroids and proteins produced in the feto-placental unit and secreted into the maternal circulation have been demonstrated to be of potential clinical importance in the identification of pregnancies in which there is a fetal disorder. The concentration of certain pregnancy markers in the maternal circulation and amniotic fluid has been shown to vary in pregnancies associated with fetal abnormalities compared to the level detected in normal pregnancies. Although such variation is not diagnostic of a fetal abnormality, analysis of certain markers in the maternal serum may be used to identify those pregnancies which are at increased risk of being associated with a particular fetal disorder. By identifying which pregnancies are at risk by this process of prenatal screening, a diagnostic test can be offered to confirm or exclude the presence of a fetal abnormality.

Biochemical analysis of markers in maternal serum requires only that a venous blood sample be taken from the mother and therefore does not hazard the pregnancy. As prenatal screening and diagnosis is intended only to identify pregnancies associated with a fetal disorder and cannot offer a cure, the only preventative measure is termination of the pregnancy.

1.3.1 PRENATAL SCREENING FOR CHROMOSOME ABNORMALITIES.

The risk of a pregnancy being associated with an autosomal trisomy increases with advancing maternal age. For this reason, advanced maternal age was originally the most important criterion on which women were offered fetal chromosome analysis for the diagnosis of autosomal trisomies. Women aged 35 years and over (around 7% of the pregnant population) were considered to be at high risk and were offered a diagnostic test. This form of screening however, proved to be less than successful (Stone et al 1989) as only about 30% of autosomal trisomies occur in women of that age group. Thus, maternal age screening has a major disadvantage in that it misses 70% of all autosomal trisomy cases.

The discovery that Down's syndrome and other autosomal trisomy pregnancies were associated with reduced levels of maternal serum AFP (Cuckle et al 1984, Merkatz et al 1984) raised the potential for improving screening for chromosome abnormalities. Several studies have shown that maternal serum AFP levels are reduced to around 70% of normal in Down's syndrome pregnancies and by using the risks derived from AFP levels and maternal age, it was estimated that screening performance could be improved to a detection rate of 37% of all autosomal pregnancies for no increase in the follow up rate accepted for maternal age screening (Cuckle et al 1987, Zeitune et al 1991).

It has also been shown that there are variations in the levels of other maternal serum markers in autosomal trisomy pregnancies. The main Down's syndrome markers are AFP, human chorionic gonadotropin (hCG) and unconjugated oestriol (UE3), and screening for Down's syndrome by measurement of some or all of these markers is well established in the United Kingdom. In such screening programmes, a woman's risk of having a pregnancy associated with Down's syndrome is estimated by combining her maternal age risk and a risk derived from the levels of the markers in the maternal serum. If a woman has a risk greater than a particular cut-off point,

diagnostic testing is advised. There is also interest in extending this type of multiple marker screening to detect trisomy 18 pregnancies.

At present, if a woman is diagnosed as having an autosomal trisomy pregnancy, the only options available are to continue the pregnancy to term or to terminate the pregnancy. In practice, most women with affected pregnancies choose to avoid the birth of a disabled child. Selective termination of affected pregnancies in the first trimester is medically safer than at later gestations and may also help to reduce the emotional stress of the parents. It would therefore be desirable if screening procedures could be moved to the first trimester of pregnancy enabling termination of pregnancy to be carried out at an earlier stage. For this reason, the variation in the levels of many maternal serum markers in the first trimester of autosomal trisomy pregnancies has also been investigated (Macintosh and Chard 1992, Macintosh and Chard 1993). For many markers, the concentration changes seen in trisomic pregnancies are of a different magnitude at different stages of pregnancy.

1.4 BIOCHEMICAL MARKERS.

As a result of the immense amount of interest that biochemical screening for chromosome abnormalities has generated, numerous pregnancy markers have been investigated in maternal serum, amniotic fluid and fetal tissues from pregnancies affected by fetal Down's syndrome or other chromosome abnormalities. Complex patterns of variation of these markers have emerged, although little is known about the mechanisms which modulate the levels of these markers.

1.4.1 ALPHAFETOPROTEIN.

Alphafetoprotein (AFP) is a fetal-specific and cancer-associated glycoprotein and as such is of considerable clinical value as an indicator of fetal well-being and as a tumour marker.

AFP is a 67-74 kDa protein which belongs to a superfamily of proteins which also includes Vitamin-D binding protein and albumin (Smith and Kelleher 1980, Deutsch 1991). There is considerable structural and sequence homology between the three proteins (Deutsch 1991). The genes for Vitamin-D binding protein, albumin and AFP have been mapped to the long arm of chromosome 4 and are thought to be derived from a common ancestral gene via gene duplication and subsequent divergence. (Weitkamp et al 1966, Harper and Dugaiczyk 1983).

During fetal development AFP is synthesised initially by the yolk sac and then by the fetal liver, although its presence has been detected in other fetal tissues including the gastrointestinal tract and occasionally in the kidney and placenta (van Firth and Adinolf 1969, Gitlin et al 1972, Hustin et al 1980). Only trace amounts of AFP (2-25ng/ml) are detectable in normal adult serum (Gitlin 1975, Rouslahti and Hirai 1978). AFP exists in two distinct forms which can be distinguished by their ability to bind to Concanavalin-A (Con-A) (Ruoslahti et al 1978, Smith and Kelleher 1980). The Con-A reactive form of AFP is derived mainly from the fetal liver and the Con-A non-reactive form is derived from the yolk sac (Ruoslahti et al 1978, Smith and Kelleher 1980).

Abnormal levels of AFP in non-pregnant adult human serum can be caused by a variety of conditions including hereditary persistence of AFP, primary liver cancer, germ cell tumours, hepatitis, liver cirrhosis and tumours of the gastrointestinal tract (Rouslahti and Hirai 1978, Deutsch 1991, McVey et al 1993).

The physiological role of AFP has not yet been defined. AFP binds to a variety of molecules (oestrogens, fatty acids, bilirubin, drugs, metals) and may be involved in a number of physiological processes depending on the ligand to which it is bound (Deutsch 1991). Functions ascribed to AFP include binding and transport, immunoregulation and growth regulation (Ruoslahti and Hirai 1978, Deutsch 1991).

1.4.1.1 AFP in Normal Pregnancy.

The principle sources of AFP in the fetal circulation during early development are the yolk sac and the fetal liver, with the fetal liver becoming the major source as gestation advances (Gitlin et al 1972, Gitlin 1975). AFP levels in the fetal circulation increase to a peak of approximately 3mg/ml at around 13 weeks gestation and then decrease to term (Gitlin and Boesman 1966). Circulating AFP levels decline rapidly after 32 weeks gestation and continue to decline after birth until adult levels are reached by around two years of age (Gitlin and Boesman 1966, Gitlin 1975).

In amniotic fluid, AFP levels increase from 11 weeks gestation to a peak at 13 weeks followed by a decrease to term (Seppala and Rouslahti 1972, Crandall et al 1989, Palomaki et al 1993), similar to the concentration profile of AFP in fetal serum. Wathen et al (1991, 1993) found that amniotic fluid AFP levels were high at 8 weeks gestation with a rapid decline to 11 weeks, after which the concentration profile was identical to that previously described. Amniotic fluid AFP is derived from the fetal circulation through the unkeratinised skin of the fetus in early pregnancy and from the fetal urine once the fetal kidney starts to function (Seppala and Rouslahti 1972, Gitlin and Boesman 1966, Gitlin 1975). The relative concentrations of Con-A reactive and Con-A non-reactive AFP in amniotic fluid changes as gestation advances. The contribution of Con-A non-reactive AFP to total amniotic fluid AFP levels decreases as gestation advances, possibly due to the decline of the yolk sac at the end of the first trimester (Rouslahti et al 1979, Toftager-Larsen 1980, Mackiewicz et al 1984, Jauniaux and Moscoso 1992).

In the maternal circulation AFP levels rise steadily above non-pregnant adult base line levels at 6 to 7 weeks gestation to approximately 450ng/ml at 32 weeks gestation,

after which levels gradually decrease to term (Gitlin 1975, Rouslahti and Hirai 1978). Maternal serum AFP is derived from the fetal serum via the placenta and from the amniotic fluid through the fetal membranes (Gitlin 1975, Los et al 1985).

1.4.1.2 AFP in Complications of Pregnancy.

In twin pregnancies, maternal serum AFP levels are approximately double those found in normal singleton pregnancies (Wald et al 1975, UK Collaborative study 1977).

Brock and Sutcliffe (1972) observed that the concentration of AFP in the amniotic fluid from pregnancies affected by neural tube defects (NTD) was significantly higher than in normal pregnancies. This provided a method for the prenatal diagnosis of NTD (UK Collaborative Study 1979). The discovery that AFP levels are also elevated in the maternal circulation of NTD pregnancies (Brock et al 1974) led to the development of a prenatal screening test for NTD (UK Collaborative Study 1977).

In adult human serum, AFP is normally only present in small amounts. Hereditary persistence of AFP is an autosomal dominant trait in which affected individuals have markedly elevated serum AFP levels (McVey et al 1993). This condition does not appear to be associated with any clinical disability, but during pregnancy can give rise to abnormally high maternal serum AFP levels in cases where the fetus is not affected by a NTD.

Abnormal levels of AFP during pregnancy have also been reported in association with anterior abdominal wall defects, congenital nephrosis, fetal exomphalos, fetal esphogeal atresia, Meckel's syndrome, or threatened abortion and intrauterine fetal death (Ruoslahti and Hirai 1978, Westergaard et al 1985b, Morrow et al 1993). Maternal serum AFP concentrations have been reported to be low in women with

anembryonic pregnancies (Bennett et al 1978), although Stabile et al (1989) were unable to confirm these findings. Second trimester maternal serum AFP levels have been reported to be normal or reduced in women with insulin-dependent diabetes (Wald et al 1979, Reece et al 1987, Canick et al 1990b, Wald et al 1992a).

1.4.1.3 AFP in Chromosomally Abnormal Pregnancies.

The association between reduced maternal serum AFP levels and fetal chromosome abnormalities was first reported by Merkatz et al (1984). AFP levels were significantly reduced in second trimester maternal serum from Down's syndrome, Trisomy 18 and Trisomy 13 pregnancies. Subsequent studies have confirmed this observation, particularly in association with Down's syndrome (Fuhrmann et al 1984, Cuckle et al 1987, Tabor et al 1987, Waller et al 1990, Zeitune et al 1991, Spencer et al 1992a). In a meta-analysis of 24 published studies, including a total of 823 Down's syndrome cases, the overall second trimester maternal serum AFP level in Down's syndrome pregnancies was found to be 0.74 multiples of the median (MOM) (Wald and Cuckle 1992). A similar reduction has also been found in the first trimester of Down's syndrome pregnancies (Cuckle et al 1988, Brock et al 1990, Aitken et al 1993a, Fuhrmann et al 1993, Crandall et al 1993). The association between reduced second trimester maternal serum AFP levels and fetal Trisomy 13 and Trisomy 18 has also been confirmed (Lindenbaum et al 1987, Canick et al 1990a, Miller et al 1991, Zeitune et al 1991, Barkai et al 1993). Reduced maternal serum levels of AFP have also been reported in the first trimester of Trisomy 18 pregnancies (Johnson et al 1991, Aitken et al 1993a, Fuhrmann et al 1993).

Although Merkatz et al (1984) found apparently normal amniotic fluid AFP levels associated with fetal trisomy, other groups have reported that amniotic fluid AFP levels

in Down's syndrome pregnancies are reduced to approximately 70% of normal levels (Cowchock and Ruch 1984, Davis et al 1985, Jones et al 1986, Crandall et al 1988, Kaffe et al 1988, Zeitune et al 1989). Combining the results 10 published studies, including a total of 546 Down's syndrome pregnancies, amniotic fluid AFP levels were below the control group median in 85.3% of affected cases (Zeitune et al 1989). No such association has been found in pregnancies affected by Trisomy 18 or Trisomy 13 (Davis et al 1985, Doran et al 1986, Ashwood et al 1987, Lindenbaum et al 1987, Kaffe et al 1988, Crandall et al 1988, Zeitune et al 1989).

The concentration of AFP in serum from Down's syndrome fetuses has been reported to be normal before 20 weeks gestation, but declines more rapidly than in fetal serum from unaffected pregnancies after 20 weeks (Nicolini et al 1988, Scioscia et al 1988, Seller et al 1990). Cuckle and Wald (1986) reported that AFP levels were significantly reduced to 0.45 (MOM) in cord sera from Down's syndrome babies compared to normal.

1.4.2 HUMAN CHORIONIC GONADOTROPIN.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone which is closely related to the pituitary hormones luteinising hormone (LH), follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH) (Pierce and Parsons 1981). HCG is composed of two non-identical subunits non-covalently linked to form a heterodimer and contains approximately 30% carbohydrate by weight. The α-subunit (15kDa) is identical to that of the other glycoprotein hormones, while the β-subunit (23kDa) is non-identical and confers specific biological activity. Due to the structural homology between hCG and LH the two hormones bind to the same receptors which are present in gonadal tissues and in a number of other tissues including the placenta, uterus,

fetal membranes and decidua (Reshef et al 1990, Lei and Rao 1992). The α-subunit of hCG is coded by a single gene on chromosome 6 (Fiddes and Goodman 1981) and the β-subunit by a cluster of six genes on chromosome 19 (Policastro et al 1986, Graham et al 1987). Talmadge et al (1984) reported that only two of the β-genes were expressed in the placenta, however more recent studies suggest that at least five β-genes are transcribed in vivo (Bo and Boime 1992).

In the placenta mononucleated cytotrophoblast cells continuously differentiate and fuse to form multinucleated syncytiotrophoblast cells. Hoshina et al (1982,1983) reported that αhCG and βhCG subunit genes were expressed predominantly in the syncytiotrophoblast and in some cytotrophoblast cells. Subsequent studies have shown that the αhCG subunit is localised primarily in the cytotrophoblast, while the βhCG subunit is localised almost exclusively to the syncytiotrophoblast (Hay et al 1988, Kelly et al 1991). Synthesis of intact hCG is linked primarily to the syncytiotrophoblast and multinucleated intermediate cells (Gosseye and Fox 1984, Hay 1988), although hCG may be expressed in cytotrophoblast cells in placentae at 4-5 weeks gestation (Marou et al 1992).

The principle physiological role of hCG is the maintenance of the corpus luteum during the initial stages of pregnancy. The corpus luteum is essential for the production of hormones during the first seven weeks of pregnancy, until placental production of hormones takes over around the 8th week of pregnancy. Apart from its luteotrophic function, the role of hCG throughout the remainder of pregnancy is largely unknown. HCG may be involved in a number of processes including placental glycogen metabolism (Demers et al 1973), trophoblast differentiation (Shi et al 1993), steroid biosynthesis and regulation of placental function (Lei and Rao 1992).

1.4.2.1 HCG in Normal Pregnancy.

HCG synthesis begins in the blastocyst and can be detected in the maternal circulation around the time of implantation (Grudzinskas et al 1977). The concentration of heterodimeric HCG (intact hCG) in the maternal circulation rises rapidly to a peak at 8-10 weeks gestation and then declines to a plateau at 18-20 weeks gestation after which the concentration remains relatively constant to term (Braunstein et al 1976, Kletzky et al 1985, Ozturk et al 1987). HCG is also present in the maternal circulation in the form of its free αhCG (FαhCG) and βhCG (FβhCG) subunits. Maternal serum FαhCG increases from low levels in early pregnancy to around 30-40% of total hCG levels at term (Cole et al 1984, Ozturk et al 1987). FβhCG accounts for less than 5% of total hCG in maternal serum and levels increase to a peak at 8-9 weeks gestation and decline thereafter (Cole et al 1984, Ozturk et al 1987). The concentration of hCG in the maternal circulation is influenced by the sex of the fetus. From approximately 18 weeks gestation, maternal serum levels of hCG have been reported to be higher in women carrying a female fetus (Crosignani et al 1972, Boroditsky et al 1975, Obiekwe and Chard 1982, Leporrier et al 1992, Muller et al 1993).

The concentration of intact hCG in amniotic fluid is lower than in the maternal circulation (Ozturk et al 1988, Iles et al 1992). Amniotic fluid intact hCG levels increase from nine weeks gestation to a peak at 11-14 weeks followed by a marked decrease to low levels which are maintained to term (Clements et al 1976, Ozturk et al 1988). In fetal serum, hCG levels are lower than in amniotic fluid and maternal serum and show a changing profile similar to that in the maternal circulation (Crosignani et al 1972, Clements et al 1976). The relative concentrations of intact hCG in maternal serum, amniotic fluid and fetal serum suggest that hCG is secreted from the

syncytiotrophoblast directly into the maternal circulation and from there into the amniotic fluid across the fetal membranes. Amniotic fluid FαhCG and FβhCG levels decrease from around 15 weeks gestation until 23 weeks gestation (Ozturk et al 1988). In contrast to intact hCG, the concentration of FαhCG and FβhCG in the amniotic fluid is approximately 10 times higher than in the maternal circulation, although there is no evidence of increased breakdown of hCG into its subunits in amniotic fluid (Ozturk et al 1988, Iles et al 1992). This suggests that, unlike intact hCG, the free subunits of hCG may reach the amniotic fluid via an alternative route or the there are other sources hCG subunits in the amniotic fluid.

1.4.2.2 HCG in Complications of Pregnancy.

HCG is a product of the trophoblast and as such is an indicator of the presence of viable trophoblastic tissue. The measurement of hCG in serum or urine is used as an indicator of pregnancy. Abnormal levels of hCG are associated with a number of pregnancy complications. Low maternal serum levels of hCG have been reported in association with ectopic pregnancy, threatened abortion and fetal death (Bischof et al 1983d, Salem et al 1984, Grudzinskas et al 1986, Muller et al 1993a). In women with insulin-dependent diabetes mellitus intact hCG levels are normal or slightly reduced (Canick et al 1990b, Wald et al 1992a). In twin pregnancies, maternal serum intact hCG levels are elevated to approximately two times the levels in singleton pregnancies (Canick et al 1990b, Neibolo et al 1991). Elevated maternal serum levels of hCG have also been reported in association with gestational trophoblastic disease, intrauterine growth retardation and fetal death (Grudzinskas et al 1986, Tanaka et al 1993, Muller et al 1993a). Henderson et al (1992) reported a significant reduction in the expression of free α- and β-subunits in trophoblast tissue from pregnancies with early embryonic failure.

1.4.2.3 Intact hCG in Chromosomally Abnormal Pregnancies.

Maternal serum levels of intact hCG are elevated to approximately twice normal levels in the second trimester of pregnancies with a Down's syndrome fetus (Bogart et al 1987, Wald et al 1988a, Norgaard-Pedersen et al 1990, Crossley et al 1991a, Haddow et al 1992, Ryall et al 1992, Spencer et al 1992a, Stone et al 1993). A meta-analysis of 18 published studies, including a total of 559 Down's syndrome pregnancies, revealed an overall second trimester maternal intact hCG level of 2.05 MOM (Wald and Cuckle 1992). In the first trimester, although intact hCG levels are marginally raised in association with Down's syndrome, the magnitude of the change is much less than that observed in the second trimester (Cuckle et al 1988, Bogart et al 1989, Brock et al 1990, Johnson et al 1991, Kratzer et al 1991b, van Lith et al 1992, Aitken et al 1993a, Crandall et al 1993).

In contrast to Down's syndrome, second trimester intact hCG levels are markedly reduced in pregnancies associated with Trisomy 18 (Arab et al 1988, Canick et al 1990a, Crossley et al 1991b, Miller et al 1991, Barkai et al 1993). Kratzer et al (1991b) and Aitken et al (1993a) reported that maternal serum intact hCG levels were significantly reduced in the first trimester of Trisomy 18 pregnancies, while Van Lith et al (1992) reported that levels were not significantly different from normal. In Trisomy 13 pregnancies, second trimester maternal serum hCG levels have been reported as normal (Crossley et al 1991b), elevated (Muller et al 1993a) and reduced (Suchy and Yeager 1990). In the first trimester, maternal serum intact hCG levels have been reported to be low in association with fetal Trisomy 13 (Kratzer et al 1991b).

In amniotic fluid from Down's syndrome pregnancies, intact hCG levels are significantly higher than in unaffected pregnancies (Cuckle et al 1991, Wolf et al 1992, Spencer et al 1993a).

1.4.2.4 Free αhCG and βhCG Subunits in Chromosomally Abnormal Pregnancies.

FβhCG levels are elevated to more than twice normal levels in second trimester maternal serum from Down's syndrome pregnancies (Macri et al 1990, Crossley et al 1991a, Spencer 1991a, Ryall et al 1992, Spencer et al 1992a, Stone et al 1993). Maternal serum FβhCG levels are also increased in the first trimester of Down's syndrome pregnancies, the magnitude of increase being similar to that in the second trimester (Ozturk et al 1990, Spencer et al 1992b, Aitken et al 1993a, Macri et al 1993a).

Some studies have shown that FαhCG levels in maternal serum are significantly elevated in the second trimester of Down's syndrome pregnancies (Bogart et al 1987, Bogart et al 1989, Kratzer et al 1991b, Ryall et al 1992). However, in a study of 36 Down's syndrome cases, Spencer (1993) was unable to confirm this observation. Bogart et al (1989) reported that FαhCG levels in the first trimester of Down's syndrome pregnancies were not significantly different from normal. In contrast, data from Ozturk et al (1990) suggest that FαhCG levels are slightly elevated, while Kratzer et al (1991b) found that FαhCG levels to be significantly reduced in the first trimester of Down's syndrome pregnancies

Reduced maternal serum levels of FβhCG (0.37 MOM) have been reported in association with Trisomy 18 in the second trimester of pregnancy (Spencer et al 1993b), and with Trisomy 18 and Trisomy 13 in the first trimester (Ozturk et al 1990, Spencer et al 1992b, Aitken et al 1993a, Macri et al 1993a). Kratzer et al (1991b) reported that first trimester maternal serum FαhCG levels were reduced in Trisomy 13 pregnancies but normal in Trisomy 18 pregnancies. In contrast, Ozturk et al (1990)

found that FαhCG levels were elevated in Trisomy 18 pregnancies in the first trimester. Bogart et al (1989) found that second trimester serum levels of FαhCG were normal in two cases of Trisomy 18 and elevated in one of two cases of Trisomy 13.

Both FβhCG and FαhCG levels have been found to be elevated in amniotic fluid from Down's syndrome pregnancies (Wolf et al 1992, Spencer et al 1993a).

1.4.3 PREGNANCY ASSOCIATED PLASMA PROTEIN-A.

Pregnancy associated plasma protein-A (PAPP-A) is a glycoprotein with a molecular weight of approximately 750-820kDa and is composed of two identical subunits of equal molecular weight (Bischof 1979). The gene encoding PAPP-A has been mapped to chromosome 9q33.1 (Silahtaroglu et al 1993). The syncytiotrophoblast of the placenta is believed to be the principle source of circulating PAPP-A. Immunohistochemical studies demonstrating that PAPP-A is localised to the syncytiotrophoblast support this theory (Lin and Halbert 1976, Wahlstrom et al 1981, Gosseye and Fox 1984, Tornehave et al 1984). However, PAPP-A has also been shown to be present in other tissues including the villous cytotrophoblast and decidua (DuBerg et al 1982, Dobashi et al 1984, Schindler et al 1984), and in non-pregnant females and in male seminal plasma (Bischof et al 1983b, Bersinger and Klopper 1984). Schindler and Bischof (1984) demonstrated the histochemical localisation of PAPP-A in the decidua, fetal adrenal cells, fetal renal tubules, fetal intestinal mucosa, adult proliferative endometrial cells and red cell precursors. However, they found that the use of different preparations of PAPP-A antibody revealed significant differences in immunohistochemical staining.

In a recent study, Oxvig et al (1993) proposed that circulating PAPP-A was present in the form of a disulphide-bridged complex between PAPP-A and the pro-form of eosinophil major basic protein (proMBP). Eosinophil major basic protein (MBP) is found in the core of the eosinophil granule. During pregnancy, a protein immunologically identical to MBP is found in placenta, amniotic fluid and maternal serum (Maddox et al 1983, Maddox et al 1984, Vernof et al 1984). Levels of the pregnancy associated MBP in the maternal circulation increase in a sigmiodal fashion with advancing gestation (Wagner et al 1993). Oxvig et al (1993) reported that certain commercial preparations of anti-PAPP-A polyclonal antibody used in the analysis of PAPP-A levels in pregnancy and in immunohistochemical studies were poly-specific, binding to both MBP and its pro-form. Bueler and Bersinger (1989) have also demonstrated the cross-reactivity of some batches of commercially available anti-PAPP-A antibodies, in that antiserum marketed by Dakopatts was capable of binding to human haptoglobin. Cross-reactivity of commercial PAPP-A antibodies may partly be responsible for the controversy surrounding the site of synthesis of PAPP-A.

The physiological role of PAPP-A during pregnancy is not known. PAPP-A has been reported to exert inhibitory effects on immune and coagulation systems and it has been proposed that PAPP-A may play a role in the protection of the fetal allograft (Bischof et al 1979, Bischof et al 1982b, Bischof et al 1983a/c, Bischof et al 1984, Stabile et al 1988)

1.4.3.1 PAPP-A in Normal Pregnancy.

PAPP-A is synthesised in the syncytiotrophoblast of the placenta and secreted directly into the maternal circulation where levels increase in a sigmoidal fashion to term (Sutcliffe et al 1980, Folkersen et al 1981, Bischof et al 1982a). The rate of production

of PAPP-A may be a direct reflection of the total mass of the trophoblast and the blood flow in the intervillous space (Gordon and Chard 1979, Chard 1986).

The concentration of PAPP-A in the amniotic fluid is lower than that in the maternal circulation and the change in amniotic fluid PAPP-A levels with gestation reflects the concentration profile of PAPP-A in the maternal circulation (Bischof et al 1982a). This suggests that amniotic fluid PAPP-A is derived primarily from the maternal circulation across the fetal membranes.

1.4.3.2 PAPP-A in Complications of Pregnancy.

PAPP-A is of potential value in the prenatal diagnosis of a rare condition known as Cornelia de Lange (CdL) syndrome. Westergaard et al (1983a) reported the apparent absence of PAPP-A from the maternal circulation and from trophoblast tissue in pregnancies which resulted in the birth of a child with CdL syndrome. Support for this observation is provided in a subsequent study by Graham et al (1988).

Reduced levels of PAPP-A in the maternal circulation have also been associated with threatened abortion, early pregnancy failure, ectopic pregnancy, insulin dependent diabetes and trophoblastic disease (Sutcliffe et al 1982, Masson et al 1983, Westergaard et al 1983b, Westergaard et al 1985b, Grudzinskas et al 1986, Stabile et al 1988). Elevated maternal serum levels of PAPP-A have been reported in association with twin pregnancy, pre-eclampsia and ante-partum haemorrhage (Klopper and Hughes 1980, Westergaard et al 1985a, Grudzinskas et al 1986).

1.4.3.3 PAPP-A in Chromosomally Abnormal Pregnancies.

Maternal serum levels of PAPP-A are significantly reduced in Down's syndrome pregnancies in the first trimester, with median PAPP-A levels reported in the range of 0.23 to 0.63 MOM (Wald et al 1992b, Aitken et al 1993b, Brambati et al 1993, Hurley et al 1993, Muller et al 1993b). Maternal serum PAPP-A levels in the second trimester of Down's syndrome pregnancies are not significantly different from normal (Cuckle et al 1992, Wald and Voller 1992, Knight et al 1993). There are no published studies on amniotic fluid levels of PAPP-A in Down's syndrome pregnancies.

1.4.4 PREGNANCY SPECIFIC BETA 1 GLYCOPROTEIN.

Pregnancy specific beta 1 glycoprotein (SP1) is a 90kDa glycoprotein which is synthesised by the syncytiotrophoblast and secreted into the maternal circulation during pregnancy (Bohn 1979). SP1 belongs to the immunoglobulin superfamily and exhibits strong homology with carcinoembryonic antigen and neurone-cellular adhesion molecule (Streydio et al 1988, Barnett et al 1990, Leslie et al 1990, Zheng et al 1990). A family of genes encoding SP1 has been mapped to chromosome 19 (Streydio et al 1990). Immunohistochemical studies have shown that SP1 is localised to the syncytiotrophoblast of the placenta and synthesis of SP1 has been demonstrated in trophoblast cultures (Lin and Halbert 1976, Gosseye and Fox 1984, Chou and Zilberstein 1990). Differential expression of SP1 genes has also been demonstrated in the chorionic and amniotic membranes as well as in the trophoblast (Plouzek et al 1993). SP1 may be present at extremely low concentrations in the serum of healthy non-pregnant individuals (Grudzinskas et al 1979, Sorensen 1984). Elevated levels of SP1 can be detected in the serum of individuals with trophoblastic

tumours and other malignant non-trophoblastic tumours (Searle et al 1978, Grudzinskas et al 1979, Sorensen 1984).

The physiological role of SP1 is not known. Considering the structural homology between SP1 and cellular adhesion molecules, SP1 may act as placental cellular adhesion molecule important in the maintenance of placental structural integrity. SP1 may also be involved in immunosuppression, carbohydrate metabolism and play a role as a carrier protein (Grudzinskas et al 1979, Sorensen 1984).

1.4.4.1 SP1 in Normal Pregnancy.

SP1 can be detected in the maternal circulation soon after conception (18-23 days) (Grudzinskas et al 1977). SP1 is secreted from the syncytiotrophoblast into the maternal circulation where levels increase in a sigmiodal fashion to term (Schultz-Larsen 1978, Guibal et al 1980, Braunstein et al 1980). The concentration of SP1 in the maternal circulation at term is approximately 100-290mg/l which is considerably higher than the levels of other placental proteins (Bohn 1979, Sorensen 1984). It is thought that the concentration of SP1 in the maternal circulation throughout gestation reflects the growth of the placenta and trophoblastic mass (Gordon and Chard 1979, Braunstein et al 1980). The concentration of SP1 in the amniotic fluid is approximately 1% of that in the maternal serum, while fetal serum levels are approximately 0.1% of maternal serum levels (Grudzinskas et al 1978). SP1 is present in maternal urine at approximately 1% of maternal serum levels (Grudzinskas et al 1978). The increase in amniotic fluid SP1 levels with gestation reflects the concentration profile of SP1 in the maternal circulation, suggesting that amniotic fluid SP1 may be derived from the maternal circulation across the fetal membranes.

1.4.4.2 SP1 in Complications of Pregnancy.

Low levels of SP1 in the maternal circulation in early pregnancy are predictive of pregnancy failure following threatened abortion (Schultz-Larson and Herz 1978, Ho and Jones 1980, Masson et al 1983, Sterzik et al 1986). Lower than normal serum SP1 levels have also been reported associated with ectopic pregnancy, fetal hypoxia, intrauterine growth retardation, anembryonic pregnancy and pre-eclampsia (Gordon et al 1977, Bennett et al 1978, Ho and Jones 1980, MacDonald et al 1983, Sterzik et al 1989, Silver et al 1993). Elevated maternal serum SP1 levels are associated with twin pregnancy and choriocarcinoma (Searle et al 1978, Westergaard et al 1985a).

1.4.4.3 SP1 in Chromosomally Abnormal Pregnancies.

Maternal serum concentrations of SP1 were first investigated in relation to Down's syndrome by Bartels and Lindemann (1988). SP1 levels were found to be elevated to approximately twice normal levels (2.10 MOM) in Down's syndrome pregnancies in the second trimester. Subsequent studies have confirmed that SP1 levels are elevated in Down's syndrome pregnancies (Wald et al 1989, Bartels et al 1990, Petrocik et al 1990, Graham et al 1992, Aitken et al 1994), however the degree of elevation appears to be less than in the initial study by Bartels and Lindemann (1988). A meta-analysis of five published studies, including a total of 213 Down's syndrome pregnancies, revealed an overall second trimester maternal serum SP1 level of 1.54 MOM in the affected cases (Wald and Cuckle et al 1992). In the first trimester, maternal serum SP1 levels are significantly reduced in association with Down's syndrome. Brock et al (1990) reported a median maternal serum level of 0.79 MOM in 21 Down's syndrome pregnancies at 7-14 weeks gestation, while Macintosh et al (1993) found maternal serum SP1 levels to be reduced to 0.40 MOM in 14 Down's syndrome pregnancies at 6-12 weeks gestation.

Bartels and Lindemann (1988) reported that in three out of four Trisomy 18 cases, maternal serum SP1 levels were elevated compared to controls. Subsequent studies suggest that maternal serum SP1 levels are not significantly different from normal in Trisomy 18 pregnancies in either the first or second trimesters (Bartels et al 1990, Graham et al 1992, MacIntosh et al 1993). Bartels et al (1990) reported maternal serum SP1 levels of 1.07 MOM and 0.36 MOM in two Trisomy 13 pregnancies, while Graham et al (1992) found that maternal serum SP1 levels in four Trisomy 13 cases were not significantly different from controls.

Bartels and Lindemann (1988) also reported that amniotic fluid levels of SP1 in Down's syndrome pregnancies were significantly elevated at 16 and 17 weeks gestation and normal at 18 and 19 weeks gestation. Previous studies have shown amniotic fluid SP1 levels to be normal or slightly reduced in association with fetal trisomy (Wurz et al 1981, Heikinheimo et al 1984).

1.4.5 ALKALINE PHOSPHATASE.

The alkaline phosphatases (ALP) are a group of isoenzymes which are present on the microvillar membranes of a number of tissues. The enzymes are attached to the outer surface of the microvillar membrane by means of a glycosyl-phosphatidylinositol anchor (GPI) that is covalently attached to the C-terminus of the peptide (Low 1988). Four structural genes encoding the alkaline phosphatase isoenzymes have now been identified. The gene encoding the tissue non-specific isoenzyme, which is expressed in bone, liver, kidney and other cells, has been mapped to chromosome 1 (Swallow et al 1984). The adult intestinal ALP gene has been mapped to chromosome 2 (q34-37), where the genes encoding the placental and germ-cell (placental-like ALP) isoenzymes are also located (Kam et al 1985, Henthorn et al 1987, Martin et al 1987). The germ-cell isoenzyme is expressed at low levels in lung, cervix, testes and thymus

(Goldstein et al 1982). The placental ALP gene exhibits considerable allelic variation which gives rise to a number of electrophoretically distinct phenotypes of placental ALP (Robson and Harris 1967, Donald and Robson 1974). There is considerable homology between the amino acid sequences of the placental and germ-cell isoenzymes (98%), and between the placental and adult intestinal isoenzymes (86.5%). Adult intestinal ALP and tissue non-specific ALP exhibit 56.6% sequence homology (Henthorn et al 1987, De Broe and Moss 1992).

The different isoenyzmes exhibit considerable heterogeneity with respect to electrophoretic mobility, inhibition characteristics and susceptibility to heat. These differences can be exploited to distinguish between the isoenzymes. Mulivor et al (1978c) demonstrated that the isoenzymes can be separated into three distinct categories of tissue non-specific, placental and adult intestinal ALP using the appropriate combination of the inhibitors L-homoarginine, L-phenylalanine, L-leucine, L-leucylglycylglycine and L-phenylalanylglycylglycine. There is no difference in the inhibition characteristics of the tissue non-specific isoenzymes and differences in the electrophoretic mobilities of these isoenzymes are probably due to tissue specific variation in post-translational modifications. The placental isoenzyme can be distinguished on the basis of its resistance to heating at 65°C, a temperature at which the other isoenzymes are inactivated (Moss 1982). Placental ALP isoenzyme is also immunologically distinct from the other isoenzymes (Moss 1982).

Circulating ALP in normal adult serum is thought to be mainly of the tissue non-specific type, although a small amount of the intestinal isoenzyme is also present (Moss 1982, Mulivor et al 1985). ALP activity in body fluids may originate from the breakdown and desquamation of microvillar membranes or from the differential release of the enzyme from microvillar membranes by specific phospholipases, present in various tissues and

body fluids, which cleave the GPI anchor (Davitz et al 1987, Davitz et al 1989, Hamilton et al 1989, Raymond et al 1991, De Broe and Moss 1992, Deng et al 1992).

The ALP isoenzymes hydrolyse a variety of monophosphate esters at a high optimum pH, although the physiological significance of this role *in vivo* is not clear. ALP may have an important role in bone mineralisation due the association between reduced levels of tissue non-specific ALP and abnormal bone mineralisation in the disease hypophosphatasia (Mulivor et al 1978b). Makiya and Stigbrand (1992) proposed that due to its affinity for immunoglobulin G (IgG) molecules, placental ALP may be involved in the transport of IgG across the placenta to the fetus. ALP may also be involved in the binding and transport of substances across microvillar membranes.

1.4.5.1 ALP in Normal Pregnancy.

Mulivor et al (1978a) identified the existence of a fetal form of the intestinal ALP isoenzyme which is electrophoretically distinct from the adult intestinal form. There was no evidence for the existence of fetal forms of the tissue non-specific and liver isoenzymes. The major difference between the fetal and adult intestinal ALP was their carbohydrate content, although differences in their electrophoretic mobility after treatment with neuraminidase suggested possible differences in the protein structure. Behrens et al (1983) proposed that the fetal isoenzyme was composed of two non-identical subunits, one similar to that of the placental isoenzyme and the other, a glycosylated form of the adult intestinal ALP. This suggests that expression of fetal intestinal ALP does not arise from the existence of a separate genetic loci, but from the co-expression of the placental and adult intestinal genes during fetal development.

ALP levels in the maternal circulation during pregnancy are increased due to the contribution of the placental isoenzyme (Mulivor et al 1985). Synthesis of the placental

isoenzyme in the syncytiotrophoblast occurs from around the 7th week of gestation (Okamoto et al 1990). Low levels of placental ALP activity have been detected in maternal circulation from 8 weeks gestation and activity increases throughout pregnancy, with a marked increase after the second trimester (Okamoto et al 1990).

Muller et al (1988) reported that total ALP activity in amniotic fluid was extremely low until 13 weeks gestation at which stage ALP activity increased corresponding to the disappearance of the anal membrane. Amniotic fluid ALP activity increases to a peak at around 18 weeks gestation followed by a decline in activity to a plateau at around 24 weeks, with a further increase in activity after 28 weeks gestation (Seelen 1978, Mulivor et al 1979, Muller et al 1988, Campbell et al 1992b). Mulivor et al (1979) demonstrated that the relative contributions of the ALP isoenzymes to total ALP activity in the amniotic fluid changed considerably as pregnancy progressed. At 14-22 weeks gestation the major isoenzyme in the amniotic fluid was the intestinal isoenzyme of fetal origin (81%), with a small contribution from the tissue non-specific (15%) and placental (4%) isoenzymes. Later in pregnancy (25-44 weeks gestation) the intestinal isoenzyme was found to contribute to only 5% of total ALP activity, while the proportions of placental and tissue non-specific activity had increased (placental - 27%, tissue non-specific - 69%). High levels of ALP are present in the fetal intestine and in meconium (Dahlquist and Lindberg 1966). Intestinal ALP in the amniotic fluid may be derived from the fetal intestine as a result of desquamation of intestinal epithelia. The demonstration that a proportion of ALP activity in the amniotic fluid is bound to microvillar membrane fragments, thought to originate primarily from the fetal intestine, supports this theory (Jalanko et al 1985, Potier et al 1986). Mulivor et al (1979) proposed that in early pregnancy some placental ALP may enter the amniotic fluid directly from the placenta and that the increase in placental ALP levels with

gestation can be attributed to an increased contribution of placental ALP from the maternal circulation.

1.4.5.2 ALP in Complications of Pregnancy.

The activity of total ALP in the amniotic fluid is significantly reduced in pregnancies with a fetus affected by cystic fibrosis (Brock et al 1984, Aitken et al 1985, Mulivor et al 1987). Elevated amniotic fluid ALP activity has been reported in association with a number of fetal abnormalities including intra-uterine fetal death, abdominal wall defects, hydrops fetalis syndrome and Meckel's syndrome (Jalanko et al 1983a), while low amniotic fluid ALP activity has been reported in cases of fetal intestinal obstructions (Morin et al 1987). Brock and Barron (1988) reported that maternal plasma placental ALP activity was significantly elevated in pregnancies with subsequent low birth weight outcome.

1.4.5.3 ALP in Chromosomally Abnormal Pregnancies.

The activity of total ALP in amniotic fluid has been reported to be reduced in association with Down's syndrome and Trisomy 18 (Jalanko et al 1983a, Morin et al 1987). Brock et al (1984) reported that the ratio of phenylalanine-resistant ALP (tissue-non-specific ALP) and homoarginine-resistant ALP (placental and intestinal) was abnormal in amniotic fluid from a small proportion of Down's syndrome (11%) and Trisomy 18 (21%) pregnancies. In contrast, Giddy et al (1989) found that the activities of phenylalanine-resistant ALP and homoarginine-resistant ALP in amniotic fluid from Down's syndrome pregnancies were not significantly different from normal. Ind et al (1993) investigated the activity of the placental isoenzyme in amniotic fluid from

Down's syndrome pregnancies and found that placental ALP activity was significantly lower than normal.

Brock et al (1990) reported that the activity of placental ALP in first trimester maternal serum from Down's syndrome pregnancies was not significantly different form normal.

Urea-resistant neutrophil alkaline phosphatase (URNAP) is present in normal human polymorphonuclear leukocytes. Grozdea et al (1984) reported a significant increase in URNAP activity in women who had previously given birth to a Down's syndrome child. Cuckle et al (1990b) investigated maternal URNAP activity in 72 women with Down's syndrome pregnancies prior to therapeutic termination, and in 156 unaffected pregnancies. Maternal blood films were stained for URNAP activity and a semi-quantitative technique was used to estimate enzyme activity. The median URNAP activity the Down's syndrome pregnancies was 1.65 times higher than in the controls.

1.4.6 GAMMA GLUTAMYL TRANSFERASE.

Gamma glutamyl transferase (GGT) is an enzyme found on the external surface of cells which exhibit a high secretory or absorptive capacity. GGT activity is most prominent in the kidney, but is also present in the liver, pancreas, intestine, lung, testes, epididymis, spleen and placenta (Rosalki 1975). GGT in tissues is present primarily as a membrane bound form. It is made up of two subunits of unequal size (22kDa and 62kDa) and is bound to the microvillar membrane via a single hydrophobic anchoring sequence located at the N-terminus of the large subunit. The smaller hydrophilic subunit, on which the catalytically active site is located, extends into the extracellular compartment (Semenza 1986). GGT in tissues also exists in a soluble form which contributes to around 10% of tissue GGT activity (Szewozuk 1966). High

levels of GGT activity have been reported in bile and seminal plasma (Rosalki and Rowe 1973, Wenham et al 1982). GGT activity is also present in serum, urine and at very low levels in cerebrospinal fluid (Swinnen 1967, Beck 1978).

A number of isoenzymes of GGT have been identified which exhibit marked heterogeneity with respect to lectin affinity and electrophoretic mobility. These differences are evident between isoenzymes from different tissues and are probably due to variations in post-translational modifications (Shaw et al 1980, Huseby 1981).

The liver is likely to be the main source of GGT activity in serum due to the demonstration of similarities in electrophoretic mobility, lectin affinity and kinetic properties of the liver and serum isoenzymes (Naftalin et al 1969, Shaw et al 1980, Huseby 1981). GGT activity present in body fluids is thought to arise directly from the cells of the liver and kidney by a process of cell breakdown and desquamation (Rosalki 1975).

A cluster of genes coding for GGT has been mapped to the long arm of chromosome 22 (Bulle et al 1987). Figlewicz et al (1993) not only identified three GGT genetic loci on chromosome 22, but also GGT related sequences on chromosomes 18, 19 and 20.

As a key enzyme in the gamma glutamyl cycle, GGT is involved in the metabolism of glutathione. GGT catalyses the transfer of the gamma glutamyl moiety from glutathione (and other gamma glutamyl peptides) to other peptides, amino acids, water and to glutathione itself. The gamma amino acids formed by GGT are transported into cells where they are converted into the corresponding amino acids for reutilisation. Glutathione itself is reformed in the gamma glutamyl cycle and is involved, either directly or indirectly, in a number of biological processes including DNA and protein

synthesis, metabolism, enzyme activity, transport and cellular protection (Meister and Anderson 1983).

1.4.6.1 GGT in Normal Pregnancy.

Levels of GGT in the maternal circulation during pregnancy have been reported as normal (Lum and Gambino 1972) and reduced (Rosalki et al 1970). Rosalki (1975) reported that plasma levels of GGT decrease with advancing gestation with levels in the third trimester being almost half that of non-pregnant female controls. Other studies have shown that there is no significant change in GGT activity with gestation (Jalanko et al 1983b, Moniz et al 1984).

GGT activity in the fetal serum is approximately 10 times greater than in maternal serum and there is no significant change in activity with advancing gestation (Moniz et al 1984).

Kottegen et al (1976) demonstrated that GGT exists in two forms in the liver and small intestine depending on the stage of development. A sialic acid rich fetal GGT, which does not bind to Concanavalin-A (Con-A), was found in the fetal liver and small intestine and in undifferentiated cryptal cells of the adult small intestine. A sialic acid poor adult GGT, which bind Con-A, was present in the adult liver and small intestine.

In the amniotic fluid, GGT activity is extremely low until the 13th week of gestation when there is a sharp increase in the levels of enzyme activity (Muller et al 1988). GGT activity reaches a maximum around 15-16 weeks gestation, after which activity decreases to a plateau which is maintained to term (Jalanko et al 1983b, Moniz et al 1984, Muller et al 1988). Moniz et al (1984) reported that amniotic fluid contained at least two isoenzymes of GGT, the main isoenzyme having the same electrophoretic

mobility as that of the fetal serum. The contribution of the adult form of GGT to the total GGT activity decreased as gestation advanced. In the amniotic fluid, a high proportion of GGT activity was shown to be bound to a particulate fraction containing microvillar membrane fragments thought to originate primarily from the fetal intestinal epithelia (Jalanko et al 1985, Potier et al 1986). This indicates that the fetal intestine may be a major source of amniotic fluid GGT activity through cell breakdown and desquamation of the intestinal epithelia. Jalanko et al (1983b) demonstrated that considerable GGT activity was present in a number of fetal tissues including the intestine, kidney, liver, placenta and fetal membranes. Second trimester bile and meconium contained the highest GGT activities. All of these sources may contribute to amniotic fluid GGT activity.

1.4.6.2 GGT in Complications of Pregnancy.

GGT levels are significantly reduced in amniotic fluid from pregnancies with a fetus affected by cystic fibrosis (Carbarns et al 1983, Brock et al 1984, Aitken et al 1985, Mulivor et al 1987). Abnormal amniotic fluid GGT activity has also been associated with a number of other fetal abnormalities including Meckel's syndrome, abdominal wall defects, neural tube defects, intestinal and biliary atresia and congenital nephrosis (Carbarns et al 1983, Jalanko et al 1983b, Buamah et al 1984, Morin et al 1987, Muller et al 1988).

1.4.6.3 GGT in Chromosomally Abnormal Pregnancies.

GGT activity in amniotic fluid from Down's syndrome, Trisomy 18 and Trisomy 13 pregnancies is significantly lower than the levels found in unaffected pregnancies (Jalanko and Aula 1982, Brock et al 1984, Buamah et al 1984, Morin et al 1987, Macek et al 1987, Jones ad Evans, Giddy et al 1988, Zeitune et al 1989). Combining

the results of eight published studies, amniotic fluid GGT activity was below the control group median in 91% of Down's syndrome pregnancies (n=234), 96.4 % of Trisomy 18 pregnancies (n=56) and 94.1% of Trisomy 18 pregnancies (n=17) (Zeitune et al 1989).

1.5 OTHER MARKERS IN CHROMOSOMALLY ABNORMAL PREGNANCIES.

1.5.1 UNCONJUGATED OESTRIOL.

Oestriol, the major oestrogen produced during pregnancy, is synthesised in the placenta from the fetal precursor 16α-hydroxy-dehydroepiandrosterone-sulphate. The concentration of oestriol in maternal serum and amniotic fluid increases throughout pregnancy (Belisle and Tulchinsky 1988, Russell 1989). Oestriol undergoes sulphate or glucuronide conjugation in the maternal liver, however, a small proportion (~10%) of oestriol remains unconjugated (UE_3).

The concentration of UE_3 is reduced in maternal serum from Down's syndrome pregnancies in second trimester (Canick et al 1988, Wald et al 1988b, Del Junco et al 1989, Osanthanondh et al 1989, Heyl et al 1990, Norgaard-Pedersen et al 1990, MacDonald et al 1991, Haddow et al 1992, Mancini et al 1992, Phillips et al 1992, Ryall et al 1992, Spencer et al 1992a, Crossley et al 1993). In a meta analysis of 11 published studies, including a total of 363 Down's syndrome pregnancies, the overall median second trimester maternal serum UE_3 level was 0.73 MOM in the affected cases (Wald and Cuckle 1992). Maternal serum UE_3 levels are also reduced in the first trimester of Down's s syndrome pregnancies, with median levels in the range of 0.35-0.73 MOM (Cuckle et al 1988, Brock et al 1990, Crandall et al 1991, Aitken et al 1993a, Crandall et al 1993). In Trisomy 18 pregnancies, maternal serum UE_3 levels

are reduced in both the first and second trimesters (Canick et al 1990, Aitken et al 1993a, Barkai et al 1993, Crandall et al 1993, Crossley et al 1993).

Cuckle et al (1991) reported that the concentration of UE_3 in second trimester amniotic fluid from Down's syndrome pregnancies was reduced to 0.50 MOM.

1.5.2 INHIBIN.

Inhibin is produced by the testes and the ovary and suppresses the secretion of follicle-stimulating hormone (FSH) from the pituitary. Inhibin is a 32kDa dimeric protein composed of two non-identical subunits, an α-subunit and one of two β-subunits (βA and βB). Thus two forms of inhibin exist: inhibin-A (α-βA) and inhibin-B (α-βB) (Mather et al 1992). The placenta is the most likely source of circulating inhibin during pregnancy (Petraglia et al 1987, Mather et al 1992). The concentration of inhibin in the maternal circulation increases to a peak at 8-10 weeks gestation, followed by a decrease to a plateau between 14 and 30 weeks, with a subsequent increase so that the highest levels are reached at term (Abe et al 1990, Tabei et al 1991). Maternal serum concentrations of inhibin are elevated in the second trimester of Down's syndrome pregnancies (Van Lith et al 1992, Spencer et al 1993c).

1.5.3 CANCER ANTIGEN 125.

Cancer antigen 125 (CA125) is a tumour associated protein which can be detected in maternal serum and amniotic fluid during pregnancy (Niloff et al 1984, O'Brien et al 1986, Campbell et al 1992). The concentration of CA125 in maternal serum is high during the first trimester and then decreases during the second trimester to low levels at term (Niloff et al 1984). Amniotic fluid CA125 levels are high in early pregnancy and

decrease towards term (Niloff et al 1984, Kobayashi et al 1989). The decidua contains high concentrations of CA125 and is the most likely source of CA125 during pregnancy (O'Brien et al 1986, Kobayashi et al 1989, Jacobs and Bast 1989). CA125 is also present at high concentrations in the amnion and chorion (O'Brien et al 1986, Kobayashi et al 1989)

The data on maternal serum CA125 concentrations in Down's syndrome pregnancies are variable. Van Lith et al (1991b) reported that first trimester maternal serum CA125 levels were reduced in Down's syndrome pregnancies. However, other studies reported that maternal serum CA125 levels were not significantly different from normal in either the first or second trimesters of Down's syndrome pregnancies (Spencer 1991b, Norton and Golbus 1992, Van Blerk et al 1992, Van Lith et al 1993). In contrast, Hogdall et al (1992) reported that CA125 levels were increased in first and second trimester maternal serum from Down's syndrome pregnancies. Van Blerk et al (1992) reported that amniotic fluid CA125 levels in Down's syndrome pregnancies were not significantly different from normal. In contrast, Borri et al (1993) reported that amniotic fluid CA125 levels were significantly increased in Down's syndrome pregnancies.

1.5.4 PROGESTERONE.

Progesterone is a steroid hormone which is secreted by the corpus luteum and by the placenta. Maternal serum concentrations of progesterone increase in a sigmoidal fashion throughout pregnancy. In amniotic fluid, the highest concentrations of progesterone are reached between 10 and 20 weeks gestation, with a gradual decline thereafter (Belisle and Tulchinsky 1988).

Cuckle et al (1990a) reported that progesterone levels were significantly increased to 1.20 MOM in second trimester maternal serum from 77 Down' syndrome pregnancies. Two other studies have also reported that maternal serum progesterone levels are elevated in the second trimester of Down's syndrome pregnancies (Knight et al 1989, Kratzer et al 1991b). In contrast, Ryall et al (1992) reported that second trimester maternal serum progesterone levels in Down's syndrome pregnancies were not significantly different from in unaffected pregnancies. Kratzer et al (1991b) reported that first trimester maternal serum progesterone levels in Down's syndrome pregnancies were not significantly different from normal, whereas in Trisomy 18 and Trisomy 13 pregnancies, progesterone levels were significantly reduced.

1.5.5 HUMAN PLACENTAL LACTOGEN.

Human placental lactogen (hPL) is a glycoprotein hormone which is made in the syncytiotrophoblast of the placenta. The concentration of hPL in the maternal circulation increases in a sigmoidal fashion throughout pregnancy (Guibal et al 1980, Kilman and Feinberg 1992). The concentration of hPL in amniotic fluid is lower than in maternal serum and increases gradually throughout pregnancy (Belisle and Tulchinsky 1988). Maternal serum concentrations of hPL have been reported to be elevated in Down's syndrome pregnancies (Knight et al 1989, Ryall et al 1992).

1.6 POSSIBLE CAUSES OF MARKER CHANGES IN CHROMOSOMALLY ABNORMAL PREGNANCIES.

From the above it is clear that a complex pattern of variation in the concentration of biochemical markers exists in maternal serum and amniotic fluid from trisomic

pregnancies. However, the mechanisms which give rise to these changes in Down's syndrome, Trisomy 18 and Trisomy 13 pregnancies are poorly understood.

It was proposed initially that fetal and placental immaturity was the cause of the abnormal levels of AFP, UE_3 and hCG in the second trimester of Down's syndrome pregnancies, reduced AFP and UE_3, and elevated hCG levels being characteristic of fetal and placental production at an earlier gestation (Canick et al 1988, Wald et al 1988). However, the elevation of SP-1 levels in the second trimester of Down's syndrome pregnancies contradicts this theory. Librach et al (1988) reported that there was no difference in the weights of Down's syndrome fetuses compared to unaffected fetuses in the second trimester. Kucera (1971) measured the prenatal growth of 563 Down's syndrome fetuses from 29 weeks of gestation to term. There was no evidence of abnormal growth of Down's syndrome fetuses compared to normal until the last few weeks of term. Shepard et al (1989) found no evidence of reduced placental weight in association with Down's syndrome pregnancies. Waller et al (1992) investigated levels of AFP in maternal serum from 54 women who subsequently gave birth to a Down's syndrome child. They found no significant association between maternal serum AFP levels and birth weight in Down's syndrome pregnancies.

In Trisomy 18 pregnancies the size of the placenta is generally smaller than in normal pregnancies (Shepard et al 1989) and placental insufficiency may be a cause of low serum AFP and hCG levels in Trisomy 18 pregnancies. However, normal serum SP-1 levels in both the first and second trimesters of Trisomy 18 pregnancies suggest the explanation may be more complex.

Merkatz et al (1984) postulated that altered trophoblast production or reduced clearance by the placenta, rather than reduced fetal synthesis, may be responsible for low maternal serum AFP levels in Down's syndrome pregnancies. However, this was

based on their finding that amniotic fluid AFP levels were normal. As described previously, subsequent studies have shown that amniotic fluid AFP levels are reduced in Down's syndrome pregnancies. Voitlander and Vogel (1985) proposed that due to the reduction in serum albumin levels in Down's syndrome patients and the close proximity of the AFP and albumin genes on chromosome 4, the reduction of AFP and albumin in Down's syndrome (both prenatally and postnatally) are caused by disruption of a common regulatory mechanism. Some authors have suggested that low maternal serum and amniotic fluid AFP levels in Down's syndrome pregnancies may be due to reduced or delayed synthesis of AFP by the fetal liver (Cuckle et al 1984, Davis et al 1985, Crandall et al 1988). Some support for this theory was obtained from the results of a study by Cuckle and Wald (1986), which showed that AFP levels were significantly reduced in cord blood at term from 22 babies with Down's syndrome. However, Nicolini et al (1988) and Scioscia et al (1988) both reported that second trimester fetal serum AFP levels were normal in cases of Down's syndrome. Seller (1990) also measured serum levels of AFP in Down's syndrome fetuses and found that levels were normal prior to 20 weeks gestation, after which serum AFP levels declined more rapidly in Down syndrome fetuses than in normal fetuses. Kronquist et al (1990a) examined fetal hepatic AFP in a total of 28 cases of Down's syndrome and 47 unaffected controls. There was no difference in the structure of the mRNA transcript or in the average mass or charge of the AFP polypetide in the Down's syndrome and unaffected cases. This suggests that mRNA processing and post-translational modifications of the AFP polypeptide are not altered in Down's syndrome. The mean level of AFP per mg of protein in fetal liver homogenates was significantly reduced in cases of fetal Down's syndrome at 17-23 weeks gestation. However, further investigation, including affected cases at 12.7-14.9 weeks gestation, revealed that this reduction was significant only at 17-19 weeks gestation (Kronquist et al 1994). Jones et al (1988) found that there was no significant difference in the percentage contributions of Con-A binding (fetal liver origin) and Con-A non-binding (yolk sac

origin) AFP in amniotic fluid from normal and Down's syndrome pregnancies. These results suggest that it is unlikely that reduced fetal synthesis of AFP is the fundamental cause of low maternal serum and amniotic fluid levels of AFP in Down's syndrome pregnancies noted in the first and second trimesters. Other mechanisms have been proposed including impaired kidney function and abnormal membrane or placental passage (Nicolini et al 1988, Scioscia et al 1998, Seller 1990, van Lith et al 1991).

Nicolini et al (1988) reported that AFP levels in fetal serum may be reduced in association with Trisomy 18 (0.78 MOM), suggesting that reduced hepatic synthesis of AFP may be the underlying cause of lower than normal maternal serum AFP levels in Trisomy 18 pregnancies. AFP levels in the amniotic fluid of Trisomy 18 pregnancies may be restored to normal by a lack of fetal swallowing giving rise to reduced turnover of AFP (Zeitune et al 1989).

HCG, PAPP-A, SP1 and placental ALP are all products of the placenta and it is possible that there may be a common mechanism which may affect the levels of these markers in Down's syndrome pregnancies. Chard (1991) proposed that in Down's syndrome pregnancies there is a general reduction in the levels of products of fetal origin and a general increase in products of placental origin. However, subsequent studies have shown that there is considerable variation in the changes in the maternal serum concentrations of placental markers in Down's syndrome pregnancies. Intact hCG is elevated in the second trimester of Down's syndrome pregnancies but only slightly increased in the first trimester, while FβhCG is elevated in both the first and second trimesters. Maternal serum PAPP-A and SP1 levels are reduced in the first trimester Down's syndrome pregnancies, however, in the second trimester SP1 levels are elevated while PAPP-A levels are unchanged. Maternal serum placental ALP does not appear to be altered in the first trimester of Down's syndrome pregnancies. Lack of appropriate decline of intact hCG levels after 10 weeks gestation may be the cause of

elevated levels in the second trimester (Bogart et al 1989). Kratzer et al (1990a) investigated the bioactivity of hCG in maternal serum from Down's syndrome and unaffected pregnancies. There was no significant difference in the bioactivity of hCG in maternal serum from the control and Down's syndrome pregnancies in either the first or second trimesters. This suggests that abnormal regulation of hCG synthesis, rather than the production of an abnormal hCG molecule, is more likely to be the cause of altered hCG levels in Down's syndrome pregnancies. Other possible mechanisms for the variation of placental markers in trisomic pregnancies include abnormal synthesis, altered metabolic clearance, altered trophoblastic mass and morphological changes giving rise to changes in the secretory and synthetic capacity of the placenta (Bartels et al 1990, Graham et al 1992). Abnormal placental morphology has been described in association with fetal trisomy, in particular maturation of placental villi appears to be impaired in trisomic placentae (Honore et al 1976, Rochelson et al 1990, Oberweis et al 1993).

GGT activity is substantially decreased in amniotic fluid from pregnancies with a fetus affected by autosomal trisomy or CF. Heeley and Fagan (1984) proposed that the abnormalities associated with CF and Trisomy 18 were caused by a common defect due to the elevated levels of blood immunoreactive trypsin in CF and Trisomy 18 infants and the presence of common pathohistological lesions. Gosden and Gosden (1984) investigated the possibility that the reduction in microvillar enzyme activity association with autosomal trisomy and CF was further evidence for a common defect. They proposed that a common defect was unlikely and that in CF and Trisomy 13 pregnancies reduced amniotic fluid microvillar enzyme activity was caused by disruptions in the pathways by which the enzymes reach the amniotic fluid. In Trisomy 18 fetuses there was a deficiency of microvilli in the epithelial cells of the intestine, trachea and proximal renal tubules. Brock et al (1984) proposed that renal abnormalities, which occur in 70% of Trisomy 18 cases, may be partially responsible

for the reduction in amniotic fluid microvillar enzyme activity in Trisomy 18 pregnancies. However, such defects are uncommon in Down's syndrome fetuses. Morin et al (1987) reported that intestinal abnormalities were present in the majority of Down's syndrome and Trisomy 18 cases which had low amniotic fluid microvillar enzyme activity.

Thus, no clear understanding has emerged of the mechanisms which give rise to the various changes observed in maternal serum and amniotic fluid marker levels in Down's syndrome and other chromosomally abnormal pregnancies.

1.7 AIMS.

The aim of this study was to investigate the physiological basis of the changes in marker concentrations associated with chromosomally abnormal pregnancies by:

1. Measurement of endogenous levels of AFP, intact hCG, FβhCG, PAPP-A, SP1, total and placental ALP and GGT in placental tissue from Down's syndrome, Trisomy 18 and Trisomy 13 pregnancies.

2. Measurement of endogenous levels of AFP and GGT in fetal liver and GGT in fetal ileum from Down's syndrome, Trisomy 18 and Trisomy 13 pregnancies.

3. Measurement of each of these markers in corresponding tissues from unaffected pregnancies to allow a comparison of the variation in marker levels between affected and unaffected pregnancies.

4. Investigation of the localisation of AFP, hCG, PAPP-A, SP1 and placental ALP in placental tissue from Down's syndrome and unaffected pregnancies by immunohistochemical methods.

5. Analysis of AFP, intact hCG, FβhCG, PAPP-A, SP1, total and placental ALP levels in corresponding second trimester maternal serum samples from Down's syndrome, Trisomy 18 and Trisomy 13 pregnancies from which fetal tissues were obtained.

6. Analysis of AFP, intact hCG, FβhCG, PAPP-A, SP1, total ALP, placental ALP and GGT levels in corresponding second trimester amniotic fluid samples from Down's syndrome, Trisomy 18 and Trisomy 13 pregnancies from which fetal tissues were obtained.

7. Investigation of the relationship between the levels of these markers in fetal tissues and in corresponding maternal serum and amniotic fluid from the affected pregnancies.

8. Relating the biochemical results to the pathology of the affected cases.

CHAPTER 2.

MATERIALS AND METHODS.

2.1 BIOCHEMICAL MARKERS IN FETAL TISSUES.

2.1.1 FETAL TISSUES.

2.1.1.1 Down's Syndrome.

Between May 1992 and March 1996 samples of fetal liver, fetal ileum and placenta were obtained following the therapeutic termination of 67 pregnancies in which prenatal diagnosis by fetal chromosome analysis had confirmed the presence of Down's syndrome. Parental consent was obtained in all cases and the use of fetal material in this study was approved by the Yorkhill NHS Trust Ethics Committee. The indication for amniocentesis or chorionic villous sampling in 62 cases was a high risk on serum screening, in one case the indication was advanced maternal age alone and in 3 cases fetal abnormalities were detected on ultrasound scan (US). Reports of post mortem examinations were available for 55 of the Down's syndrome fetuses and confirmation of the prenatal diagnosis was obtained by chromosome analysis of cultured fetal cells (rib, pericardium or facia lata). Of the 67 Down's syndrome cases, 25 had a 47,XX,+21 karyotype and 37 had a 47,XY,+21 karyotype. Five of the cases had chromosome translocations (47,XY,inv(5)(q13.1,q35),+21; 46,XX,-21,+t(21q,21q); 46,XY, der(14:21)(q10,q10),+21; 46,XY,-14,t(14q,21q); 47,XX,+21, t(4q,10q). Where possible, the gestation of the fetus at termination of pregnancy was determined from the date of last menstrual period (LMP). If the LMP was uncertain or absent, or if the gestation by US was more than one week over or two weeks under the gestation by LMP, the gestation at termination was calculated from the gestation at the time of screening (or diagnostic testing) as determined by US. The gestation of the Down's syndrome cases ranged from 10-24 weeks. A summary of the fetal tissues available at each gestation is given in Table 2.1.1.

Table 2.1.1 Summary of fetal tissues available for study from Down's syndrome and control tissues at each week of gestation.

Gestation	Unaffected controls			Down's syndrome		
	Placenta	Liver	Ileum	Placenta	Liver	Ileum
10	-	-	-	1	-	
11	-	-	-	-	-	
12	2	2	2	-	-	
13	5	6	5	-	1	1
14	9	12	8	2	2	2
15	4	7	8	-	-	-
16	12	16	15	3	4	3
17	7	7	7	2	2	1
18	5	6	6	5	7	6
19	6	9	9	8	7	6
20	2	1	1	14	13	12
21	-	-	-	6	10	10
22	-	2	2	5	5	4
23	-	-	-	4	2	1
24	-	-	-	1	1	1
Total	52	65	63	51	54	47

2.1.1.2 Trisomy 13 and Trisomy 18.

During this study fetal tissues were also collected from one Trisomy 13 and four Trisomy 18 pregnancies following prenatal diagnosis and subsequent termination of pregnancy. A summary of the fetal tissues available for study from these trisomic pregnancies is given in Table 2.1.2.

2.1.1.3 Unaffected Controls.

Samples of fetal liver, fetal ileum and placenta were obtained following termination of apparently normal pregnancies for psycho-social reasons. The fetuses were sexed and the gestation at termination was determined from the average foot measurement. The gestational age of the control fetuses ranged from 12-22 weeks gestation. A summary of the control fetal tissues available at each gestation is given in Table 2.1.1.

2.1.1.4 Termination of Pregnancy.

Fetal tissues were obtained from trisomic and apparently normal pregnancies following induction of labour by administration of Gemeprost (Cervigem) vaginal pessaries either alone or in combination with Mifepristone (RU486).

2.1.2 COLLECTION AND STORAGE OF FETAL MATERIAL.

Fetal tissues were obtained with minimum delay, within 24 hours if possible, after termination of pregnancy. Three sections of placenta, one section of liver and one section of ileum were obtained from the majority of cases. In some cases only

Table 2.1.2 Summary of fetal tissues available for study from Trisomy 13 and Trisomy 18 pregnancies.

Case no.	Gestation	Placenta	Liver	Ileum
T13/1	23	+	+	+
T18/1	23	+	+	+
T18/2	22	+	+	+
T18/3	21	+	+	+
T18/4	19	-	+	+

placental tissue or only liver and ileum could be obtained. The tissues were wrapped in aluminium foil, snap frozen in liquid nitrogen and stored frozen at -70°C.

2.1.3 PREPARATION OF FETAL MATERIAL FOR MARKER ANALYSIS.

2.1.3.1 Placenta.

Placental tissue was removed from frozen storage (-70°C) and allowed to thaw at room temperature. The tissue was washed in phosphate buffered saline (PBS) (pH 7.2) to remove excess blood. A cross-section of placenta (0.2g) was removed and homogenised in 1ml of extraction buffer (10mM sodium phosphate buffer containing 120mM sodium chloride, 2.7mM potassium chloride and 0.1% Triton X-100, pH 7.4) using a Potter- Elvehjem homogeniser. The homogenate was sonicated on ice at an amplitude of 4μm (2 x 30s), centrifuged at 10000g for 15 minutes and the resulting supernatant was used for analysis.

2.1.3.2 Liver.

Fetal liver was recovered from frozen storage (-70°C) and allowed to thaw at room temperature. A portion of tissue (0.2g) was removed and homogenised in 1ml of extraction buffer. The extraction procedure was the same as described for placental tissue except that the liver homogenate was not sonicated as this was found to inactivate the enzymes in the sample.

2.1.3.3 Ileum.

Fetal ileum was recovered from frozen storage (-70°C) and allowed to thaw at room temperature. A portion of tissue (50mg) was removed and any meconium was gently

squeezed out. The tissue was homogenised in 1ml of extraction buffer using the same procedure as described for placental tissue.

2.1.4 MARKER ANALYSIS IN FETAL TISSUES.

2.1.4.1 Total Protein Estimation.

Total protein in the tissue extracts was measured using the method of Lowry et al (1951). This method is based on the reaction of certain amino acids (principally tyrosine, tryptophan and to a lesser extent cysteine and histidine) with a mixture sodium molybdate, tungstate and phosphoric acid with a copper catalyst. The chelation of copper to the amino acid backbone facilitates the reduction of the mixed metalloacids (Folin-Ciocalteu Phenol Reagent) producing a blue coloured solution which absorbs light strongly at 750nm.

2.1.4.1.1 Total Protein Assay Protocol.

Stock solutions A (2% sodium carbonate in 0.1M sodium hydroxide), B1 (2% copper sulphate in distilled water) and B2 (2% sodium potassium tartrate in distilled water) were prepared and stored at 4°C until required. A solution (A/B1/B2) consisting of 98% solution A, 1% solution B1 and 1% solution B2 was prepared fresh for each assay. From a stock solution of 500μg/ml bovine serum albumin (BSA) in distilled water, serial dilutions of 500-0μg/ml BSA were prepared. Tissue extracts were diluted (placenta x50, liver x50 and ileum x10) in distilled water. All test samples and standards were assayed in duplicate. In appropriately labelled tubes, 80μl of samples and standards were added to 800μl of A/B1/B2 solution. The tubes were vortex mixed and incubated at room temperature for 10 minutes. Folin-Ciocalteu Phenol Reagent (80μl, diluted 1:1

with distilled water) was added with thorough mixing and the samples placed in the dark for a minimum of 45 minutes to allow for colour development. The absorbance of the samples at 750nm was measured against a reagent blank using a Pye-Unicam 8820 UV/VIS spectrophotometer. The absorbance of the standards at 750nm was plotted against BSA concentration (μg/ml) to provide the standard curve from which the total protein content (mg/ml) of the tissue extracts was determined.

2.1.4.2 Alphafetoprotein.

AFP was measured in placental and liver extracts using an immunoradiometric assay (Stevenson et al 1987). The assay has been in use for routine pre-natal screening in the West of Scotland since 1985. This assay employs a monoclonal anti-human AFP antibody radiolabelled with iodine-125 and a polyclonal anti-human AFP antibody bound to a Sepharose solid phase. Separation of the bound and unbound fractions was by sucrose density sedimentation. The assay was later modified to include an anti-human AFP antibody coupled to magnetisable particles so that isolation of the bound and unbound fractions was by magnetic separation. The assay was standardised using the British Standard preparation 72/227.

2.1.4.2.1 AFP Assay Protocol (Sucrose Density Sedimentation).

Before assay, placental extracts were diluted x100 in bovine serum (SAPU) and liver extracts were diluted x500 in bovine serum. Test samples, standards and quality controls were assayed in duplicate. Using a Kemble 1000 automatic sample processor, 25μl of sample was diluted with 200μl of assay buffer (0.1M EPPS buffer, pH 8.0, containing 0.1% v/v Tween 20 and 0.1% w/v sodium azide). To this was added 200μl of assay buffer containing 2.2% v/v sheep serum (SAPU), 25μg/l ^{125}I-labelled

monoclonal anti-AFP (Immunodiagnostics Group, Medical Physics, Ninewells Hospital, Dundee) and 5g/l polyclonal sheep anti-human AFP linked to Sepharose CL-4B (SAPU). The samples were incubated on an orbital shaker (400rpm) for 2 1/2 hours at room temperature. After adding 1ml of wash solution (9g/l sodium chloride and 0.1% v/v Tween 20) to each tube, 3ml of density sedimentation reagent (30g/l sucrose, 0.1% Tween 20) was pumped into the bottom of each tube using a peristaltic pump. After 15-30 minutes incubation, the supernatant containing the unbound fraction was aspirated. The bound fraction was then resuspended in 3ml of density sedimentation reagent and the process was repeated. Radioactivity in the bound fraction was measured using a Packard Cobra 5010 Gamma counter. The data was then processed using a four-parameter curve fitting routine (SASPRO calculation package, Edward and Ekins 1983). Results were expressed as KU/l.

2.1.4.2.2 AFP Assay Protocol (Magnetic Separation).

Using a Kemble 1000 automatic sample processor, 25µl of sample was diluted with 100µl of assay buffer (0.1M EPPS buffer, pH 8.0, containing 0.2% v/v Tween 20 and 1g/l sodium azide). To this was added 100µl of EPPS buffer containing 5% sheep serum, 5% bovine serum, 0.5% mouse serum and 10µg/l ^{125}I-labelled monoclonal anti-AFP (Immunodiagnostics Group, Medical Physics, Ninewells Hospital, Dundee). The samples were incubated on an orbital shaker (400rpm) for 2 hours at room temperature, after which 100µl of magnetic anti-AFP was added. The samples were then incubated for a further 30 minutes at room temperature. The tubes were washed with 0.5ml of wash buffer (9g/l sodium chloride and 0.1% v/v Tween 20) and placed on a magnetic separator for 5 minutes. The supernatant was then dumped and the process was repeated. Radioactivity in the bound fraction was measured using a Packard Cobra 5010 Gamma counter. The data was then processed using a four-

parameter curve fitting routine (SASPRO calculation package, Edward and Ekins 1983). Results were expressed as KU/l.

2.1.4.2.3 AFP Data Analysis.

The sucrose density sedimentation AFP assay had an inter-assay coefficient of variation (CV) of 6.0% and an intra-assay CV of 2.5% within the working range of 5-500KU/l. The magnetic separation assay had an inter and intra-assay CVs of less than 10% within the working range of 2-490KU/l. AFP levels in the tissue extracts were converted to units per mg of protein. Median placental and liver AFP levels at each gestation were calculated using linear regression of logarithmically transformed data. AFP levels in the unaffected and Down's syndrome tissues were converted to MOM at each gestation.

2.1.4.3 Human Chorionic Gonadotropin.

HCG was measured in placental tissue extracts using the Serono hCG MAIAclone kit (Serono Diagnostics). This assay is used for routine pre-natal screening in the West of Scotland and measures predominantly intact hCG (i.e. dimeric hCG) with only minimal recognition of the free β-subunit. The assay incorporates two monoclonal antibodies labelled with ^{125}I which bind to the intact hCG molecule and its β-subunit and one flourescein labelled monoclonal antibody which binds to a discrete site on the β-subunit forming a 'sandwich'. The hCG-antibody complex is then bound by a sheep serum monoclonal anti-flourescein antibody coupled to a magnetic solid phase, allowing rapid sedimentation in a magnetic field.

2.1.4.3.1 HCG Assay Protocol.

Placental homogenates and quality control samples were diluted x500 in horse serum containing 0.2% sodium azide. In addition to the negative (horse serum and 0.2% w/v sodium azide) and positive controls (horse serum with hCG and 0.2% w/v sodium azide) supplied with the kit, quality controls made up of pooled maternal serum (15-20 weeks gestation) were included in the assays. The pooled sera was aliquoted and stored frozen (-20°C) until use. The volumes of samples, standards and reagents used in the assay were half those given in the manufacturers protocol.

Using a Kemble 1000 automatic sample processor, 25µl of samples, standards or quality controls were added to appropriately labelled tubes along with 250µl of ^{125}I anti-hCG Reagent (flourescein and ^{125}I labelled mouse monoclonal anti-hCG antibodies in Tris buffer containing normal sheep serum, bovine serum albumin (BSA), inert dye and 0.2% w/v sodium azide). The tubes were vortex mixed and incubated at 37°C for 15 minutes. MAIAclone Separation Reagent (sheep antiserum to flourescein bound to magnetic particles in Tris buffer containing BSA and 0.1% w/v sodium azide) was mixed thoroughly and 100µl was added to each tube. The tubes were mixed gently and incubated for 5 minutes at room temperature. The rack of tubes was placed on the MAIA Magnetic Separator and the particles allowed to sediment for 2 minutes. The supernatant was decanted from the tubes by inversion of the MAIA Magnetic Separator. To reduce non-specific binding, 250µl of Wash Buffer (Tris buffer containing 0.8% w/v sodium azide, diluted x8 in distilled water) was added and each tube was mixed thoroughly by vortexing. The tubes were returned to the MAIA Magnetic Separator and the particles were allowed to sediment for a further 2 minutes, after which the supernatant was decanted from the tubes. Radioactivity in the bound

fraction was measured using a Packard Cobra 5010 Gamma counter. The data was then processed using the SASPRO calculation package (Edward and Ekins 1983). Results were expressed as IU/ml standardised against the International Reference preparation for hCG (75/537).

2.1.4.3.2 HCG Data Analysis.

The hCG assay had an inter-assay CV of 7.5% and an intra-assay CV of 3% within the range of 5-100IU/l. HCG levels in the tissue extracts were converted to IU per mg of protein. Median hCG levels at each gestation in unaffected placental tissue were calculated using an exponential regression model. HCG levels in the unaffected and Down's syndrome tissues were converted to MOM at each gestation.

2.1.4.4 Free Beta Human Chorionic Gonadotropin.

FβhCG was measured in placental tissue extracts using an ELISA kit provided by NTD Laboratories. This assay is specific for the free beta subunit and both non-competetive and competetive cross-reactivity studies have demonstrated minimal cross-reactivity with the intact molecule (0.05% wt/wt) and with other glycoprotein hormones or their β-subunits (Macri et al 1993b).

2.1.4.4.1 FβhCG Assay Protocol.

Placental tissue extracts were diluted x20 in assay buffer before assay. Before beginning the assay all placental extracts, calibrators and controls were arranged in a preparation tray according to the layout of the assay plate to allow quick and efficient transfer of samples. Assay reagents were prepared fresh for each assay.

To 25ml of assay buffer, 50µl Heterophilic Blocking Reagent (HBR) was added and mixed thoroughly. Using an eight-channel multi-pipette 100µl of the assay buffer/ HBR mixture was transferred to all assay plate wells and 20µl of tissue extract, standards or controls were added to the appropriate wells in duplicate. The assay plate was covered with an adhesive plate sealer and placed on a platform rotator for 30 minutes (+/- 5 minutes) at 200rpm. After incubation the adhesive seal was removed, the contents of the assay plate discarded and the assay plate was inverted and tapped 3 times on absorbent paper towels. Wash solution (PBS pH7.2, containing 0.05% Tween 20) was then added to all wells, discarded and the plate tapped 3 times on absorbent paper tissues. This procedure was repeated until the assay plate had been washed 5 times. After the final wash the plate was tapped 10 times on the paper towels to remove all residual wash solution. To each well of the assay plate, 100µl of Reagent 1 (20µl Stock Biotin Conjugate in 25ml assay buffer) was added. The plate was covered with an adhesive seal and incubated on a platform rotator for 30 minutes (+/- 5 minutes) at 200rpm. During this incubation, the substrate was prepared by adding 10 ortho-phenylenediamine (OPD) tablets to 25ml of Substrate Solution. The OPD/ Substrate Solution was mixed thoroughly and stored in the dark until required. After 30 minutes incubation, the assay plate was washed 5 times as described previously and 100µl of Reagent 2 (Streptavidin-Horseradish Peroxidase Conjugate) was added to each well. The plate was covered with an adhesive sealer and incubated for 4.5 minutes (+/- 15 seconds) at 200rpm. The assay plate was then washed 5 times (as described previously) and 100µl of OPD/Substrate Solution added to all wells. The assay plate was covered with an adhesive sealer and incubated on platform rotator for 8 minutes (+/- 0.5 minutes) at 200rpm. The reaction was stopped by the addition of 100µl of stop solution (1.0M sulphuric acid) to all wells. The absorbance at 492nm was measured using a Labsystems Multiscan microtitre plate reader and data analysis was carried out using the Genesis calculation package (Labsystems). Results were

expressed in ng/ml. Purified FβhCG (UCB Bioproducts, Belgium) was used as a standard.

2.1.4.4.2 FβhCG Data Analysis.

The FβhCG assay had an inter-assay CV of 7.0% at 6ng/ml and 6.0% at 40ng/ml. The concentration of FβhCG in placental tissue extracts was expressed as ng per mg of total protein. Median FβhCG levels at each gestation in unaffected placental tissue were calculated using linear regression of logarithmically transformed data. FβhCG levels in the unaffected and Down's syndrome tissues were converted to MOM at each gestation.

2.1.4.5 PREGNANCY ASSOCIATED PLASMA PROTEIN A.

PAPP-A was measured in placental tissue extracts using an ELISA kit provided by NTD Laboratories (Spencer at al 1994). The anti-PAPP-A antibody (DAKO, A230, Lot No. 082) used in the assay showed no significany cross-reactivity when tested against non-pregnant normal human plasma (DAKO specification sheet) and does not significantly cross react with haptoglobin or MBP (personal communication, Dr JN Macri).

2.1.4.5.1 PAPP-A Assay Protocol.

Placental tissue extracts were diluted x5 in assay buffer. Before beginning the assay all placental extracts, calibrators and controls were arranged in a preparation tray according to the layout of the assay plate to allow quick and efficient transfer of samples. Assay reagents were prepared fresh for each assay.

Using an eight-channel multi-pipette, 100µl of assay buffer was transferred to all assay plate wells and 20µl of tissue extract, standards or controls were then added to the appropriate wells in duplicate. The assay plate was covered with an adhesive plate

sealer and placed on platform rotator for 30 minutes (+/- 5 minutes) at 200rpm. After incubation the adhesive seal was removed, the contents of the assay plate discarded and the assay plate was inverted and tapped 3 times on absorbent paper towels. Wash solution (PBS pH7.2, containing 0.05% Tween 20) was then added to all wells, discarded and the plate tapped 3 times on absorbent paper tissues. This procedure was repeated until the assay plate had been washed 5 times. After the final wash the plate was tapped 10 times on the paper towels to remove all residual wash solution. To each well of the assay plate, 100µl of Reagent 1 (10µl Stock Reagent 1 in 12.5ml assay buffer) was added. The plate was covered with an adhesive seal and incubated on a platform rotator for 30 minutes (+/- 5 minutes) at 200rpm. Working Substrate/ Chromogen Solution was prepared by adding 7ml of 3,3',5,5' tetramethylbenzidine (TMB)/ Peroxidase Substrate Solution A to 7ml of Peroxidase Solution B. The Working Substrate/ Chromogen Solution was mixed thoroughly and 100µl added to all wells. The plate was covered with an adhesive sealer and incubated for 12 minutes at 200rpm. After incubation 100µl of Stop Solution (1M sulphuric acid) was added to all wells. The absorbance at 450nm was measured using a Labsystems Multiscan plate reader and data analysis was carried out using the Genesis calculation package (Labsystems). A third trimester pregnancy sera pool standardised against PAPP-A in the World Health Organisation 78/610 reference preparation for pregnancy associated proteins was used as a standard. Results were expressed as IU/l.

2.1.4.5.2 PAPP-A Data Analysis.

The PAPP-A assay had an inter-assay CV of 6.9% at 0.28IU/l and 6.5% at 1.08IU/l. The concentration of PAPP-A in placental tissue extracts was expressed as mIU per mg of total protein. Median PAPP-A levels at each gestation in unaffected placental

tissue were calculated using logarithmic regression. PAPP-A levels in the unaffected and Down's syndrome tissues were converted to MOM at each gestation.

2.1.4.6 PREGNANCY SPECIFIC BETA 1 GLYCOPROTEIN.

SP1 was measured in undiluted placental extracts by 'Rocket' immunoelectrophoresis (Teisner et al 1978). This assay is based on the electrophoretic separation of samples in an agarose gel which has a central strip containing a specific antibody. During electrophoresis the sample migrates into the antiserum strip, becoming more dilute the further it travels. When the concentration of antigen in the sample reaches the equivalent concentration of specific antibody, an insoluble antigen-antibody complex is formed. After staining, this is visualised as a 'rocket' peak, the area of which is proportional to the concentration of antigen in the test sample.

2.1.4.6.1 SP1 Assay Protocol.

The assays were standardised using a secondary standard consisting of pooled maternal serum samples collected routinely at 15-20 weeks gestation. The secondary standard was calibrated against an International reference preparation of SP1 (Statens Seruminstitut, WHO Laboratory for Biological Standards) and had a concentration of 0.028IU/ml. The internal quality control consisted of pooled sera from pregnant women between 15-20 weeks gestation. Both the secondary standard and internal quality control were aliquoted and stored at -20°C until use.

Immunoelectrophoresis plates were prepared using 1% (w/v) agarose diluted in a 1:1 solution of gel buffer (7.5mM barbitone, 42.5mM sodium barbitone and 1.6mM calcium lactate in distilled water, pH 8.6) and distilled water. The agarose solution was heated

to boiling and 10ml of the molten solution was poured onto a glass plate (8 x 8cm). When the agarose had solidified, a central strip representing 1/4 of the area of the plate was removed and replaced with 2.5ml of molten agarose containing 25μl of rabbit anti-human SP1 polyclonal antibody (DAKO). Using a pasteur pipette, 14 wells were punched in the agarose in a row directly below the antibody strip following a template. A second row of adjoining wells was made directly below and overlapping the first row, each double well being large enough to accommodate 4μl of sample. Placental extracts, SP1 standard and internal quality control were applied to the wells in the agarose using a SGE microsyringe.

SP1 standard and internal quality control were applied in duplicate on each plate, while placental extracts were assayed in duplicate on separate plates. In order to minimise possible effects caused by fluctuation in experimental conditions, such as uneven distribution of antibody in the antibody strip, duplicate samples were placed in different positions on each plate.

A flat-bed electrophoresis tank was filled with tank buffer (3mM barbitone, 1.7mM sodium barbitone and 2.0mM calcium lactate in distilled water, pH 8.6) and electrophoresis was carried out overnight at 4°C at 2.0 volts/cm across the plate. After electrophoresis, the agarose was overlaid with moist filter paper and dried in a warm air stream. The plates were then immersed in coomassie brilliant blue stain (2g coomassie brilliant blue in 1l destain solution consisting of 100ml acetic acid, 450ml methanol and 450ml distilled water) until peaks could be observed in the antiserum strip.

The area under a peak is proportional to the concentration of SP1 in the sample applied to that well, but with narrow 'rocket' peaks, peak height may be used with little

error. The concentration of SP1 was calculated using the following equation. Results were expressed as IU/ml.

$$\text{SP1 conc.} = \frac{\text{test peak height (mm)}}{\text{standard peak height (mm)}} \times \text{standard conc.}$$

2.1.4.6.2 SP1 Data Analysis.

The SP1 assay had an inter-assay CV of 6.6% and an intra-assay CV of 7.6% at a concentration of 0.026IU/ml. The concentration of SP1 in placental tissue extracts was expressed as IU per mg of total protein. Median SP1 levels at each gestation in unaffected placental tissue were calculated using linear regression. SP1 levels in the unaffected and Down's syndrome tissues were converted to MOM at each gestation.

2.1.4.7 ALKALINE PHOSPHATASE.

Total and placental ALP were measured in placental tissue extracts using a fluorometric end point assay with 4-methylumbelliferyl phosphate (4-MUP) as a substrate. Placental ALP is resistant to heating at 65°C for at least 30 minutes, a temperature at which other ALP isoenzymes are rapidly inactivated. Placental ALP can be determined by measuring the residual ALP activity following heat inactivation at 60°C for 1 hour (Aitken et al 1996).

2.1.4.7.1 Preparation of 4-Methylumbelliferone Standard Curve.

A solution of 200µM 4-methylumbelliferone (4-MU, Sigma) was prepared by dissolving 7.05mg of 4-MU in approximately 200µl of acetone and then making up this solution to

200ml with AMPD buffer (0.1M 2-amino-2-methyl-1,3-propandiol buffer containing 0.28M sodium chloride and 0.5mM magnesium chloride, pH 9.8). A stock standard concentration of 4000nM 4-MU was prepared by diluting 4ml of the 200μM 4-MU solution in 196ml 0.4M glycine sodium hydroxide buffer (pH 10.4). A series of standards from 4000nM-500nM 4-MU were then prepared with 0.4M glycine sodium hydroxide buffer. The standards were aliquoted and stored frozen (-20°C) until use. At the beginning of each assay, the 4-MU standards were recovered from frozen storage, allowed to thaw at room temperature and mixed thoroughly. The 1000nM 4-MU standard was used to standardise a Perkin Elmer MPF-44B fluorescence spectrophotometer to 90% of full scale deflection on the chart recorder at signal gain 1 (excitation 365nm and emission 448nm). The fluorescence of the standards was measured against a glycine sodium hydroxide buffer blank and a standard curve of fluorescence against 4-MU concentration was prepared.

2.1.4.7.2 Measurement of Total and Placental Alkaline Phosphatase.

Placental extracts were diluted x10 in extraction buffer before assay. A pool of supernatant from 25 unaffected control placental homogenates was collected, aliquoted and stored at -20°C for use as an internal quality control. All samples were assayed in duplicate. Each test sample was divided into two aliquots and one aliquot was incubated at 60°C for 1 hour. ALP activity in both the heated (placental ALP) and unheated samples (total ALP) was then determined. A reaction mixture containing 10μl test sample, 90μl PBS (pH 7.2) and 100μl of substrate solution (5mM 4-MUP in 0.1M AMPD buffer) was incubated at 37°C for 1 hour. A reagent blank containing 10μl extraction buffer instead of test sample was included in all assays. After incubation the reaction was stopped by adding 2.3ml of 0.4M glycine sodium hydroxide buffer (pH 10.4) and the fluorescence was measured using a Perkin Elmer MPF-44B

fluorescence spectrophotometer standardised to 90% of full scale deflection on the chart recorder at signal gain 1 (excitation 365nm, emission 448nm and slit width 7). The concentration of 4-MU liberated in the reaction was determined using the 4-MU standard curve and the ALP activity (nmoles/hr/ml) calculated using the following equation.

$$\text{ALP activity} = \frac{\text{4-MU liberated (nM) x dilution factor}}{\text{incubation time (hr) x 1000}}$$

Where:

Dilution factor	=	250.
Incubation time	=	1 hour.
1000	=	conversion from litres to ml.

2.1.4.7.3 ALP Data Analysis.

ALP activity in placental extracts was expressed as nmoles per hour per mg of total protein. The total ALP assay had an inter-assay CV of 7.1% and an intra-assay CV of 6.0% at 933nmoles/hr/mg, while the placental ALP assay had an inter-assay CV of 3.4% and an intra-assay CV of 3.3% at 1045nmoles/hr/mg. The Wilcoxon Signed-Rank Test was used to compare total and placental ALP levels in the placental homogenates. Median placental ALP levels at each gestation in unaffected placental tissue was calculated using linear regression of logarithmically transformed data. Placental ALP levels in the unaffected and Down's syndrome tissues were converted to MOM at each gestation.

2.1.4.8 GAMMA GLUTAMYL TRANSFERASE.

GGT was measured in extracts of placental, fetal liver and fetal ileum by kinetic enzyme assay using γ-GT Reagent from Sigma Diagnostics. The reconstituted γ-GT Reagent contains L-γ-glutamyl-3-carboxy-4-nitroanilide (4.56mM) and glycylglycine (60mM). GGT catalyses the transfer of the glutamyl group from L-γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine to produce L-γ-glutamylglycylglycine and 5-amino-2-nitrobenzoate which absorbs light at 405nm.

2.1.4.8.1 GGT Assay Protocol.

ACCUTROL™ Normal Control Serum (Sigma Diagnostics) was used as a quality control for the GGT assay. This is a lyophilised human serum preparation which, when reconstituted in 5ml of distilled water, has a known range of GGT activity. The ACCUTROL™ Normal Control Serum was assayed in the same manner as the tissue extracts and all samples were assayed in duplicate.

γ-GT Reagent was reconstituted in the appropriate volume of distilled water. A glass cuvette containing 1ml of γ-GT Reagent was placed in the temperature controlled chamber of a Pye Unicam 8820 Spectrophotometer set at 37°C for a minimum of 5 minutes to ensure that the γ-GT Reagent is warmed to assay temperature. After appropriate dilution in distilled water (placenta-neat, liver x10, ileum x2), 40µl of tissue extract was added and mixed thoroughly. The absorbance reading at 405nm was set to zero and the reagent mixture was incubated for 5 minutes at 37°C to allow for the initial lag phase of the reaction. The absorbance at 405nm was then recorded at 30 second intervals for 5 minutes. GGT activity is directly proportional to the rate of

increase of absorbance at 405nm and was be calculated using the following equation. Results were expressed as U/l.

$$\text{GGT activity (U/l)} = \frac{\Delta A \text{ per min} \times TV \times 1000}{9.5 \times SV \times LP}$$

Where:

ΔA per min = Change in absorbance per minute at 405nm.

TV = Total reaction mixture volume.

SV = Sample volume.

LP = Lightpath (1cm).

9.5 = Millimolar absorptivity of 5-amino-2-nitrobenzoate at 405nm.

1000 = Converts units per ml to units per litre.

2.1.4.8.2 GGT Data Analysis.

The GGT assay had an inter-assay CV of 9.9% and an intra-assay CV of 4.3% at a concentration of 32U/l. GGT activity in the tissue homogenates was expressed as units per mg of total protein. In unaffected placental tissue, median GGT levels at each gestation were calculated using linear regression. Median GGT levels were calculated using the S regression model for fetal liver extracts and by linear regression of logarithmically transformed data for fetal ileum extracts. GGT levels in Down's syndrome and unaffected tissues were converted to MOM at each gestation.

2.2 BIOCHEMICAL MARKERS IN MATERNAL SERUM.

2.2.1 MATERNAL SERUM SAMPLES.

The Duncan Guthrie Institute of Medical Genetics (Yorkhill NHS Trust, Glasgow) provides routine second trimester maternal serum screening for neural tube defects and Down's syndrome in the West of Scotland. Blood samples are collected from pregnant women at 15 to 20 weeks gestation on attendance at antenatal clinics. The blood is allowed to clot and after centrifugation, the serum is aliquoted. One aliquot is used for the analysis of screening markers (AFP and intact hCG) and the other aliquot is stored frozen at -20°C.

2.2.1.1 Down's syndrome.

Sixty-two of the Down's syndrome pregnancies from which fetal tissues were collected were identified as 'high risk' through routine maternal serum screening. The number of serum samples at each week of gestation from 15 to 20 weeks gestation is shown in Table 2.2.1. Maternal serum samples from 58 of the Down's syndrome pregnancies were available from frozen storage for further marker analysis.

2.2.1.2 Trisomy 18.

Maternal serum, collected for routine maternal serum screening, was available for two of the Trisomy 18 pregnancies (T18/3 - 16 weeks gestation, T18/4 - 15 weeks gestation) from which fetal tissues were obtained post termination.

Table 2.2.1 Summary of Down's syndrome and unaffected control maternal serum samples at each week of gestation.

Gestation	Maternal serum	
	Down's syndrome	Unaffected controls
15	18	20
16	22	20
17	17	20
18	3	20
19	-	16
20	2	18
Total	62	114

2.2.1.3 Unaffected Controls.

Serum samples randomly selected from 114 presumably chromosomally normal pregnancies (collected routinely from 1993 to 1996) at 15 to 20 weeks gestation were recovered from frozen storage for use as controls. The number of serum samples at each week of gestation from 15 to 20 weeks gestation is shown in Table 2.2.1.

2.2.2 MARKER ANALYSIS IN MATERNAL SERUM.

2.2.2.1 Alphafetoprotein.

Serum AFP was measured prospectively using an immunoradiometric assay (described in Section 2.1.4.2) as part of the routine screening programme. Maternal serum AFP levels, expressed as MOM, were available from the Departmental maternal serum screening records for 62 of the Down's syndrome pregnancies.

2.2.2.2 Human Chorionic Gonadotropin.

Serum hCG levels were measured prospectively the Serono hCG MAIAclone kit (described in Section 2.1.4.3) as part of the routine screening programme. Maternal serum hCG levels, expressed as MOM, were available from the Departmental maternal serum screening records for 62 of the Down's syndrome pregnancies.

2.2.2.3 Free Beta Human Chorionic Gonadotropin.

Maternal serum FβhCG levels were measured using an ELISA provided by NTD Laboratories (described in Section 2.1.4.4). Control median FβhCG levels (ng/ml) at

each week of gestation from 15-20 weeks were established by the analysis of 1000 second trimester serum samples from unaffected pregnancies in a previous study by Berry et al (1997). FβhCG was measured in maternal serum from 58 of the Down's syndrome pregnancies and results were converted to MOM using the appropriate gestational medians.

2.2.2.4 Pregnancy Associated Plasma Protein A.

Maternal serum PAPP-A levels were measured using an ELISA provided by NTD Laboratories (described in Section 2.1.4.5) following a x10 dilution in assay buffer. PAPP-A levels (IU/l) in maternal serum from 58 of the Down's syndrome cases were converted to MOM using second trimester control medians (15-20 weeks) established in a study by Berry et al (1997).

2.2.2.5 Pregnancy Specific Beta 1 Glycoprotein.

Maternal serum levels of SP-1 in Down's syndrome and unaffected pregnancies were measured using Rocket Immunoelectrophoresis. The technique used was identical to that described in Section 2.1.4.6, except that 30μl of anti-human SP-1 antibody were used in the antiserum strip and only 2μl of test samples, standards and quality controls were loaded into single wells beneath the antiserum strip.

2.2.2.5.1 SP-1 Data Analysis.

The maternal serum SP-1 assay had an inter-assay CV of 7.8% and an intra-assay CV of 6.4% at a concentration of 27.2IU/l. Median serum SP-1 levels (IU/l) in the unaffected pregnancies were calculated for each week of gestation from 15 to 20

weeks. Linear regression of the logarithmically transformed medians, weighted for the number of samples at each week of gestation, provided the regression equation from which smoothed gestational medians were calculated. SP-1 levels in the affected and unaffected samples were converted to MOM.

2.2.2.6 Alkaline Phosphatase.

Total and placental ALP levels were measured in maternal serum using a modified version of the technique described in Section 2.1.4.7.

2.2.2.6.1 Measurement of Total and Placental ALP.

A pool of second trimester maternal serum was aliquoted and stored at -20°C for use as an internal quality control. Affected, unaffected and quality control samples were recovered from frozen storage and allowed to thaw. Each sample was divided into two aliquots and one aliquot was heat inactivated at 60°C for 90 minutes. Total ALP was measured by adding 10µl of unheated sample to 90µl PBS (pH 7.2) and 100µl of substrate solution. The reaction mixture was incubated at 37°C for 30 minutes after which the reaction was stopped by the addition of 2.3ml of glycine sodium hydroxide buffer (pH 10.4). Placental ALP activity was measured by adding 20µl of heated sample to 80µl PBS (pH 7.2) and 100µl of substrate solution. The reaction mixture was incubated at 37°C for one hour after which the reaction was stopped as described above. A reagent blank containing 100µl PBS and 100µl of substrate solution was included in both the total and placental ALP assays. Fluorescence was measured using a Perkin Elmer MPF-44B fluorescence spectrophotometer and the concentration of 4-MU liberated was determined using a 4-MU standard curve, as described in

Section 2.1.4.7. ALP activity (nmoles/hr/ml) was calculated using the following equation.

$$\text{ALP activity} = \frac{\text{4-MU liberated (nM) x dilution factor}}{\text{incubation time (hr) x 1000}}$$

Where:

Dilution factor = 250 for unheated samples.
125 for heated samples.

Incubation time (hr) = 0.5 for unheated samples.
1 for heated samples.

1000 = conversion from litres to ml.

2.2.2.6.2 ALP Data Analysis.

The total ALP assay had an inter-assay CV of 10.3% and an intra-assay CV of 3.6% at 1046nmoles/hr/ml, while the placental ALP assay had an inter-assay CV of 34.2% and an intra-assay CV of 18.8% at 36.3nmoles/hr/ml. Median total and placental ALP levels (nmoles/hr/ml) in the unaffected pregnancies were calculated for each week of gestation from 15 to 20 weeks. Linear regression of the logarithmically transformed medians, weighted for the number of samples at each week of gestation, provided the regression equation from which smoothed gestational medians were calculated. Total and placental ALP levels in the affected and unaffected samples were converted to MOM.

2.3 BIOCHEMICAL MARKERS IN AMNIOTIC FLUID.

2.3.1 AMNIOTIC FLUID SAMPLES.

The Duncan Guthrie Institute of Medical Genetics receives amniotic fluid samples collected from women who are potential carriers of chromosomally abnormal fetuses. The amniotic fluid samples are centrifuged and the pelleted fetal cells are used for fetal chromosome analysis. The supernatant is stored frozen at -20°C.

2.3.1.1 Down's Syndrome.

Thirty-four of the Down's syndrome pregnancies from which fetal tissues were collected were prenatally diagnosed following amniocentesis. The number of amniotic fluid samples at each week of gestation from 15 to 21 weeks gestation is shown in Table 2.3.1. Amniotic fluid samples from 33 of these pregnancies were available for further marker analysis.

2.3.1.2 Trisomy 18.

One of the Trisomy 18 pregnancies (T18/4) from which fetal tissues were collected was prenatally diagnosed following amniocentesis at 17 weeks gestation. Amniotic fluid from this pregnancy was available for further marker analysis.

Table 2.3.1 Summary of Down's syndrome and unaffected control amniotic fluid samples at each week of gestation.

Gestation	Maternal serum	
	Down's syndrome	Unaffected controls
15	2	20
16	5	20
17	9	20
18	11	18
19	5	20
20	1	20
21	1	14
Total	34	132

2.3.1.3 Unaffected Controls.

Amniotic fluid samples from 132 chromosomally normal pregnancies at 15 to 20 weeks gestation were randomly selected from stored samples collected from 1992 to 1996 and used as controls. The number of amniotic fluid samples at each week of gestation is shown in Table 2.3.1.

2.3.2 MARKER ANALYSIS IN AMNIOTIC FLUID.

2.3.2.1 Alphafetoprotein.

Amniotic fluid AFP levels were measured using Rocket Immunoelectrophoresis as part of the routine analysis of amniotic fluid samples for the detection of neural tube defects.

2.3.2.1.1 AFP Assay Protocol.

The technique used to measure AFP in amniotic fluid was similar to the described for that measurement of SP1 in placental homogenate (Section 2.1.4.6). The assays were standardised using a commercial AFP standard (Behring). The working standard had a concentration 28.4MU/l. An amniotic fluid pool was used as an internal quality control. Lyophylised anti-human AFP antibody, supplied by SAPU, was reconstituted in 2ml of distilled water. Immunoelectrophoresis plates were prepared as described in Section 2.1.4.6.1, except that the antiserum strip was prepared using 2.5ml of molten agarose containing 15µl anti-human AFP polyclonal antibody. A series of single wells, each accommodating 2µl of sample, were punched in the agarose beneath the antiserum strip. AFP standard, quality control and amniotic fluid test samples were applied in

duplicate on two separate plates. Electrophoresis and staining of plates was carried out as described in Section 2.1.4.6.1. The concentration of AFP in the amniotic fluid was determined using the following equation and the average of the quadruplicate results was calculated. Results were expressed as MU/l.

$$\text{AFP conc.} = \frac{\text{test peak height (mm)}}{\text{standard peak height (mm)}} \times \text{standard conc.}$$

2.3.2.1.2 AFP Data Analysis.

The amniotic fluid AFP assay typically has an inter-assay CV of 8.9% and intra-assay CV of 10.4% at a concentration of 25.4MU/l. The concentration of AFP in the amniotic fluid was expressed in MU/l. AFP levels in amniotic fluid from 34 Down's syndrome pregnancies were converted to MOM using control gestational medians established from the routine analysis of several hundred amniotic fluid specimens at each gestation.

2.3.2.2 Human Chorionic Gonadotropin.

HCG levels in amniotic fluid from Down's syndrome and unaffected pregnancies were measured using the Serono hCG MAIAclone kit (described in Section 2.1.4.3) following a x50 dilution in horse serum.

2.3.2.2.1 HCG Data Analysis.

Median amniotic fluid hCG levels (IU/ml) in the unaffected pregnancies were calculated for each week of gestation from 15 to 21 weeks. Linear regression of the

logarithmically transformed medians, weighted for the number of samples at each week of gestation, provided the regression equation from which smoothed gestational medians were calculated. HCG levels in the affected and unaffected samples were converted to MOM.

2.3.2.3 Free Beta Human Chorionic Gonadotropin.

Levels of FβhCG in amniotic fluid from Down's syndrome and unaffected pregnancies were measured using an ELISA provided by NTD Laboratories (described in Section 2.1.4.4) following a x10 dilution in assay buffer.

2.3.2.3.1 FβhCG Data Analysis.

Median amniotic fluid FβhCG levels (ng/ml) in the unaffected pregnancies were calculated for each week of gestation from 15 to 21 weeks. Linear regression of the logarithmically transformed medians, weighted for the number of samples at each week of gestation, provided the regression equation from which smoothed gestational medians were calculated. FβhCG levels in the affected and unaffected samples were converted to MOM.

2.3.2.4 Pregnancy Associated Plasma Protein A.

Levels of PAPP-A in amniotic fluid from Down's syndrome and unaffected pregnancies were measured using an ELISA provided by NTD Laboratories (described in Section 2.1.4.5).

2.3.2.4.1 PAPP-A Data Analysis.

Median amniotic fluid PAPP-A levels (IU/l) in the unaffected pregnancies were calculated for each week of gestation from 15 to 21 weeks. Linear regression of the logarithmically transformed medians, weighted for the number of samples at each week of gestation, provided the regression equation from which smoothed gestational medians were calculated. PAPP-A levels in the affected and unaffected samples were converted to MOM.

2.3.2.5 Pregnancy Specific Beta 1 Glycoprotein.

The concentration of SP1 was measured in amniotic fluid from trisomic and unaffected pregnancies was measured by a time resolved immunofluorometric assay (Qin et al 1997) using a rabbit anti-SP1 antibody (A131, DAKO, Denmark) labelled with Eu^{3+}-chelate (Wallac OY, Finland). Analysis of amniotic fluid SP1 concentration was carried out in the Department of Clinical Biochemistry, Statens Seruminstitut, Denmark.

2.3.2.5.1 SP1 Data Analysis.

The amniotic fluid SP1 intra-assay CV of less than 10% and inter-assay CV of 12.4% at 2.7mIU/l, 9.3% at 7.5mIU/l and 7.2% at 12.8mIU/l. Median amniotic fluid SP1 levels (mIU/l) in the unaffected pregnancies were calculated for each week of gestation from 15 to 21 weeks. Linear regression of the logarithmically transformed medians, weighted for the number of samples at each week of gestation, provided the regression equation from which smoothed gestational medians were calculated. SP1 levels in the affected and unaffected samples were converted to MOM.

2.3.2.6 Alkaline Posphatase.

Total and placental ALP was measured in amniotic fluid from Down's syndrome and unaffected pregnancies using a modified version of the enzyme assay described in Section 2.1.4.7.

2.3.2.6.1 Measurement of Total and Placental ALP.

A pool of amniotic fluid samples was aliquoted and stored at -20°C for use as an internal quality control. Affected, unaffected and quality control samples were recovered from frozen storage and allowed to thaw. Each sample was divided into two aliquots and one aliquot was heat inactivated at 60°C for 1 hour. Total ALP was measured by adding 10µl of unheated sample to 90µl PBS (pH 7.2) and 100µl of substrate solution. The reaction mixture was incubated at 37°C for 1 hour after which the reaction was stopped by the addition of 2.3ml of glycine sodium hydroxide buffer (pH 10.4). Placental ALP activity was measured by adding 40µl of heated sample to 60µl PBS (pH 7.2) and 100µl of substrate solution. The reaction mixture was incubated at 37°C for one hour after which the reaction was stopped as described above. A reagent blank containing 100µl PBS and 100µl of substrate solution was included in both the total and placental ALP assays. Fluorescence was measured using a Perkin Elmer MPF-44B fluorescence spectrophotometer and the concentration of 4-MU liberated was determined using a 4-MU standard curve, as described in Section 2.1.4.7. ALP activity was calculated using the following equation.

$$\text{ALP activity} = \frac{\text{4-MU liberated (nM) x dilution factor}}{\text{incubation time (hr) x 1000}}$$

Where:

Dilution factor	=	250 for unheated samples.
		62.5 for heated samples.
Incubation time	=	1 hour.
1000	=	conversion from litres to ml.

2.3.2.6.2 ALP Data Analysis.

The total ALP assay had an inter-assay CV of 8.9% and an intra-assay CV of 7.5% at 267nmoles/hr/ml, while the placental ALP assay had an inter-assay CV of 26.9% and an intra-assay CV of 16.1% at 14.0nmoles/hr/ml. Median total and placental ALP levels (nmoles/hr/ml) in the unaffected pregnancies were calculated for each week of gestation from 15 to 20 weeks. Linear regression of the logarithmically transformed medians, weighted for the number of samples at each week of gestation, provided the regression equation from which smoothed gestational medians were calculated. Total and placental ALP levels in the affected and unaffected samples were converted to MOM.

2.3.2.7 Gamma Glutamyl Transferase.

GGT was measured in amniotic fluid from Down's syndrome and unaffected pregnancies using the γ-GT Reagent from Sigma Diagnostics (described in Section 2.1.4.8). ACCUTROL™ Normal Control Serum (Sigma Diagnostics) was used as a quality control. For the measurement of GGT in amniotic fluid the sample volume was reduced to 20μl in 1ml of γ-GT Reagent.

2.3.2.7.1 GGT Data Analysis.

The amniotic fluid GGT assay had an inter-assay CV of 7.7% and an intra-assay CV of 4.9% at 29.4U/l. GGT levels (U/l) in the unaffected pregnancies were calculated for each week of gestation from 15 to 20 weeks. Linear regression of the logarithmically transformed medians, weighted for the number of samples at each week of gestation, provided the regression equation from which smoothed gestational medians were calculated. GGT levels in the affected and unaffected samples were converted to MOM.

2.4 IMMUNOHISTOCHEMISTRY.

Placental tissue from 16 Down's syndrome and 16 unaffected control pregnancies was selected for immunohistochemical analysis. Placental tissue from each of the Down's syndrome pregnancies was matched to an unaffected control sample of the same gestational age and approximate length of time in frozen storage. The placental localisations of AFP, intact hCG, PAPP-A, placental ALP and SP-1 were investigated using a two-step indirect immunoperoxidase technique. In this technique, a specific unlabelled primary antibody is bound to antigen present in the tissue. A Horseradish Peroxidase (HRP) conjugated secondary antibody, directed against the immunoglobulin of the animal species in which the primary antibody has been raised is then applied. The peroxidase can then be visualised by development with the chromogen diaminobenzidine (DAB) and urea hydrogen peroxide to produce a brown product which is insoluble in alcohol, xylene and other inorganic solvents.

2.4.1 PREPARATION OF FROZEN SECTIONS FOR IMMUNOSTAINING.

Placental tissue was recovered from frozen storage at -70°C. Frozen sections were cut at 4μm using a cryostat at -25°C and picked up on Polysine slides (BDH) and air dried at room temperature for a minimum of 1 hour. At this stage sections can be wrapped in aluminium foil and stored at -20°C for a maximum of 7 days. Sections were fixed in acetone for 20 minutes and then air dried at room temperature.

2.4.2 INDIRECT IMMUNOPEROXIDASE TECHNIQUE FOR FROZEN SECTIONS.

All of the antibodies used in this study were obtained from DAKO. The primary antibodies were diluted to the appropriate working concentration in 0.01M PBS containing 1% normal human serum (NHS): rabbit anti-human AFP polyclonal - 1:50, rabbit anti-human hCG (β chain) polyclonal - 1:2000, rabbit anti-human PAPP-A polyclonal - 1:500, rabbit anti-human SP-1 polyclonal - 1:2000 or mouse anti-human placental ALP monoclonal - 1:5. The specificity of each of the antibodies has been ascertained by crossed-immunoelectrophoresis and/ or ELISA against non-pregnant human plasma and no significant cross-reaction was observed (DAKO specification sheet). The anti-human chorionic gonadotropin antibody shows a slight cross-reaction (approximately 3%) with LH. The matched Down's syndrome and unaffected sections were stained in the same batch to minimise possible variations due to any differences in experimental conditions and to allow comparison of the stained sections. Negative case controls, obtained by omitting primary antibody from the staining procedure, were included for each case to allow monitoring of non-specific staining. A positive control slide of normal mid-trimester placenta, and in some cases term placenta, was also included in each batch.

Fixed sections were washed in 0.01M PBS (pH 7.4) containing 0.3% Triton X-100 for 5 minutes. Appropriately diluted primary antibody was applied to the sections and incubated for 30 minutes at room temperature. The sections were washed in 0.01M PBS/ Triton X-100 for 5 minutes. A normal goat serum protein block (BioGenex) was applied to the section and incubated for 15 minutes. The slides were then drained and HRP-conjugated secondary antibody diluted 1/50 in 0.01M PBS (pH 7.4) containing 10% normal human serum was applied and incubated for 30 minutes. If the primary antibody was a rabbit anti-human polyclonal antibody, goat anti-rabbit immunoglobulin polyclonal/ HRP was used and if the primary antibody was a mouse anti-human monoclonal antibody, goat anti-mouse immunoglobulin polyclonal/ HRP was used. Slides were washed in 0.01M PBS/ Triton X-100 for 5 minutes and then in water for 5 minutes. The Sigma FAST DAB peroxidase substrate was prepared during the wash steps. Peroxidase substrate was applied to the sections and the colour was developed for approximately 3-5 minutes. The sections were washed in water for 5 minutes and then counterstained with Mayer's haematoxylin. The sections were placed in acid alcohol (0.25% concentrated hydrochloric acid in methylated spirit), in water and then in Scott's tap water (0.04M sodium bicarbonate, 0.16M anhydrous magnesium sulphate in distilled water) before being rinsed finally in water.

2.4.3 DEHYDRATION AND MOUNTING OF STAINED SECTIONS.

Sections were dehydrated through 3 changes of methylated spirits (3 x 5 minutes) and two changes of xylene (2 x 5 minutes). The sections were then mounted using Acrytol mounting medium (Surgipath).

2.4.4 QUALITATIVE AND SEMI-QUANTITATIVE ANALYSIS OF STAINED SECTIONS.

Stained sections were examined using an Olympus light microscope at x20 and x40 magnification to compare the localisation of each marker in the Down's syndrome and control placental sections. Semi-quantitative analysis of the stained sections was carried out by scoring the intensity of staining to determine if there was any difference between the Down's syndrome and unaffected control placental sections. The slides were coded and examined using an Olympus light microscope (x20 and x40 magnification). The staining intensity was scored blind by two people independently using the guidelines described in Table 2.4.1. and the results were pooled. The slides were then decoded and any differences in staining intensities between matched Down's syndrome and unaffected placental sections were investigated using the Sign test.

2.5 STATISTICAL METHODS.

Statistical analysis was carried out using SPSS for Windows.

2.5.1 COEFFICIENT OF VARIATION.

The coefficient of variation (CV) is the standard deviation (SD) expressed as a percentage of the mean (X). The inter-assay CV and intra-assay CV for each of the assays was calculated using the following equation.

$$CV = (100 \times SD / X)\%$$

Table 2.4.1 Guidelines for scoring intensity of staining of placental sections.

Score	Degree of staining
-	No staining present.
+/-	Light staining but patchy and not on all villi.
+	Light staining on all villi and around circumference of villi.
++	Medium to strong staining on all villi..
+++	Strong to very strong staining on all villi and around circumference of villi.

2.5.2 KOLMOGOROV-SMIRNOV TEST.

The Kolmogorov-Smirnov (K-S) test is used to determine how well a random sample of data fits a specific theoretical distribution. The test is based on a comparison of the sample cumulative distribution function with the hypothetical distribution function. The K-S test was used to determine whether the distributions of marker levels, both raw data and logarithmically transformed data, in unaffected tissues fitted a normal distribution.

2.5.3 MEDIANS.

The median is defined as the middle value of a group of observations when the data are arranged in order of increasing magnitude. When there is an even number of observations, the median is the average of the two middle observations. The position of the median is defined as (n+1)/2.

2.5.4 DETERMINATION OF MEDIANS BY REGRESSION ANALYSIS.

Due to the limited number of unaffected control tissues at each gestation, median marker levels were calculated using regression analysis. Regression analysis is a way of summarising the relationship between two quantitative variables by means of an equation. A regression equation allows that, for variables X and Y, a value of Y can be calculated for any given value of X. The equations for the regression models used in the analysis of the control data are described below. Median marker levels in the unaffected controls at each gestational week were calculated using the appropriate regression equation. Regression analysis, weighted according to the number of

samples at each week of gestation, was also used to calculate smoothed gestational medians for the control maternal serum and amniotic fluid data.

Linear: $Y = a + bX$

Logarithmic: $Y = a + b\ln(X)$

Exponential: $Y = a(e^{bX})$

S-curve: $Y = e^{(a + b/X)}$

2.5.5 MULTIPLES OF THE MEDIAN.

Marker levels in both Down's syndrome and unaffected control tissues were converted to multiples of the control median (MOM) of the appropriate gestational group.

MOM = marker level/ median

2.5.6 THE MANN-WHITNEY TEST.

The Mann-Whitney test is used to compare two independent samples. It is a non-parametric test which is valid no matter what the form of the population distributions. It tests the hypothesis that the population distributions of the two independent samples is the same. The observations from both samples are combined and ranked from smallest to largest. If the groups have the same distributions then the sample distribution of ranks should be similar. If one of the groups has a higher proportion of high or low ranks, there is reason to suspect that the two underlying distributions are

different. If the significance level (p-value) is greater then 0.05, the hypothesis that the samples have the same distribution is not rejected.

2.5.7 THE WILCOXON SIGNED RANK TEST.

The Wilcoxon Signed-Rank test is a non-parametric test used with two related samples to test the hypothesis that the distributions of the two variables are the same. No assumptions are made about the shape of the distributions. The test was used to compare the levels of total and placental ALP in each of the placental homogenates. The differences between the pairs are ranked without considering the signs of the differences. Average ranks are assigned in the case of ties. The sums of the ranks for the positive and negative differences are then calculated. If the distributions of the two variable are the same, the number and magnitude of the positive and negative differences should be similar. If the observed significance level is greater than 0.05, the hypothesis that the distributions are the same is not rejected.

2.5.8 CORRELATION.

The degree of association between two variables can be quantified using the Pearson correlation coefficient (r). It can be calculated from the equation below, where S_x and S_y are the standard deviations of the x and y variables respectively.

$$r = \frac{\sum(x-x)(y-y)}{(n-1)S_xS_y}$$

The value of r (+1 $\rightarrow$ -1) indicates the strength of the relationship between the two variables. The largest absolute value of 'r' is 1, which occurs when all of the points on

a scatter plot lie on a straight line. A value of zero indicates that there is no linear relationship. When 'r' is positive, there is a positive correlation between the two variables and when 'r' is negative, there is a negative correlation between the two variables. Significance testing is based on the hypothesis that the correlation coefficient is zero i.e. there is no correlation between the two variables. If the observed significance level is greater than 0.05, the hypothesis that there is no correlation between the two variables is not rejected.

2.5.9 THE SIGN TEST.

The sign test is a non-parametric test used with two related samples to test the hypothesis that the distributions of the two variables are the same. No assumptions are made about the shape of the distributions. This test was used to compare the scores, given on the basis of staining intensity, for each of the matched pairs Down's syndrome and unaffected placental sections after immunohistochemical analysis. The differences between the scores are calculated for each matched pair. The number of positive and negative differences is then determined. If the distributions of the two variables are the same, the numbers of positive and negative differences should be similar. If the observed significance level is greater than 0.05, the hypothesis that the distributions are the same is not rejected.

CHAPTER 3.

RESULTS.

3.1 MARKER LEVELS IN FETAL TISSUES.

Marker levels in the fetal tissues were expressed relative to the amount of total protein in the tissue extract. Regression analysis was used to estimate median marker levels at each gestation in the unaffected control tissues for two reasons. Firstly, the gestational ranges of the unaffected control and Down's syndrome pregnancies at termination are different. The majority of the unaffected pregnancies were terminated at 14-19 weeks gestation, while the majority of the Down's syndrome pregnancies were terminated at 18-23 weeks gestation. Secondly, there is an insufficient number of control samples at each week of gestation to allow the calculation of gestation-specific medians as described in Section 2.5.3. Using regression analysis it is possible to fit a curve which best describes the relationship between marker levels and gestation and which can be extrapolated to cover the gestations for which there is little or no control data. Median marker levels at each week of gestation can be calculated from the equation of the regression line.

Raw control data and logarithmically transformed control data were each analysed using the K-S test to determine which best fitted a normal distribution. Then, using either the raw data or the logarithmically transformed data, the most suitable regression model for each of the markers in the control tissues was established. To allow for the change in marker concentrations with gestation, all analyte values were converted to multiples of the control group median at each gestation (MOM). The median MOM for each marker in the control tissues should be close to 1.0 MOM if the chosen regression model accurately describes the data.

3.1.1 AFP LEVELS IN PLACENTAL TISSUE.

3.1.1.1 Unaffected Controls.

The concentration profile of AFP with gestation in placental tissue from 52 unaffected control pregnancies at 12 to 20 weeks gestation is shown in Figure 3.1.1. Median AFP levels, determined by linear regression of logarithmically transformed data (AFP median = $10^{(4.49-0.12 \times \text{gestation})}$), are given in Table 3.1.1. Median AFP levels decrease by an average of about 25% from week to week as gestation advances. The overall median AFP level, in MOM, in control placental tissue estimated using the regressed median values was 0.99 MOM.

3.1.1.2 Down's Syndrome.

AFP levels, expressed as MOM, in placental tissue from 51 Down's syndrome pregnancies at 10 to 24 weeks gestation are shown in Figure 3.1.2. In the majority of Down's syndrome pregnancies AFP levels were greater than 1.0 MOM and there appeared to be a trend of rising levels with advancing gestation. The median AFP level in the affected pregnancies was 2.43 MOM. AFP levels in the Down's syndrome and control pregnancies were compared using the Mann-Whitney test. AFP was significantly higher in placental tissue from Down's syndrome pregnancies than in control placental tissue ($p<0.001$).

3.1.1.3 Trisomy 13 and Trisomy 18.

AFP levels (MOM) in placental tissue from one Trisomy 13 and three Trisomy 18 pregnancies are shown in Figure 3.1.2. The level of AFP in placental tissue from one

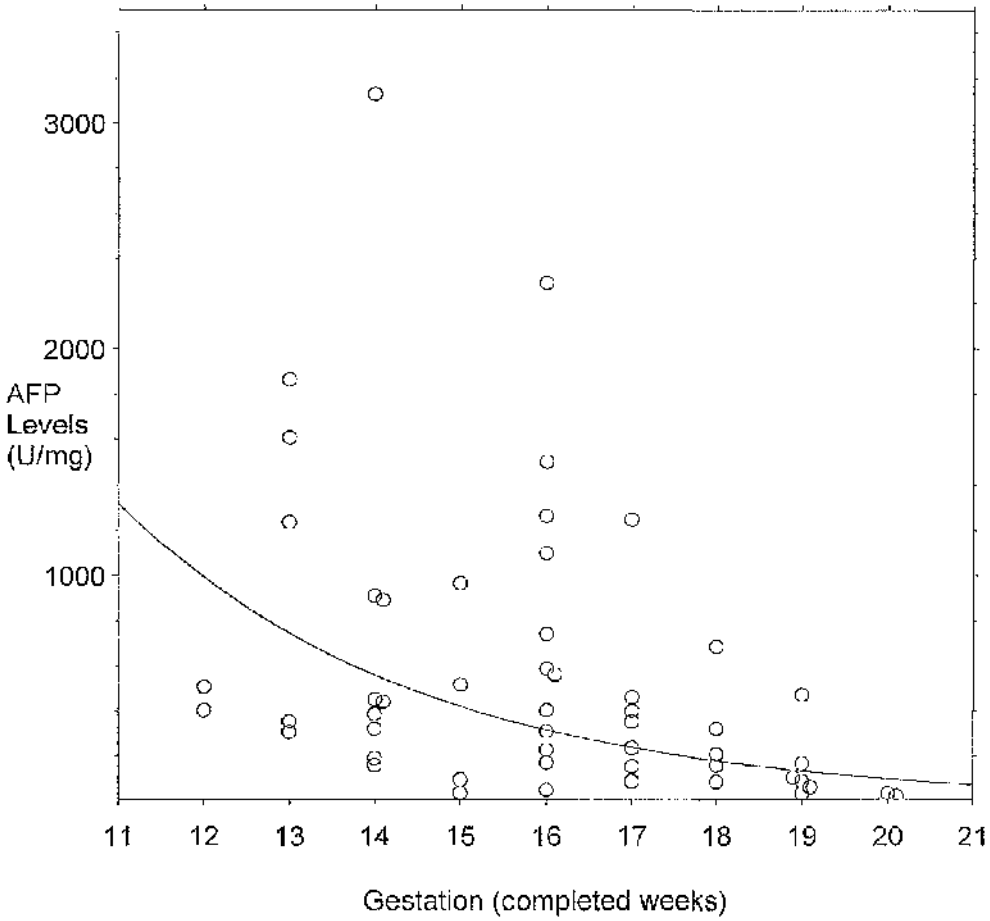

Figure 3.1.1 AFP levels (U/mg) in placental tissue from 52 unaffected control pregnancies at 12 to 20 weeks gestation. Smoothed medians across the gestational range were calculated by linear regression of logarithmically transformed data.

Table 3.1.1 Regressed median levels of AFP in placental tissue (U/mg) and fetal liver (KU/mg) from unaffected control pregnancies at each week of gestation from 10 to 24 weeks.

Gestation	Median AFP levels	
	Placenta (U/mg)	Liver (KU/mg)
10	1756.31	7.77
11	1319.78	6.71
12	991.74	5.79
13	745.25	5.00
14	560.02	4.32
15	420.82	3.73
16	316.23	3.22
17	237.63	2.78
18	178.57	2.4
19	134.18	2.07
20	100.83	1.79
21	75.77	1.54
22	56.94	1.33
23	42.79	1.15
24	31.15	0.99

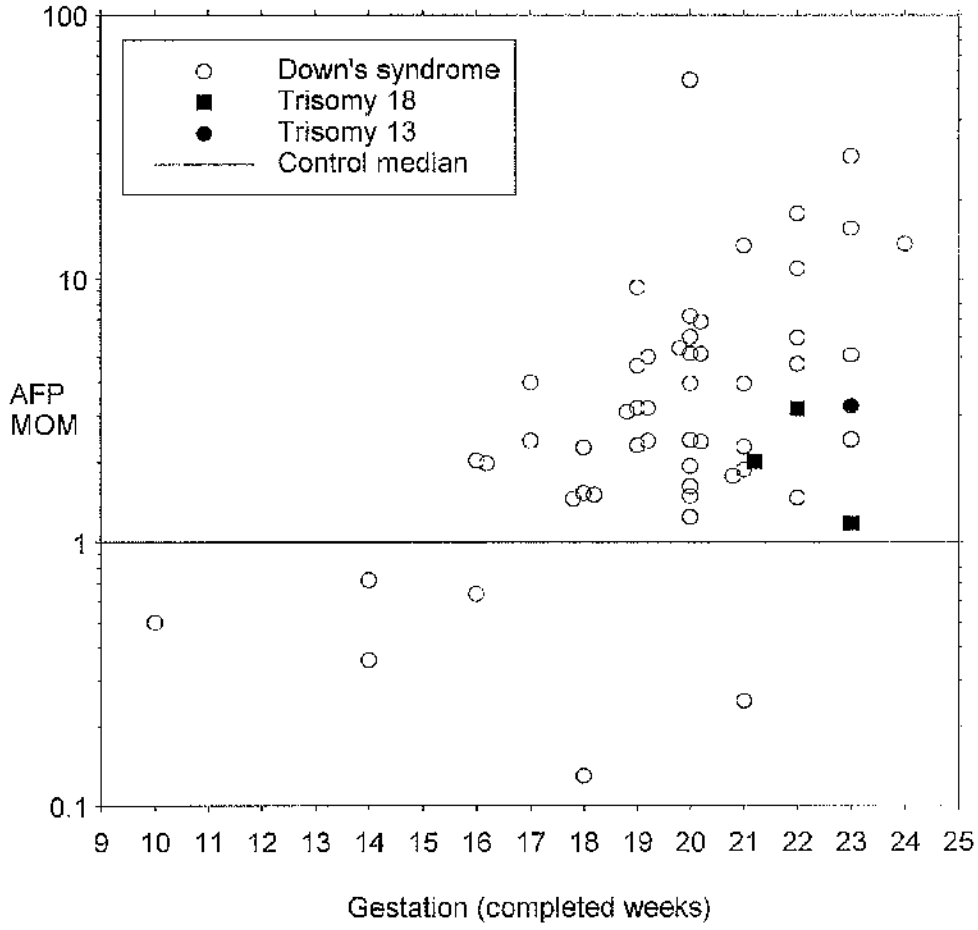

Figure 3.1.2 AFP levels (MOM) in placental tissue from 51 Down's syndrome pregnancies at 10 to 24 weeks gestation, and in one Trisomy 13 and three Trisomy 18 pregnancies.

Trisomy 13 pregnancy was 3.25 MOM. In placental tissue from three Trisomy 18 pregnancies AFP levels were 1.18 MOM, 3.18 MOM and 2.01 MOM.

3.1.2 AFP LEVELS IN FETAL LIVER.

3.1.2.1 Unaffected Controls.

The concentration profile of AFP in fetal liver from 68 unaffected pregnancies at 12 to 22 weeks gestation is shown in Figure 3.1.3. Median AFP levels at each gestation were determined by linear regression of logarithmically transformed data (AFP median = $10^{(1.53-0.0638 \times \text{gestation})}$) and are shown in Table 3.1.1. Median AFP levels decrease as gestation advances from 12 to 22 weeks. Hepatic AFP levels are 10-20 times higher than placental AFP levels at the corresponding gestations between 15 and 20 weeks. The median AFP level in fetal liver from unaffected control pregnancies was 1.01 MOM.

3.1.2.2 Down's syndrome.

The concentrations of AFP, expressed as MOM, in fetal liver samples from 54 Down's syndrome pregnancies at 13 to 24 weeks gestation are illustrated in Figure 3.1.4. AFP levels in the Down's syndrome tissue extracts were distributed evenly above and below the control group median. The median level of AFP in the Down's syndrome pregnancies was 0.97 MOM. There was no statistically significant difference in AFP levels in fetal liver from Down's syndrome pregnancies compared with the normal control pregnancies (Mann-Whitney, p=0.26).

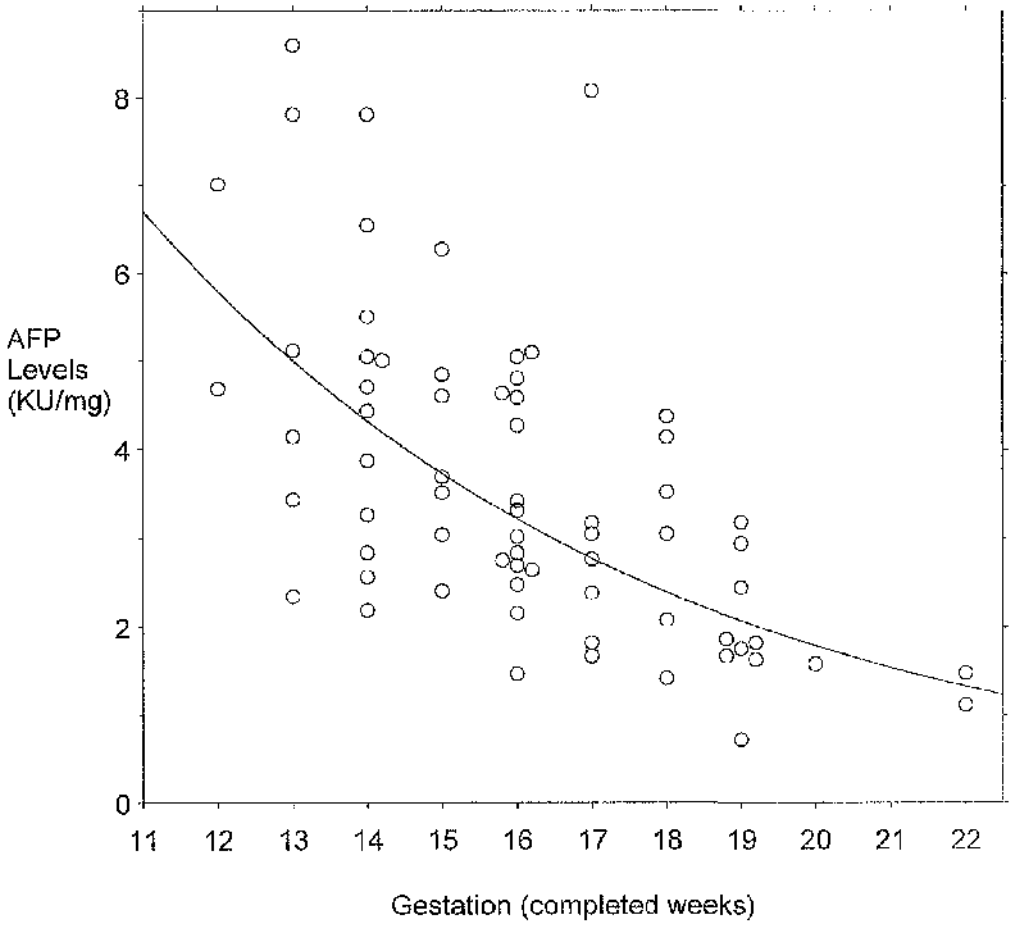

Figure 3.1.3 AFP levels (KU/mg) in fetal liver from 68 unaffected control pregnancies at 12 to 22 weeks gestation. Smoothed medians across the gestational range were calculated by linear regression of logarithmically transformed data.

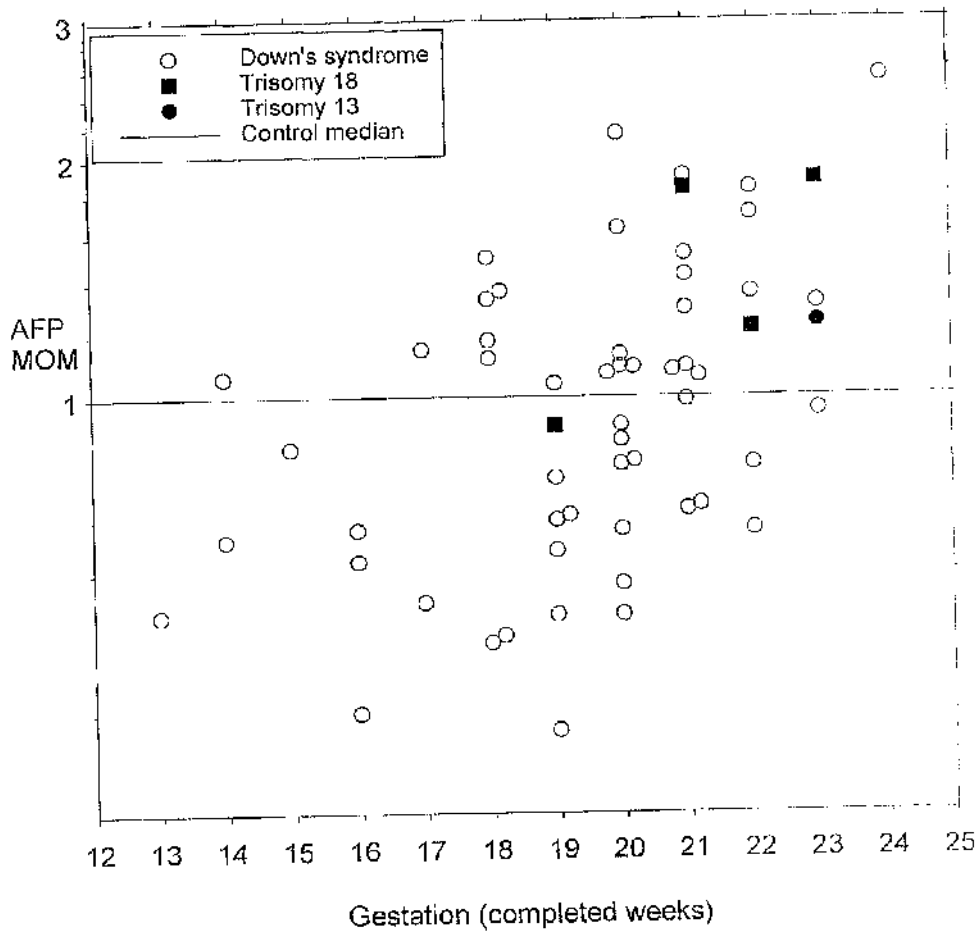

Figure 3.1.4 AFP levels (MOM) in fetal liver from 54 Down's syndrome pregnancies at 13 to 24 weeks gestation, and in one Trisomy 13 and four Trisomy 18 pregnancies.

3.1.2.3 Trisomy 13 and Trisomy 18.

AFP levels (MOM) in fetal liver from the Trisomy 13 and Trisomy 18 pregnancies are shown in Figure 3.1.4. The level of AFP in fetal liver from one Trisomy 13 pregnancy was 1.24 MOM. In fetal liver from four Trisomy 18 pregnancies AFP levels were 1.88 MOM, 1.22 MOM, 1.83 MOM and 0.92 MOM.

3.1.3 INTACT HCG LEVELS IN PLACENTAL TISSUE.

3.1.3.1 Unaffected Controls.

The concentration profile of hCG in placental tissue from 52 unaffected control pregnancies at 12 to 20 weeks gestation is shown in Figure 3.1.5. Median hCG levels at each week of gestation, determined using an exponential regression model (intact hCG median = $221\exp^{(-0.319 \times \text{gestation})}$), are given in Table 3.1.2. Intact hCG levels decreased as gestation advances from 12 to 20 weeks. The overall median level of intact hCG in placental tissue from unaffected pregnancies was 1.19 MOM.

3.1.3.2 Down's Syndrome.

Intact hCG levels, expressed as MOM, in placental tissue from 51 Down's syndrome pregnancies at 10 to 24 weeks gestation are shown in Figure 3.1.6. In each of the Down's syndrome pregnancies placental hCG levels were above the control group median. Intact hCG levels in placental tissue from Down's syndrome pregnancies were significantly higher than in unaffected pregnancies (Mann-Whitney, $p < 0.001$), with a median value of 4.06 MOM in the Down's syndrome pregnancies.

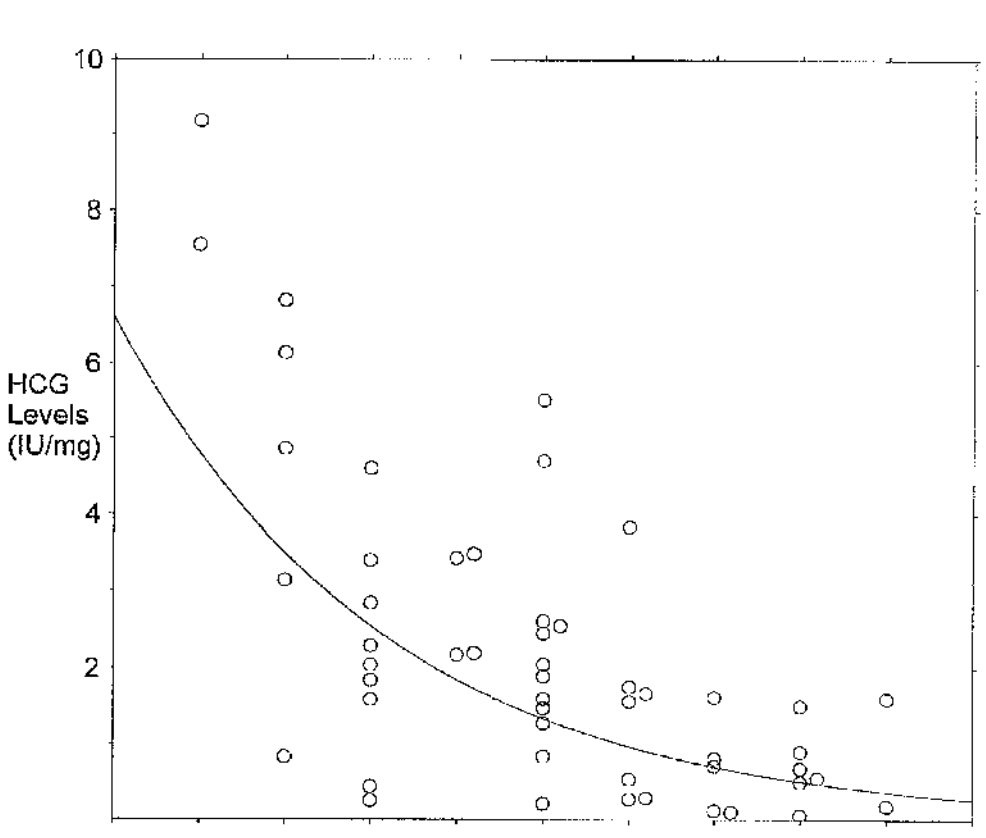

Figure 3.1.5 Intact hCG levels (IU/mg) in placental tissue from 52 unaffected control pregnancies at 12 to 20 weeks gestation. Smoothed medians across the gestational range were calculated using an exponential regression model.

Table 3.1.2 Regressed median levels of intact hCG (IU/mg) in placental tissue from unaffected control pregnancies at each week of gestation from 10 to 24 weeks.

Gestation	Median intact hCG levels (IU/mg)
10	9.10
11	6.61
12	4.81
13	3.49
14	2.54
15	1.85
16	1.34
17	0.98
18	0.71
19	0.52
20	0.37
21	0.27
22	0.2
23	0.14
24	0.10

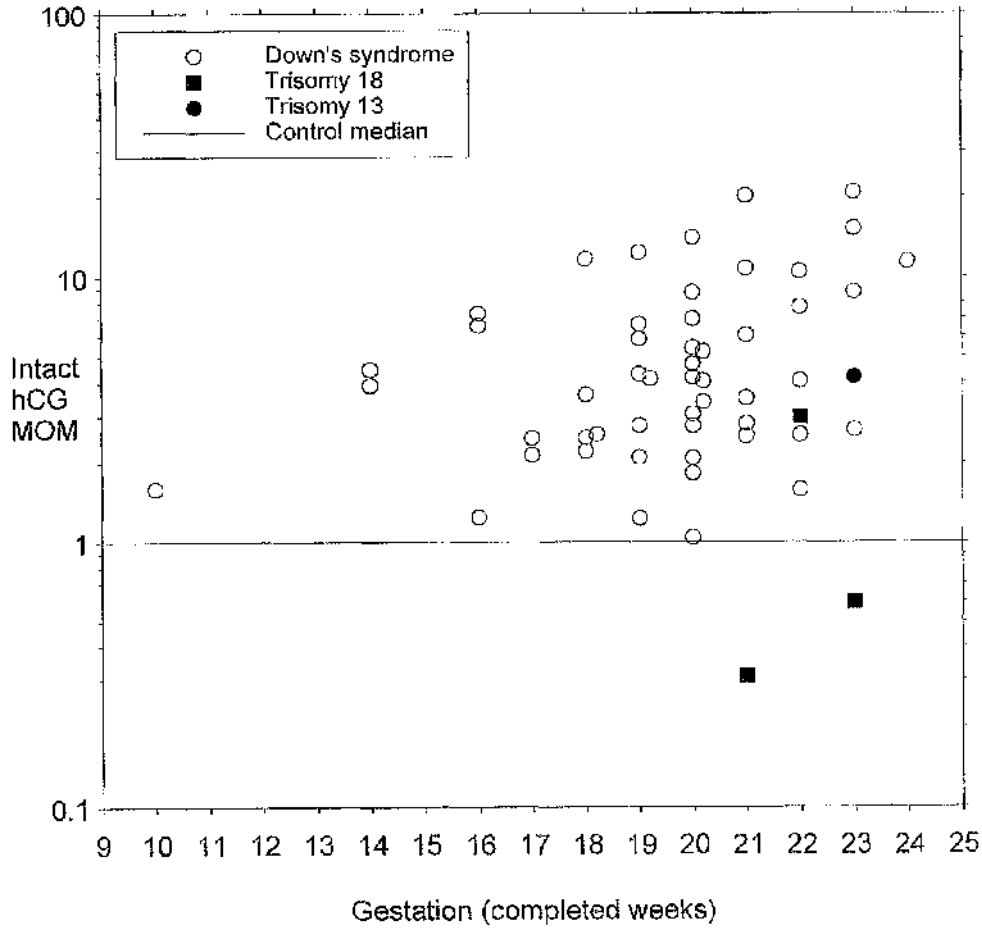

Figure 3.1.6 Intact hCG levels (MOM) in placental tissue from 51 Down's syndrome pregnancies at 10 to 24 weeks gestation, and in one Trisomy 13 and three Trisomy 18 pregnancies.

3.1.3.3 Trisomy 13 and Trisomy 18.

Intact hCG levels (MOM) in placental tissue from the Trisomy 13 and Trisomy 18 pregnancies are shown in Figure 3.1.6. The level of intact hCG in placental tissue from one Trisomy 13 pregnancy was 4.20 MOM. In placental tissue from three Trisomy 18 pregnancies intact hCG levels were 0.59 MOM, 2.97 MOM and 0.31 MOM.

3.1.4 FβHCG LEVELS IN PLACENTAL TISSUE.

3.1.4.1 Unaffected Controls.

The concentration profile of the free beta subunit of hCG in placental tissue from 52 unaffected pregnancies is illustrated in Figure 3.1.7. Gestational medians were calculated by linear regression of logarithmically transformed data (FβhCG median = $10^{(3.78-0.133 \times \text{gestation})}$) and are given in Table 3.1.3. Placental levels of FβhCG decreased with gestation from 12 to 20 weeks. The overall median level of FβhCG in placental tissue from unaffected placental tissue is 1.05 MOM.

3.1.4.2 Down's Syndrome.

Placental levels of FβhCG, expressed as MOM, in 51 Down's syndrome pregnancies at 10 to 24 weeks gestation are shown in Figure 3.1.8. The majority of the Down's syndrome cases had placental FβhCG levels above the control group median. FβhCG levels in placental tissue from Down's syndrome pregnancies were significantly higher than in unaffected pregnancies (Mann-Whitney, $p<0.001$). The overall median level of FβhCG in placental tissue from Down's syndrome pregnancies was 3.40 MOM.

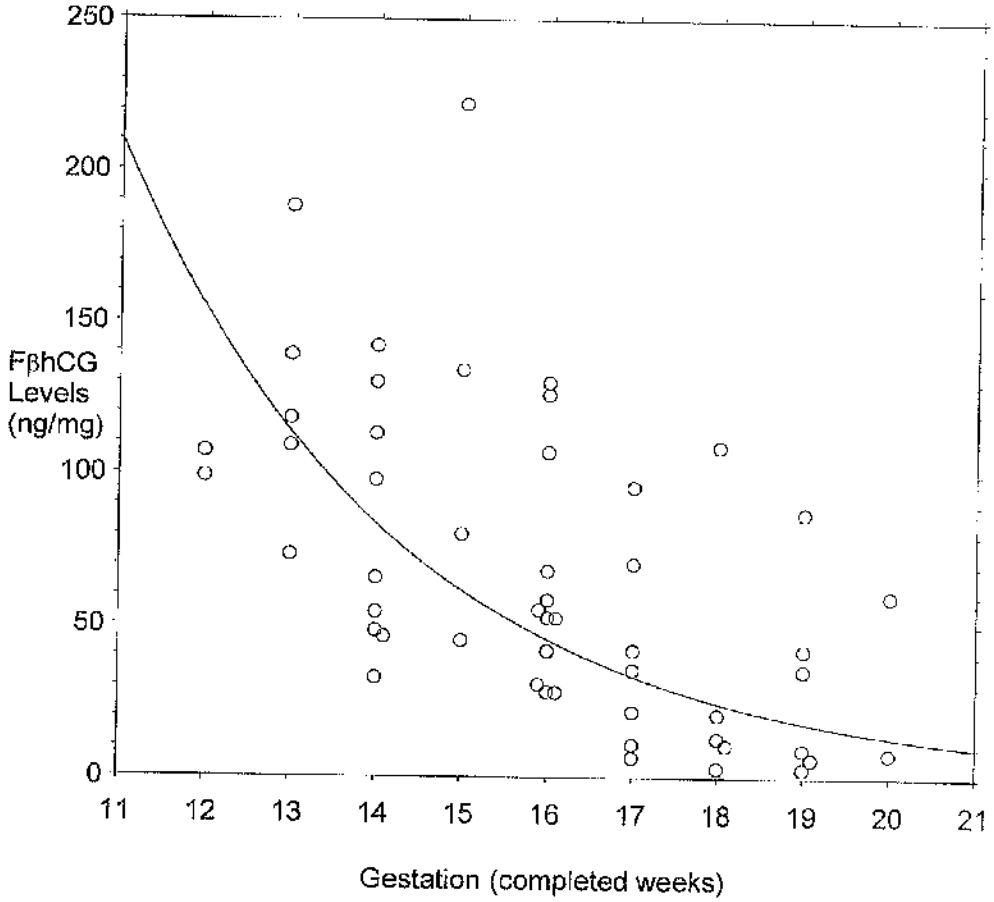

Figure 3.1.7 FβhCG levels (ng/mg) in placental tissue from 52 unaffected control pregnancies at 12 to 20 weeks gestation. Smoothed medians across the gestational range were calculated by linear regression of logarithmically transformed data.

Table 3.1.3 Regressed median levels of FβhCG (ng/mg) in placental tissue from unaffected control pregnancies at each week of gestation from 10 to 24 weeks.

Gestation	Median FβhCG levels (ng/mg)
10	285
11	210
12	154
13	114
14	83.6
15	61.6
16	45.3
17	33.4
18	24.6
19	18.1
20	13.3
21	9.80
22	7.22
23	5.31
24	3.91

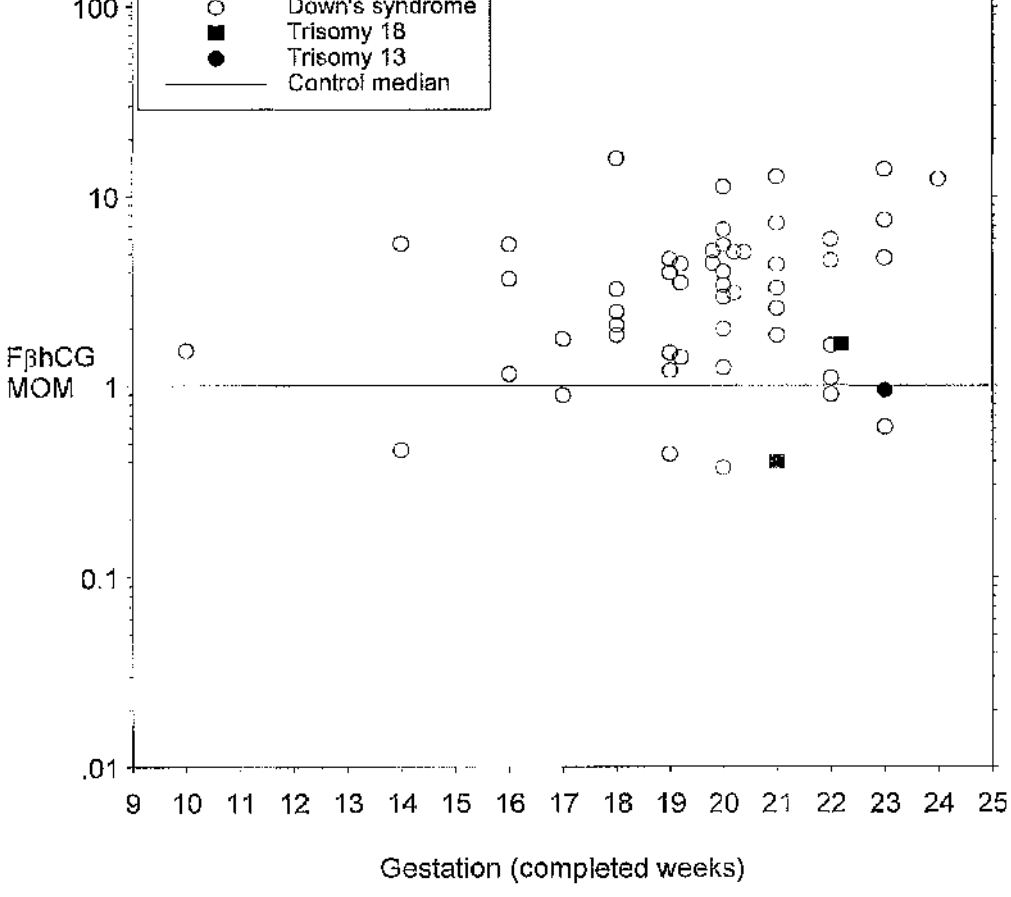

Figure 3.1.8 FβhCG levels (MOM) in placental tissue from 51 Down's syndrome pregnancies at 10 to 24 gestation, and in one Trisomy 13 and three Trisomy 18 pregnancies.

3.1.4.3 Trisomy 13 and Trisomy 18.

FβhCG levels (MOM) in placental tissue from the Trisomy 13 and Trisomy 18 pregnancies are shown in Figure 3.1.8. The level of FβhCG in placental tissue from one Trisomy 13 pregnancy was 0.95 MOM. In placental tissue from three Trisomy 18 pregnancies FβhCG levels were 1.66 MOM and 0.04 MOM in cases T18/2 and T18/3 respectively, and undetectable (below assay sensitivity) in case T18/1.

3.1.5 PAPP-A LEVELS IN PLACENTAL TISSUE.

3.1.5.1 Unaffected Controls.

The concentration profile of PAPP-A in placental tissue from 52 unaffected pregnancies at 12 to 20 weeks gestation is shown in Figure 3.1.9. Median PAPP-A levels at each gestation were determined using logarithmic regression (PAPP-A median = -0.142+0.114 ln(gestation)) and are given in Table 3.1.4. Median levels of PAPP-A in unaffected placental tissue increased gradually from 12 to 20 weeks gestation. The overall median level of PAPP-A in placental tissue from unaffected pregnancies was 0.84 MOM.

3.1.5.2 Down's Syndrome.

Placental levels of PAPP-A, expressed as MOM, in 51 Down's syndrome pregnancies at 10 to 24 weeks gestation are shown in Figure 3.1.10. PAPP-A levels were evenly distributed above and below the control group median and were not significantly different from normal (Mann-Whitney, p=0.304). The overall median placental level of PAPP-A in the Down's syndrome pregnancies was 0.96 MOM.

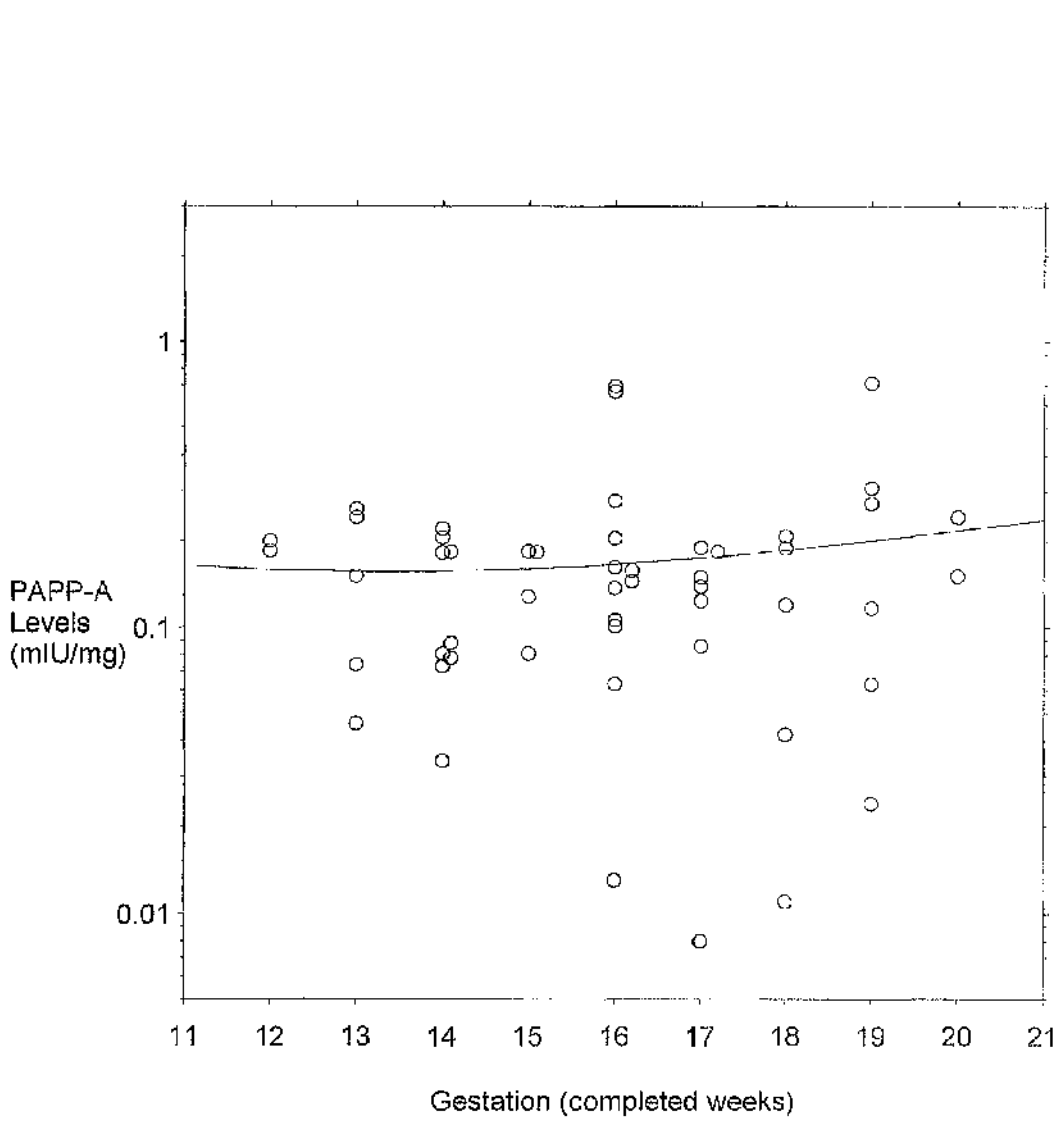

Figure 3.1.9 PAPP-A levels (mIU/mg) in placental tissue from 52 unaffected control pregnancies at 12 to 20 weeks gestation. Smoothed medians across the gestational range were calculated by logarithmic regression.

Table 3.1.4 Regressed median levels of PAPP-A (mIU/mg) in placental tissue from unaffected control pregnancies at each week of gestation from 10 to 24 weeks.

Gestation	Median PAPP-A levels
10	0.120
11	0.131
12	0.141
13	0.150
14	0.158
15	0.173
16	0.173
17	0.180
18	0.187
19	0.193
20	0.199
21	0.204
22	0.209
23	0.214
24	0.219

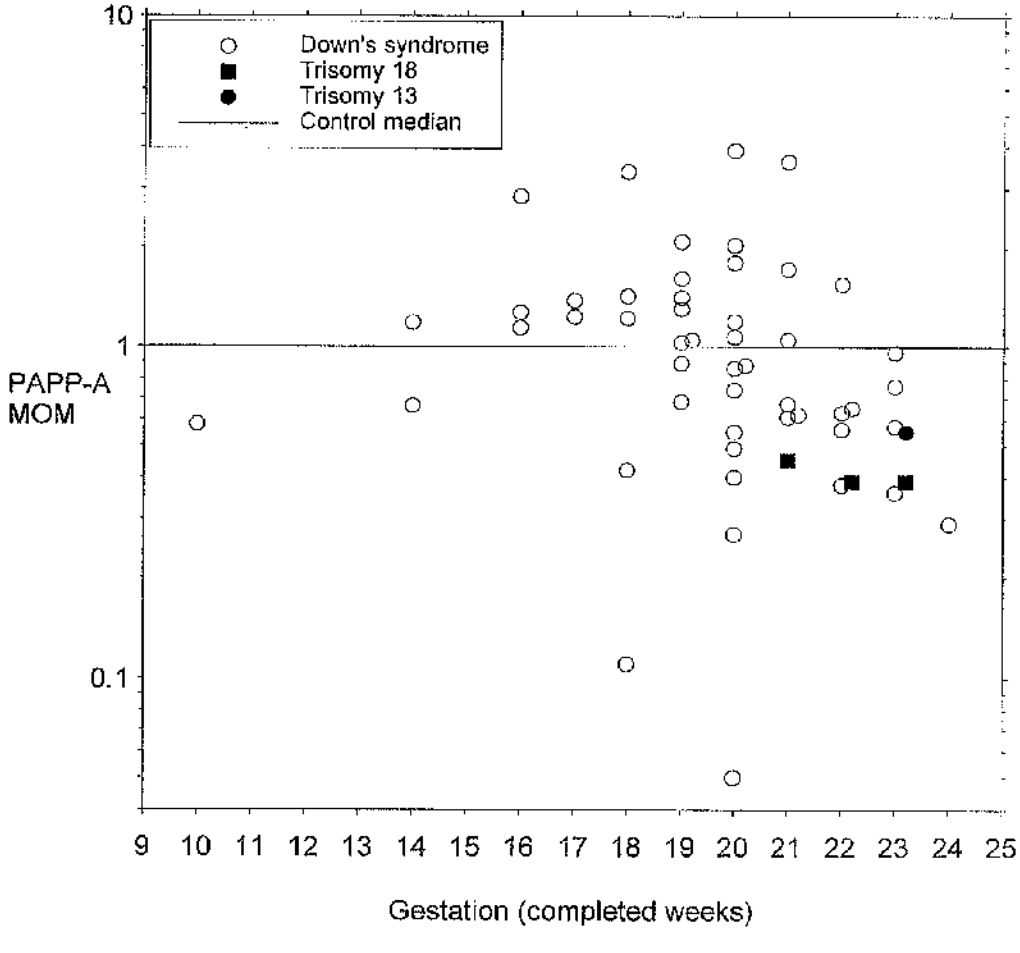

Figure 3.1.10 PAPP-A levels (MOM) in placental tissue from 51 Down's syndrome pregnancies at 10 to 24 weeks gestation, and in one Trisomy 13 and three Trisomy 18 pregnancies.

3.1.5.3 Trisomy 13 and Trisomy 18.

PAPP-A levels (MOM) in placental tissue from the Trisomy 13 and Trisomy 18 pregnancies are shown in Figure 3.1.10. The level of PAPP-A in placental tissue from one Trisomy 13 pregnancy was 0.39 MOM. In placental tissue from three Trisomy 18 pregnancies PAPP-A levels are 0.39 MOM, 0.39 MOM and 0.45 MOM.

3.1.6 SP1 LEVELS IN PLACENTAL TISSUE.

3.1.6.1 Unaffected Controls.

The concentration profile of SP1 in placental tissue from 52 unaffected pregnancies at 12 to 20 weeks gestation is shown in Figure 3.1.11. Median SP1 levels at each gestation were calculated using linear regression (SP1 mediam = $0.002\text{-}8.00\text{x}10^{-5}$ x gestation) and are given in Table 3.1.5. Median SP1 levels decreased from 12 to 20 weeks gestation. The overall median level of SP1 in placental tissue from unaffected pregnancies was 0.91 MOM.

3.1.6.2 Down's syndrome.

SP1 levels, expressed as MOM, in placental tissue from 51 Down's syndrome pregnancies at 10 to 24 weeks gestation are shown in Figure 3.1.12. The median level of SP1 in Down's syndrome placental tissue was 1.79 MOM with the majority of Down's syndrome cases having SP1 levels greater than the control group median. SP1 levels in Down's syndrome placental tissue were significantly higher than in unaffected controls (Mann-Whitney, $p < 0.01$).

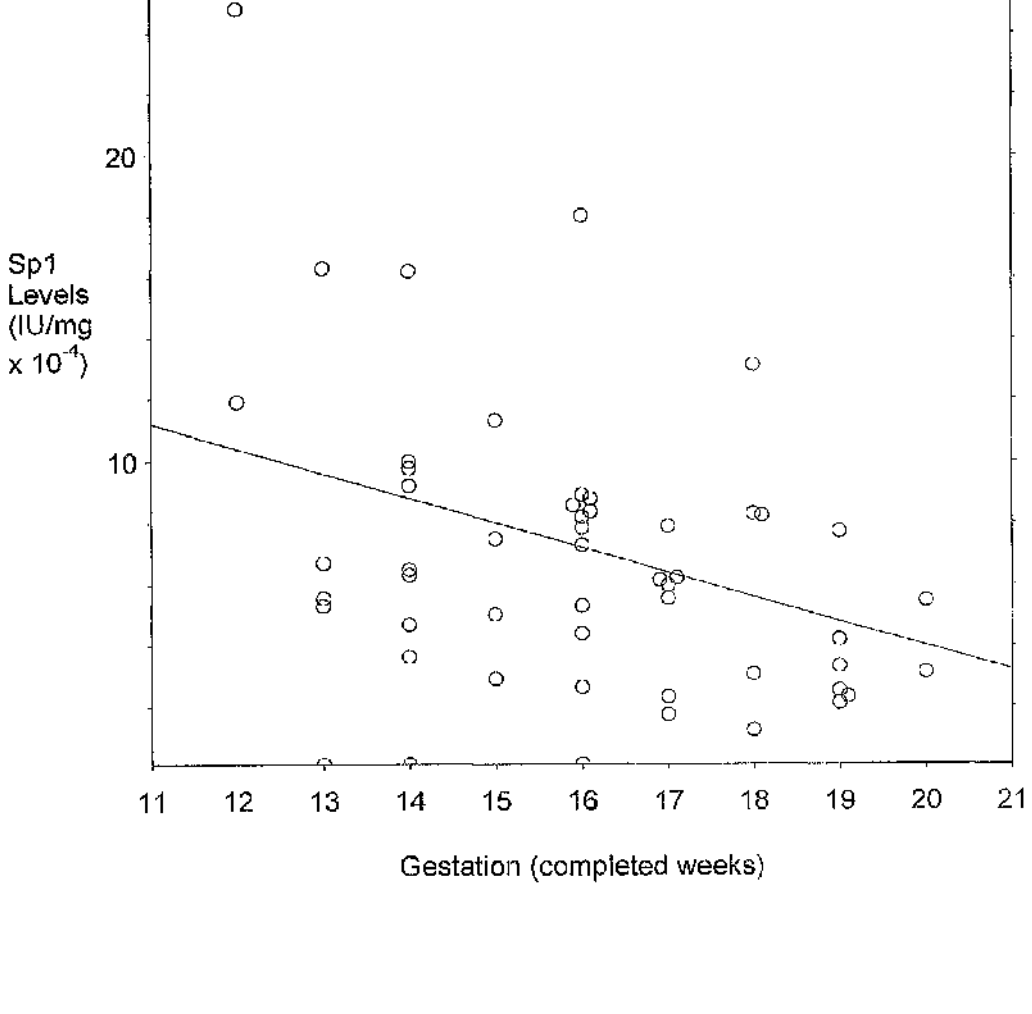

Figure 3.1.11 SP-1 levels (IU/mg x 10^{-4}) in placental tissue from 52 unaffected control pregnancies at 12 to 20 weeks gestation. Smoothed medians across the gestational range were calculated by linear regression.

Table 3.1.5 Regressed median levels of SP-1 (IU/mg x 10^{-4}) in placental tissue from unaffected control pregnancies at each week of gestation from 10 to 24 weeks.

Gestation	Median SP-1 levels (IU/mg x 10^{-4})
10	12.0
11	11.2
12	10.4
13	9.60
14	8.80
15	8.00
16	7.20
17	6.40
18	5.60
19	4.80
20	4.00
21	3.20
22	2.40
23	1.60
24	8.00

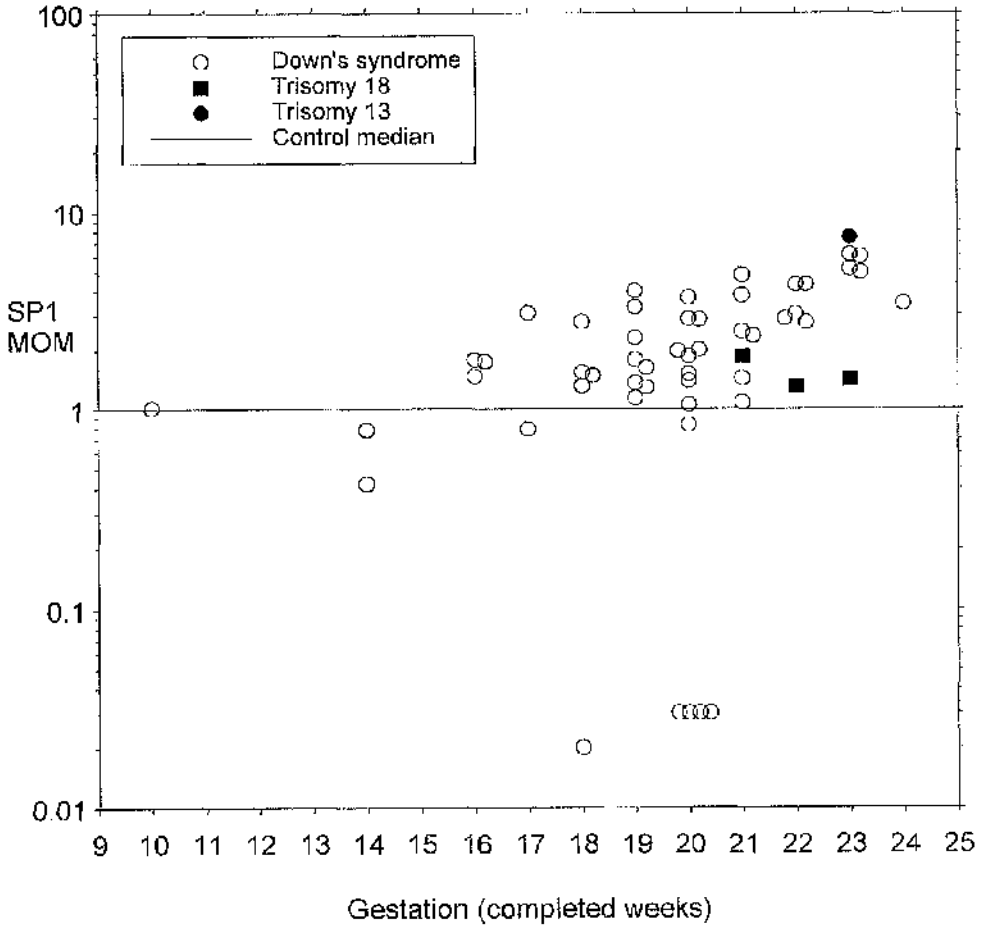

Figure 3.1.12 SP-1 levels (MOM) in placental tissue from 51 Down's syndrome pregnancies at 10 to 24 weeks gestation, and in one Trisomy 13 and three Trisomy 18 pregnancies.

3.1.6.3 Trisomy 13 and Trisomy 18.

SP1 levels (MOM) in placental tissue from the Trisomy 13 and Trisomy 18 pregnancies are shown in Figure 3.1.12. The level of SP1 in placental tissue from one Trisomy 13 pregnany was 7.44 MOM. In placental tissue from three Trisomy 18 pregnancies SP-1 levels were 1.41 MOM, 1.30 MOM and 1.85 MOM.

3.1.7 ALP LEVELS IN PLACENTAL TISSUE.

ALP activity was measured in unheated placental homogenates and in homogenates that were incubated at 60°C for 1 hour. ALP activity in the unheated samples represents total ALP activity which may be composed of bone/liver/kidney, intestinal and placental isoenzymes. Residual ALP activity in the placental homogenates after heating is due to the placental isoenzyme which is heat stable. There was little difference in ALP activity in the heated and unheated samples suggesting virtually exclusive expression of the placental isoenzyme in the placenta. The Wilcoxon Signed-Rank Test was used to compare total and placental ALP activities in each placental homogenate to determine if the distributions of the enzyme activities were the same. The results of the Wilcoxon Signed-Rank Test for the complete set of data (Down's syndrome and control samples) and for the Down's syndrome and control samples separately are presented in Table 3.1.6. There was no significant difference between total and placental ALP activities in the the placental samples from either the Down's syndrome or the unaffected pregnancies confirming that only the placental isoenzyme is expressed in placental tissue.

Table 3.1.6 Comparison of total and placental ALP levels in placental tissue from Down's syndrome and unaffected pregnancies using the Wilcoxon Signed-Rank Test.

	+ve differences	-ve differences	Ties	p-value
All data	59	50	0	0.645
Controls only	29	23	0	0.387
Down's syndrome only	26	25	0	0.603

3.1.7.1 Unaffected Controls.

The concentration profile of the placental isoenzyme of ALP in placental tissue from 52 unaffected control pregnancies at 12 to 20 weeks gestation is shown in Figure 3.1.13. Gestational medians were determined using linear regression of logarithmically transformed data (placental ALP median = $10^{(1.31+0.0681 \times \text{gestation})}$) and are given in Table 3.1.7. Median levels of placental ALP increased with advancing gestation from 12 to 20 weeks. The overall median activity of placental ALP in placental tissue from unaffected pregnancies was 1.10 MOM.

3.1.7.2 Down's Syndrome.

Placental ALP activity, expressed as MOM, in placental tissue from 51 Down's syndrome pregnancies at 10 to 24 weeks gestation are shown in Figure 3.1.14. Placental ALP activity in the Down's syndrome tissues was distributed around the control group median and were not significantly different from normal (Mann-Whitney, p=0.318). The overall median activity of placental ALP in the affected tissues was 0.91 MOM.

3.1.7.3 Trisomy 13 and Trisomy 18.

Placental ALP activity (MOM) in placental tissue from the Trisomy 13 and Trisomy 18 pregnancies are shown in Figure 3.1.14. The activity of placental ALP in placental tissue from one Trisomy 13 pregnancy was 1.43 MOM. In placental tissue from three Trisomy 18 pregnancies PALP activity was 0.70 MOM, 1.07 MOM and 2.11 MOM.

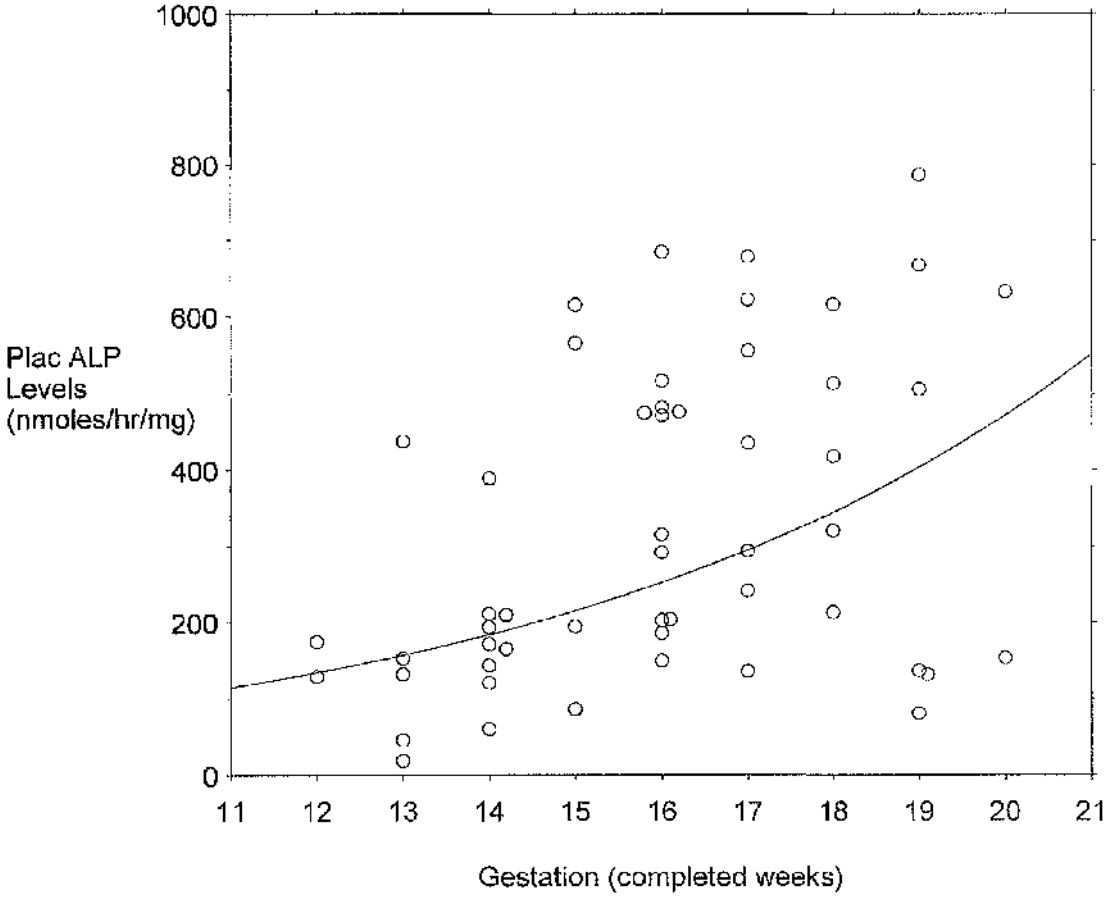

Figure 3.1.13 Placental ALP levels (nmoles/hr/mg) in placental tissue from 52 unaffected control pregnancies at 12 to 20 weeks gestation. Smoothed medians across the gestational range were calculated by linear regression of logarithmically transformed data.

Table 3.1.7 Regressed median levels of placental ALP (nmoles/hr/mg) in placental tissue from unaffected control pregnancies at each week of gestation from 10 to 24 weeks.

Gestation	Median placental ALP levels (nmoles/hr/mg)
10	98.4
11	115
12	135
13	158
14	184
15	216
16	252
17	295
18	345
19	404
20	472
21	552
22	646
23	756
24	884

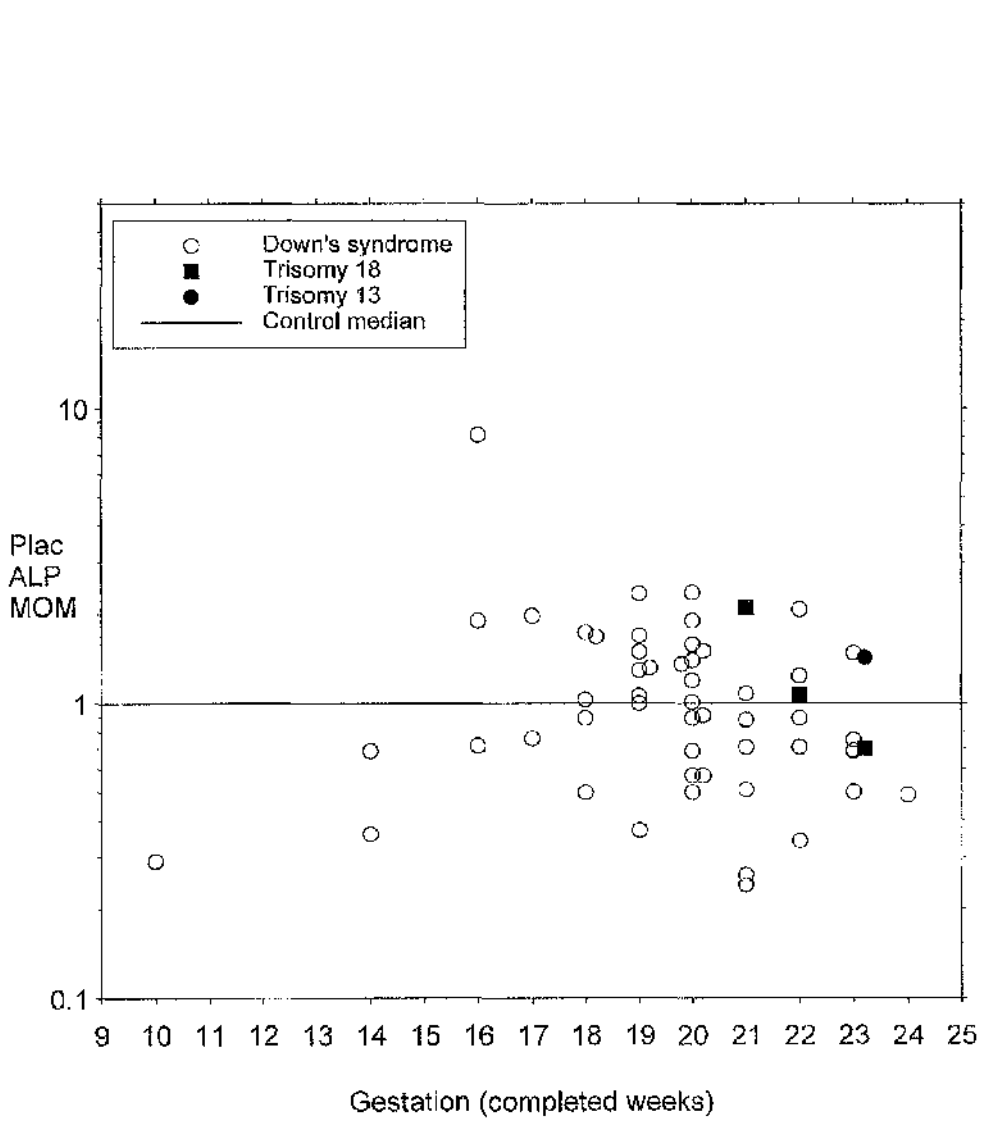

Figure 3.1.14 Placental ALP levels (MOM) in placental tissue from 51 Down's syndrome pregnancies at 10 to 24 weeks gestation, and in one Trisomy 13 and three Trisomy 18 pregnancies.

3.1.8 GGT LEVELS IN PLACENTAL TISSUE.

3.1.8.1 Unaffected Controls.

The activity profile of GGT in placental tissue from 52 unaffected control pregnancies at 12 to 20 weeks gestation is shown in Figure 3.1.15. Median levels at each week of gestation were calculated using linear regression (GGT median = $0.002\text{-}4.0\text{x}10^{-5}$ x gestation) and are given in Table 3.1.8. GGT activity decreased slightly with advancing gestation. The median level of GGT activity in placental tissue from unaffected pregnancies was 0.93 MOM.

3.1.8.2 Down's Syndrome.

GGT activity, expressed as MOM, in placental tissue from 51 Down's syndrome pregnancies at 10 to 24 weeks gestation are shown in Figure 3.1.16. GGT activity in placental tissue from Down's syndrome pregnancies was slightly higher than in unaffected pregnancies, with a median level of 1.29 MOM in the affected tissues. This increase was found to be statistically significant using the Mann-Whitney test ($p < 0.01$).

3.1.8.3 Trisomy 13 and Trisomy 18.

GGT actvity (MOM) in placental tissue from the Trisomy 13 and Trisomy 18 pregnancies are shown in Figure 3.1.16. GGT activity in placental tissue from one Trisomy 13 pregnancy was 2.68 MOM. In placental tissue from three Trisomy 18 pregnancies GGT activity was 1.20 MOM, 4.18 MOM and 2.75 MOM.

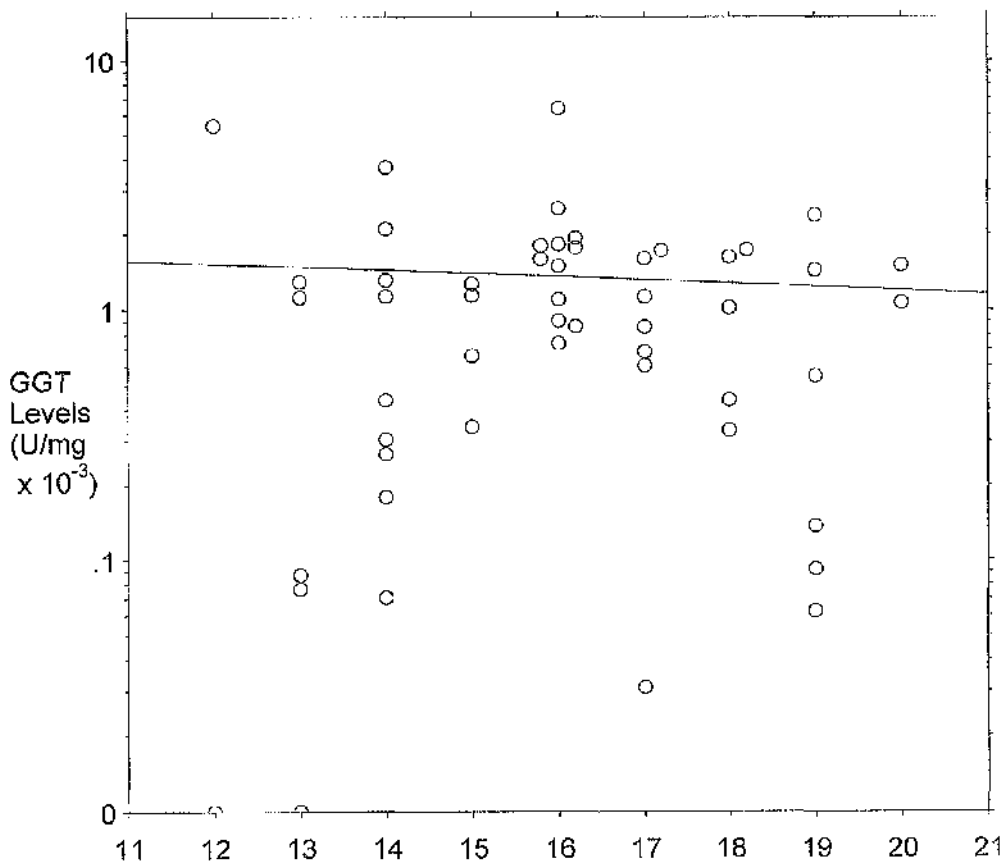

Figure 3.1.15 GGT levels (U/mg x 10^{-3}) in placental tissue from 52 unaffected control pregnancies at 12 to 20 weeks gestation. Smoothed medians across the gestational range were calculated by linear regression.

Table 3.1.8 Regressed median levels of GGT activity (U/mg x 10^{-3}) in placental tissue, fetal liver and fetal ileum from unaffected control pregnancies at each week of geststion from 10 to 24 weeks.

Gestation	Median GGT levels (U/mg x 10^{-3})		
	Placenta	Liver	Ileum
10	1.40	51.3	35.6
11	1.36	41.1	37.2
12	1.32	34.1	38.8
13	1.28	29.2	40.5
14	1.24	25.6	42.3
15	1.20	22.8	44.1
16	1.16	20.6	46.0
17	1.12	18.8	48.0
18	1.08	17.4	50.1
19	1.04	16.2	52.3
20	1.00	15.2	54.6
21	0.96	14.3	57.0
22	0.92	13.6	59.4
23	0.88	12.9	62.0
24	0.84	12.4	64.7

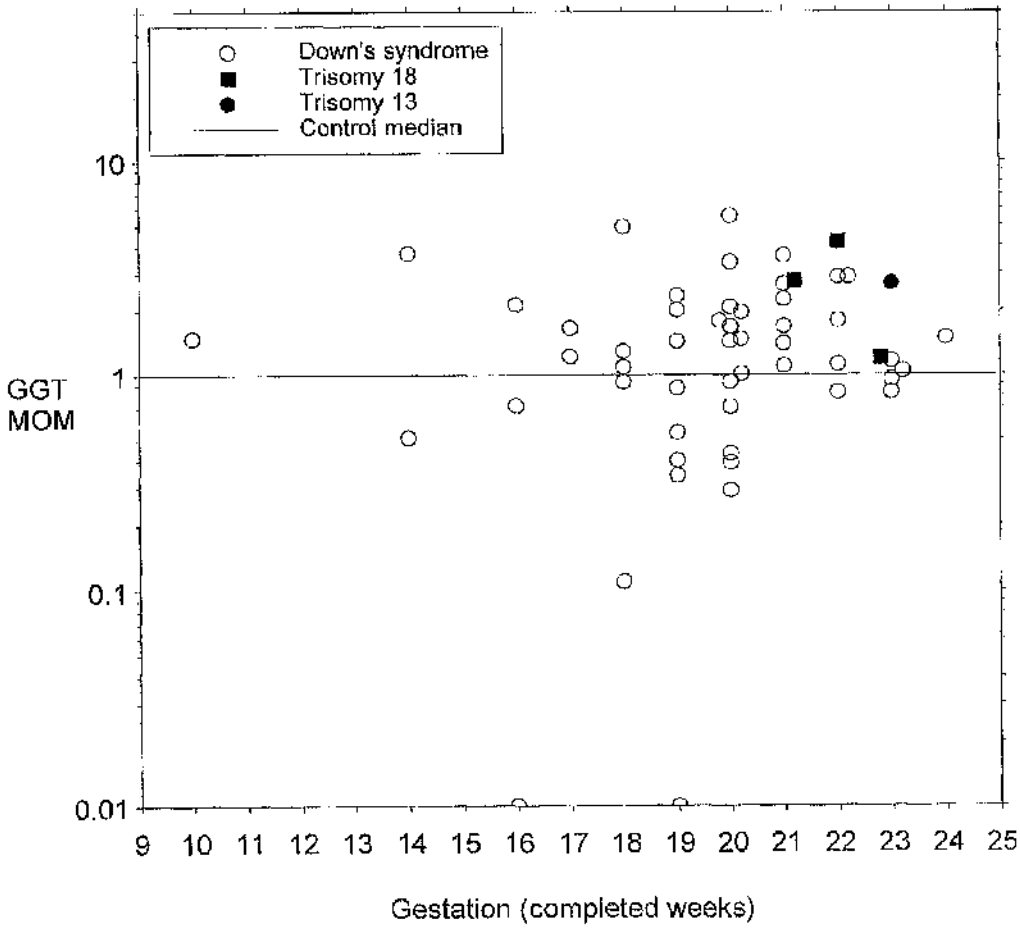

Figure 3.1.16 GGT levels (MOM) in placental tissue from 51 Down's syndrome pregnancies at 10 to 24 weeks gestation, and in one Trisomy 13 and three Trisomy 18 pregnancies.

3.1.9 GGT LEVELS IN FETAL LIVER.

3.1.9.1 Unaffected Controls.

GGT activity in fetal liver from 64 unaffected control pregnancies at 12 to 22 weeks gestation are shown in Figure 3.1.17. Median levels at each gestation, calculated using the S-phase regression model (GGT median = $\exp^{(-5.41+24.4/\text{ gestation})}$), are given in Table 3.1.8. Median GGT levels decreased with advancing gestation from 12 to 22 weeks. The median level of GGT activity in fetal liver from unaffected control pregnancies was 0.90 MOM.

3.1.9.2 Down's Syndrome.

GGT levels, expressed as MOM, in fetal liver from 53 Down's syndrome pregnancies at 13 to 24 weeks gestation are shown in Figure 3.1.18. In the majority of Down's syndrome fetuses hepatic GGT activity was greater than the control group median. The median GGT level of GGT activity in the affected cases was 2.01 MOM . GGT activity in fetal liver from Down's syndrome pregnancies was significantly higher than in unaffected pregnancies (Mann-Whitney, $p < 0.001$).

3.1.9.3 Trisomy 13 and Trisomy 18.

GGT activity (MOM) in fetal liver from the Trisomy 13 and Trisomy 18 pregnancies are shown in Figure 3.1.18. GGT activity in fetal liver from one Trisomy 13 pregnancy was 7.58 MOM. In fetal liver from four Trisomy 18 pregnancies GGT activity was 6.39 MOM, 3.97 MOM, 3.15 MOM and 1.06 MOM.

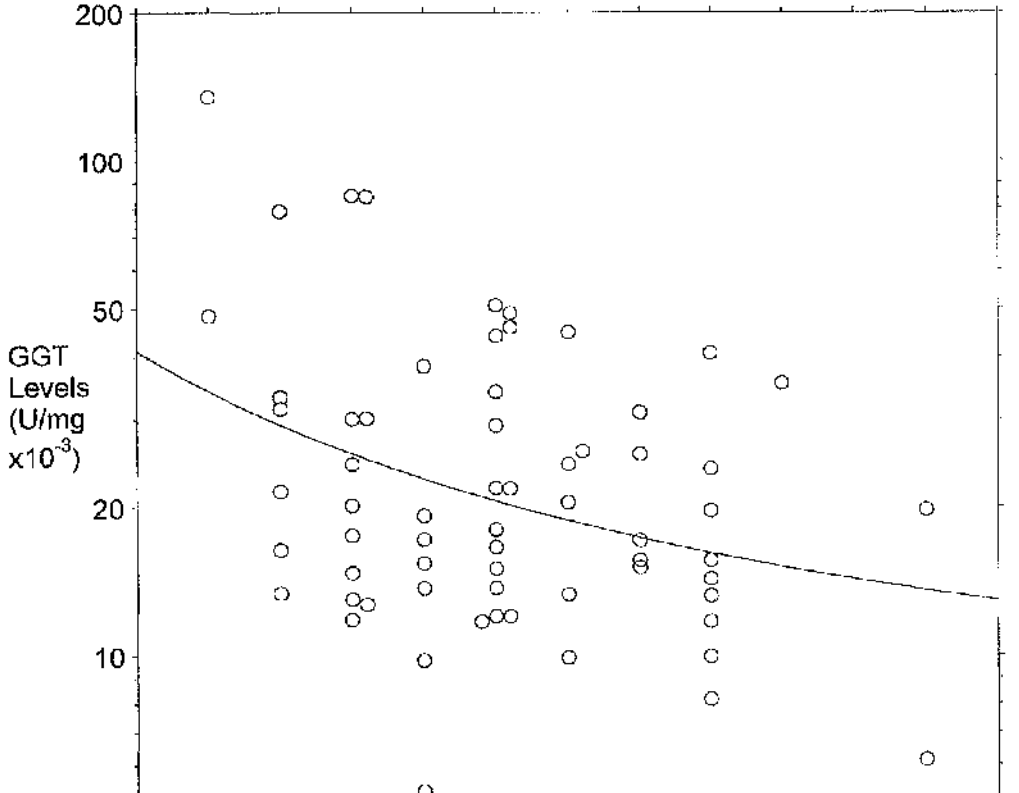

Figure 3.1.17 GGT levels (U/mg x 10^{-3}) in fetal liver from 64 unaffected control pregnancies at 12 to 22 weeks gestation. Smoothed medians across the gestational range were calculated using the S-phase regression model.

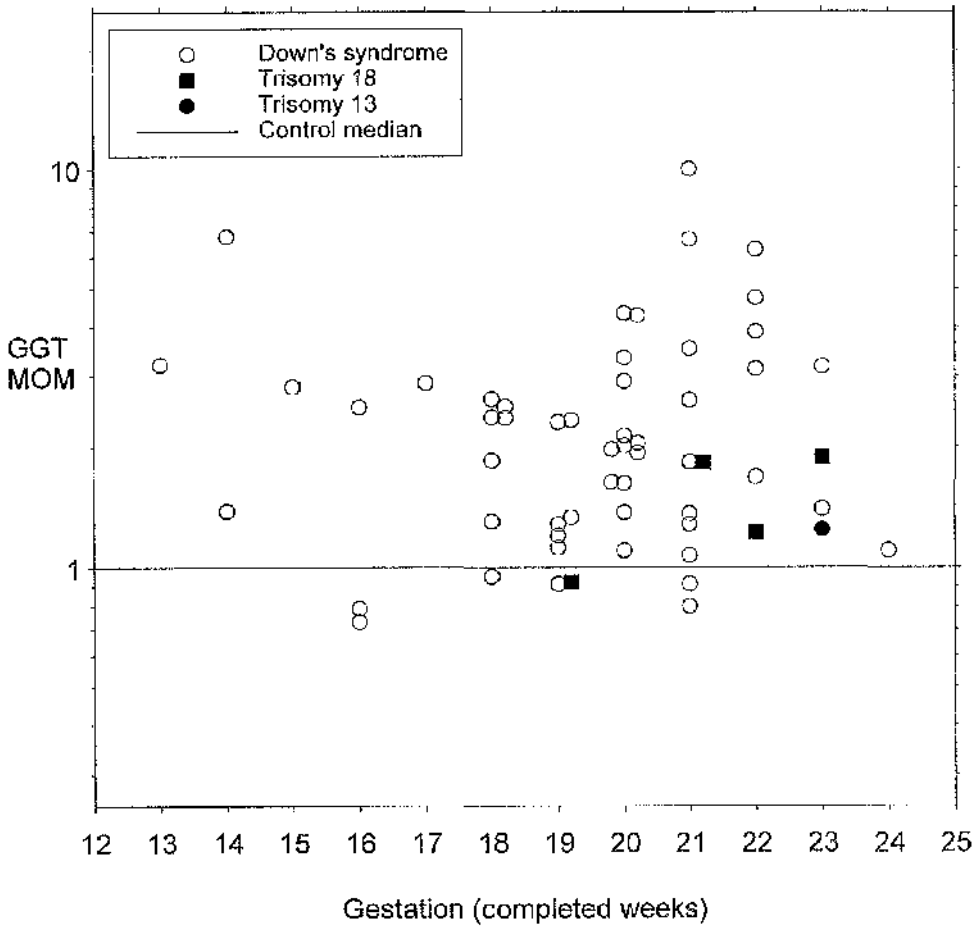

Figure 3.1.18 GGT levels (MOM) in fetal liver from 53 Down's syndrome pregnancies at 13 to 24 weeks gestation, and in one Trisomy 13 and four Trisomy 18 pregnancies.

3.1.10 GGT LEVELS IN FETAL ILEUM.

3.1.10.1 Unaffected Controls.

GGT activity in extracts of fetal ileum from 63 unaffected control pregnancies at 12 to 22 weeks gestation are shown in Figure 3.1.19. Gestational medians were determined by linear regression of logarithmically transformed data (GGT median = $10^{(-1.6330+0.0185 \times \text{gestation})}$) and are shown in Table 3.1.8. Median GGT levels increased with advancing gestation from 12 to 22 weeks. The overall median activity of GGT in fetal ileum from unaffected pregnancies was 1.02 MOM.

3.1.10.2 Down's Syndrome.

GGT activity, expressed as MOM, in fetal iluem from 47 Down's syndrome pregnancies at 13 to 24 weeks gestation are shown in Figure 3.1.20. Fetal intestinal GGT activity in Down's syndrome pregnancies was lower than in unaffected pregnancies with a median level of 0.43 MOM. This reduction was found to be statistically significant using the Mann-Whitney test ($p < 0.001$).

3.1.10.3 Trisomy 13 and Trisomy 18.

GGT activity (MOM) in fetal ileum from the Trisomy 13 and Trisomy 18 pregnancies are shown in Figure 3.1.20. The activity of GGT in fetal ileum from one Trisomy 13 pregnancies was 0.35 MOM. In fetal ileum from four Trisomy 18 pregnancies GGT levels were 0.30 MOM, 0.81 MOM, 0.52 MOM and 0.33 MOM.

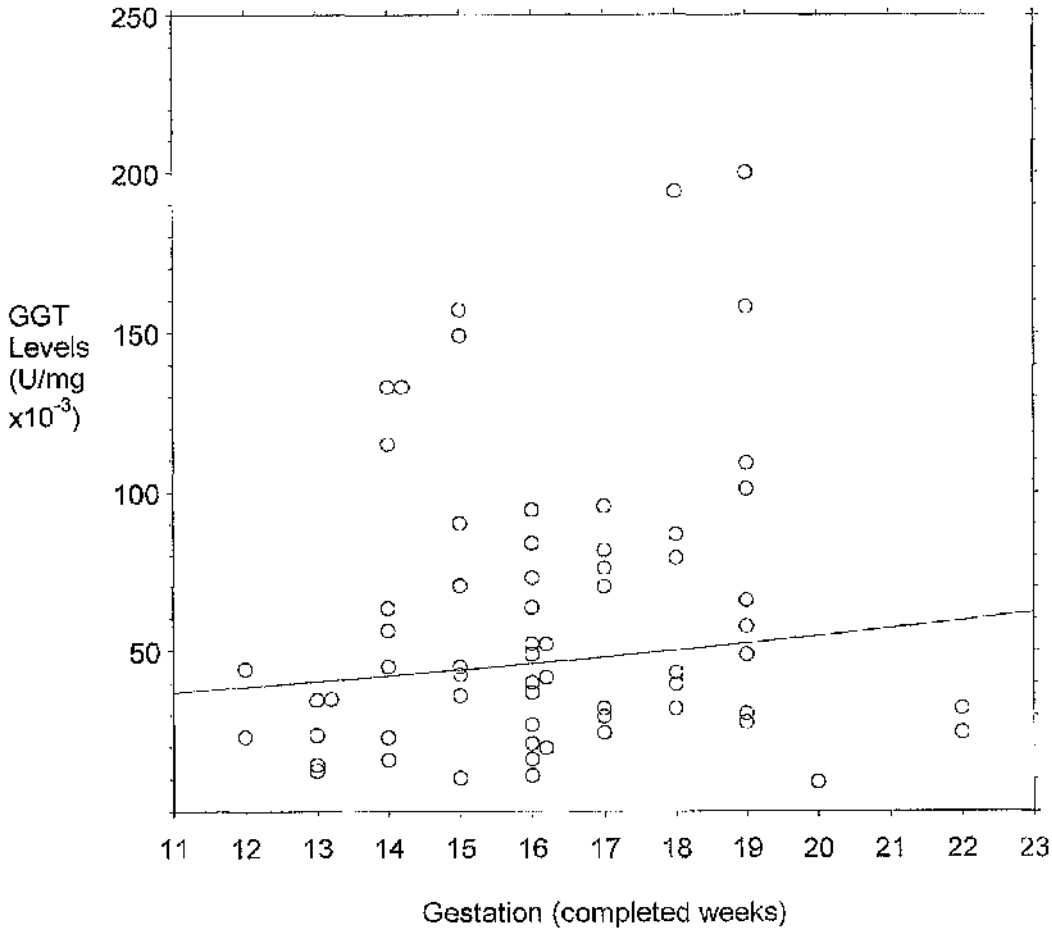

Figure 3.1.19 GGT levels (U/mg x 10^{-3}) in fetal ileum from 63 unaffected control pregnancies at 12 to 22 weeks gestation. Smoothed medians across the gestational range were calculated by linear regression of logarithmically transformed data.

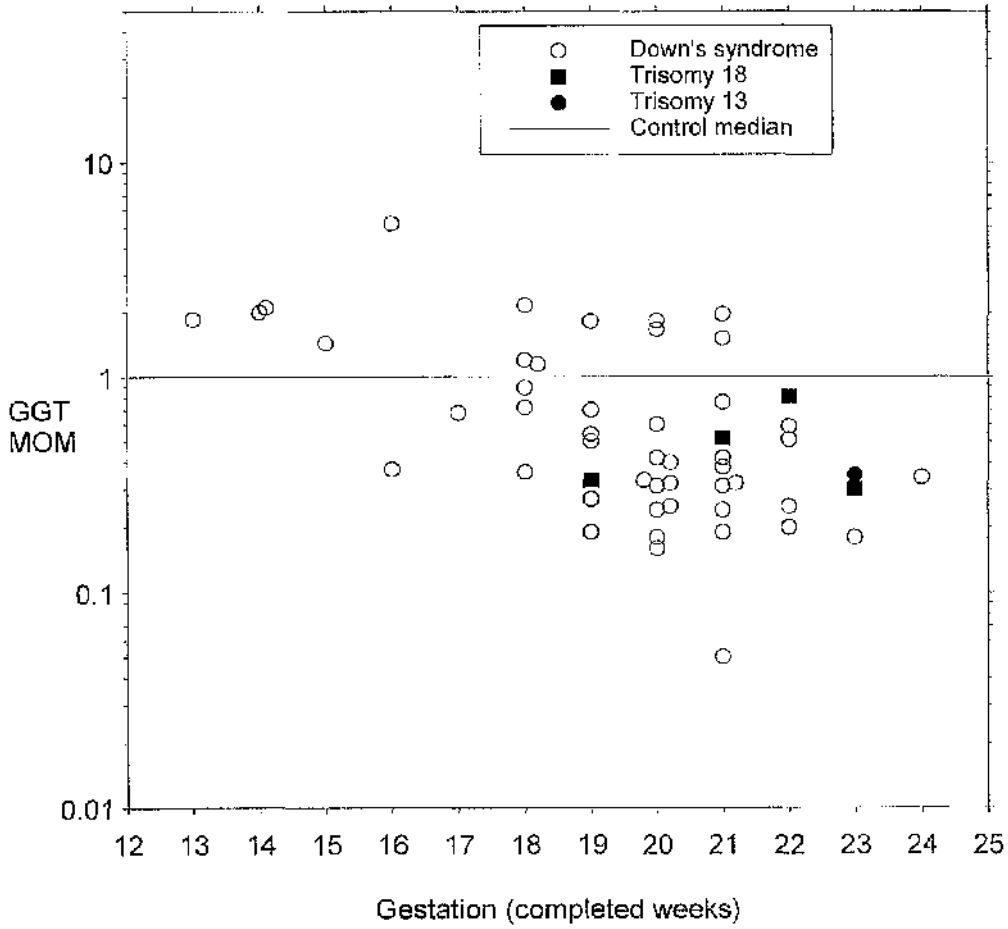

Figure 3.1.20 GGT levels (MOM) in fetal ileum from 47 Down's syndrome pregnancies at 13 to 24 weeks gestation, and in one Trisomy 13 and four Trisomy 18 pregnancies.

3.1.11 SUMMARY OF MARKER LEVELS IN FETAL TISSUES.

Median AFP levels (MOM) in placental tissue and fetal liver from Down's syndrome and unaffected control pregnancies are summarised in Table 3.1.9. AFP levels were strikingly elevated in placental tissue from Down's syndrome pregnancies, while in the fetal liver, AFP levels did not differ significantly from normal. AFP levels (MOM) in fetal liver and placental tissue from Down's syndrome, Trisomy 13 and Trisomy 18 pregnancies are shown in Figure 3.1.21.

Median levels (MOM) of the placental markers intact hCG, FβhCG, PAPP-A, SP-1 and PALP in placental tissue from Down's syndrome and unaffected control pregnancies are presented in Table 3.1.10. Intact hCG, FβhCG and SP-1 were significantly elevated in Down's syndrome placental tissue, while PAPP-A and PALP were unchanged. Levels of intact hCG, FβhCG, PAPP-A, SP-1 and PALP (MOM) in placental tissue from Down's syndrome, Trisomy 13 and Trisomy 18 pregnancies are shown in Figure 3.1.22.

Median levels of GGT (MOM) in placenta, fetal liver and fetal ileum from Down's syndrome and unaffected pregnancies are presented in Table 3.1.11. GGT was significantly elevated in placenta and in fetal liver from Down's syndrome pregnancies. In fetal ileum from Down's syndrome pregnancies GGT levels were significantly lower than normal. GGT levels (MOM) in placental tissue, fetal liver and fetal ileum from Down's syndrome, Trisomy 13 and Trisomy 18 pregnancies are shown in Figure 3.1.23.

Marker levels (MOM) in fetal tissues from the Trisomy 13 and Trisomy 18 pregnancies are presented in Table 3.1.12.

Table 3.1.9 Median AFP levels (MOM) in placental tissue and fetal liver from unaffected control and Down's syndrome pregnancies, and results of significance testing.

Tissue	Median MOM		Mann-Whitney
	Unaffected controls	Down's syndrome	p-value
Placenta	0.99	2.43	<0.001
Liver	1.01	0.97	0.259

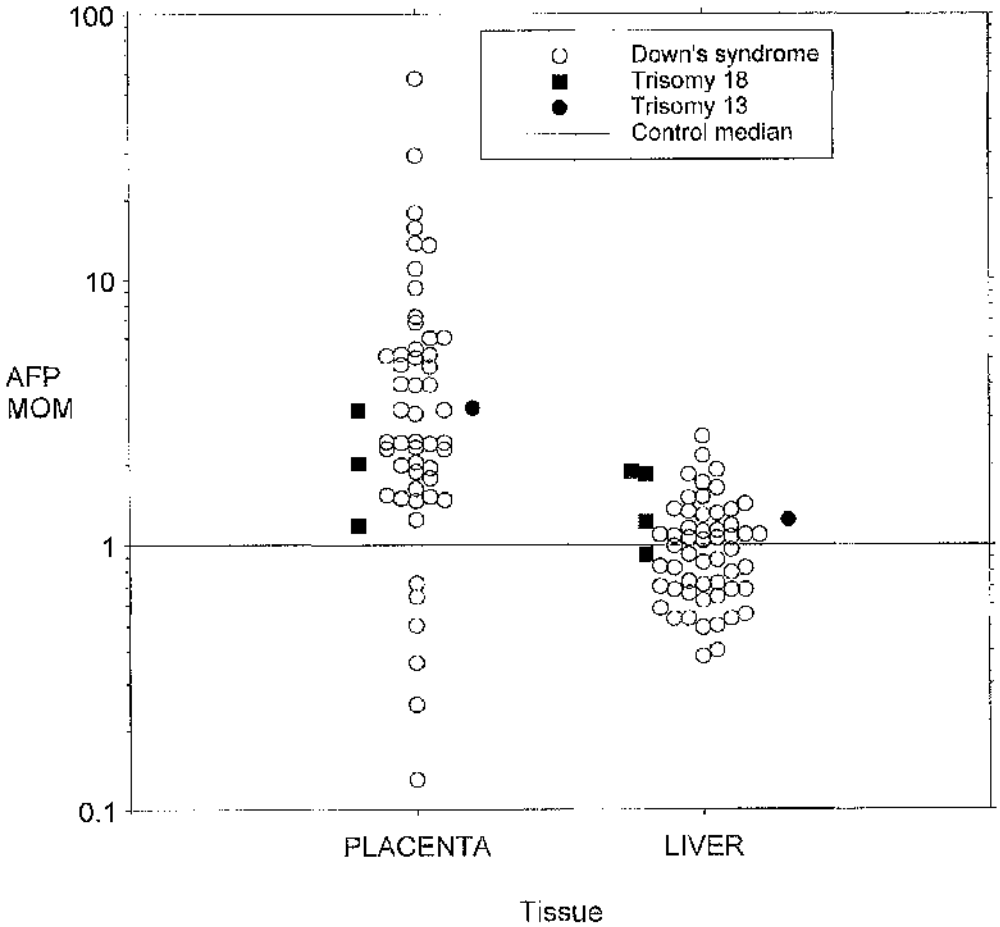

Figure 3.1.21 AFP levels (MOM) in placental tissue and fetal liver from Down's syndrome, Trisomy 18 and Trisomy 13 pregnancies.

Table 3.1.10 Median MOM levels of intact hCG, FβhCG, PAPP-A, SP-1 and PALP in placental tissue from unaffected control and Down's syndrome pregnancies and results of significance testing.

Marker	Median MOM		Mann-Whitney p-value
	Unaffected controls	Down's syndrome	
intact hCG	1.19	4.06	<0.001
FβhCG	1.05	3.40	<0.001
PAPP-A	0.84	0.96	0.304
SP-1	0.91	1.79	<0.001
Placental ALP	1.10	0.91	0.318

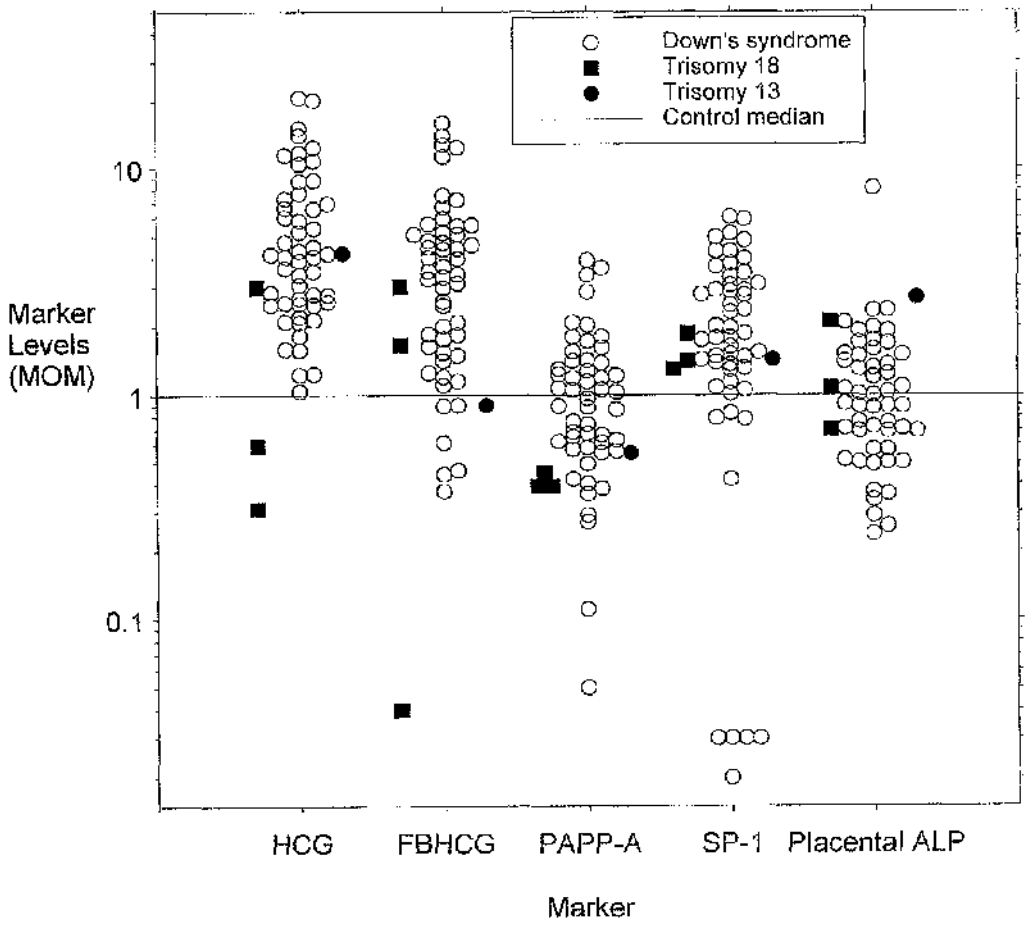

Figure 3.1.22 Levels of intact hCG, FβhCG, PAPP-A, SP-1 and placental ALP (MOM) in placental tissue from Down's syndrome, Trisomy 18 and Trisomy 13 pregnancies.

Table 3.1.11 Median levels of GGT (MOM) in placental tissue, fetal liver and fetal ileum from unaffected control and Down's syndrome pregnancies, and results of significance testing.

Tissue	Median MOM		Mann-Whitney
	Unaffected controls	Down's syndrome	p-value
Placenta	0.93	1.29	0.009
Liver	0.90	2.01	<0.001
Ileum	1.02	0.43	<0.001

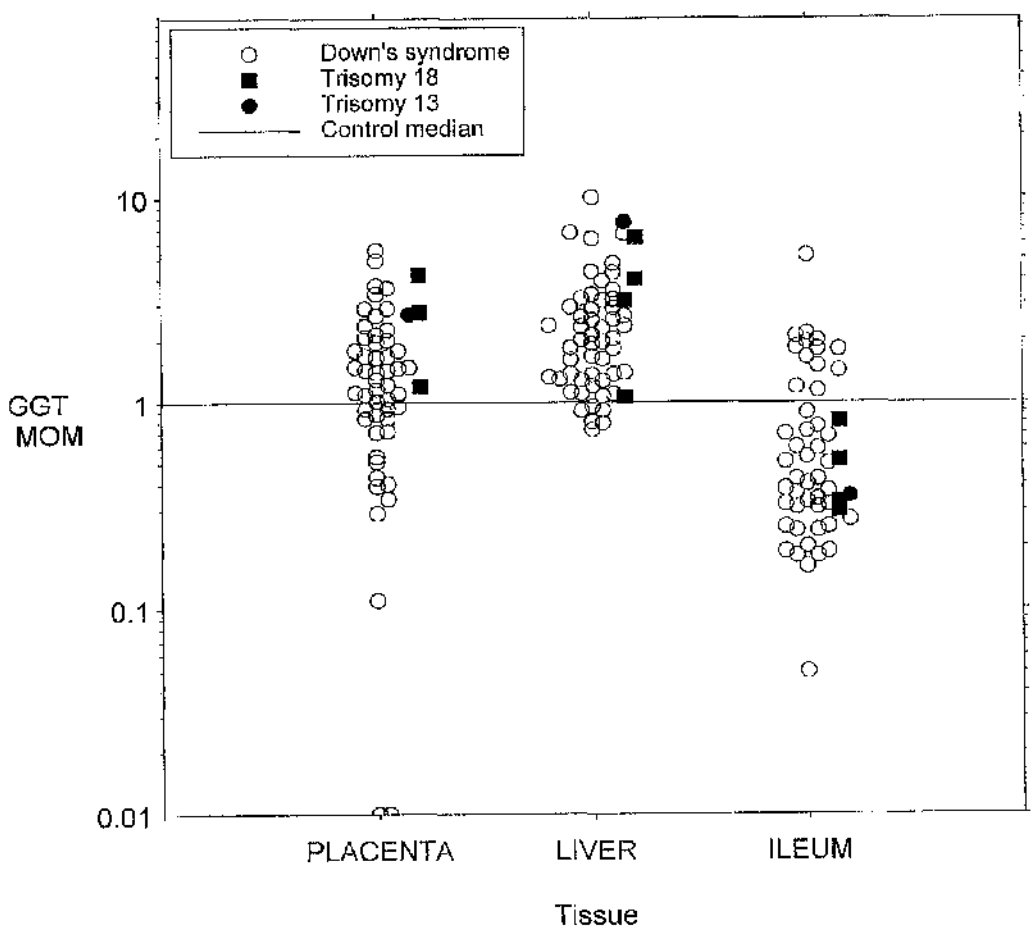

Figure 3.1.23 GGT levels (MOM) in placental tissue, fetal liver and fetal ileum from Down's syndrome, Trisomy 18 and Trisomy 13 pregnancies.

Table 3.1.12 Marker levels (MOM) in fetal and placental tissues from one Trisomy 13 and four Trisomy 18 pregnancies. No placental tissue was available from the Trisomy 18 case T18/4.

	Marker level (MOM)				
	Trisomy 13	Trisomy 18			
	1	1	2	3	4
Plac. AFP	3.25	1.18	3.18	2.01	-
Plac. hCG	4.20	0.59	2.97	0.31	-
Plac. FβhCG	0.95	ND	1.66	0.40	-
Plac. PAPP-A	0.55	0.39	0.39	0.45	-
Plac. SP1	7.44	1.41	1.30	1.85	-
Plac. PALP	1.43	0.70	1.07	2.11	-
Plac. GGT	2.68	1.20	4.18	2.75	-
Liver AFP	1.24	1.88	1.22	1.83	0.92
Liver GGT	7.58	6.39	3.97	3.15	1.06
Ileum GGT	0.35	0.30	0.81	0.52	0.33

ND - not detectable

3.2 MARKER LEVELS IN MATERNAL SERUM.

3.2.1 ALPHAFETOPROTEIN.

3.2.1.1 Down's syndrome.

Maternal serum AFP levels analysed prospectively and expressed as MOM, were available for 62 Down's syndrome pregnancies identified by maternal serum screening and are illustrated in Figure 3.2.1. The median AFP level (MOM) in maternal serum from this series of Down's syndrome pregnancies was 0.69 MOM.

3.2.1.2 Trisomy 18.

The concentrations of AFP in maternal serum were available for two Trisomy 18 pregnancies (cases T18/3 and T18/4). These were 0.55 MOM at 16 weeks gestation (T18/3) and 0.34 MOM at 15 weeks gestation (T18/4) (Figure 3.2.1).

3.2.2 HUMAN CHORIONIC GONADOTROPIN.

3.2.2.1 Down's syndrome.

HCG levels analysed prospectively and expressed as MOM, were available for the same series of 62 Down's syndrome pregnancies and are illustrated in Figure 3.2.2. Maternal serum hCG levels were above 1.00 MOM in all but two of the Down's syndrome pregnancies. The overall median serum intact hCG level in this series of affected pregnancies was 2.72 MOM.

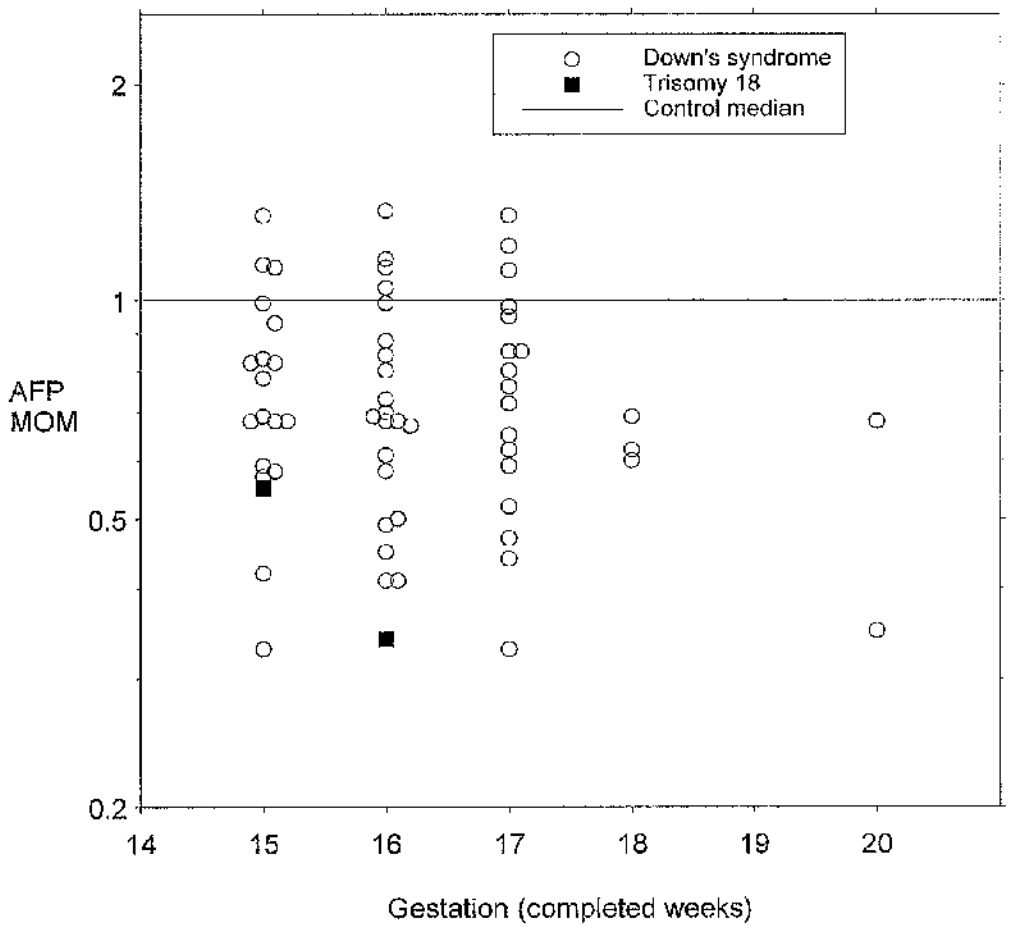

Figure 3.2.1 Levels of AFP (MOM) in maternal serum from 62 Down's syndrome pregnancies at 15 to 20 weeks gestation, and in two Trisomy 18 pregnancies.

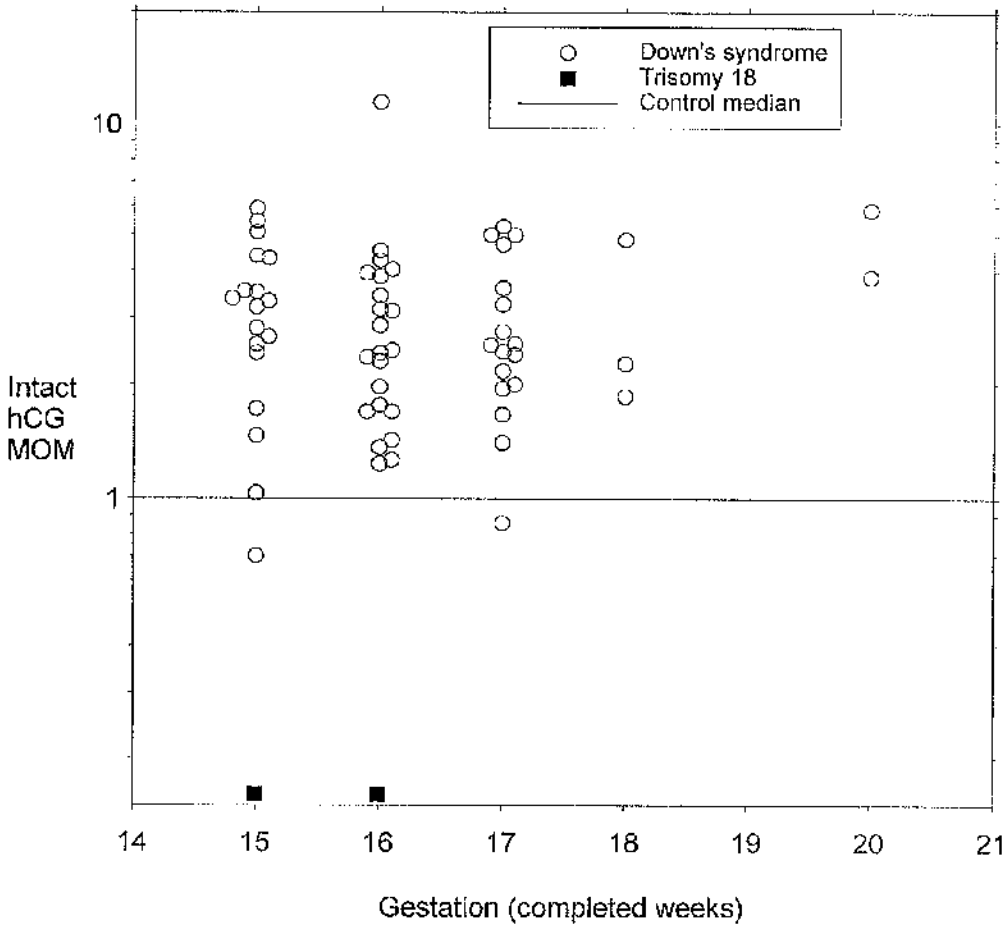

Figure 3.2.2 Levels of intact hCG (MOM) in maternal serum from 62 Down's syndrome pregnancies at 15 to 20 weeks gestation, and in two Trisomy 18 pregnancies.

3.2.2.2 Trisomy 18.

The concentrations of intact hCG in maternal serum from two Trisomy 18 pregnancies (cases T18/3 and T18/4) were 0.16 MOM at 16 weeks gestation (T18/3) and 0.17 MOM at 15 weeks gestation (T18/4) (Figure 3.2.2).

3.2.3 FREE BETA HUMAN CHORIONIC GONADOTROPIN.

3.2.3.1 Normal Ranges.

Median FβhCG levels in maternal serum from unaffected pregnancies from 15 to 20 weeks gestation have been established in this Department using the FβhCG assay described in this study (Berry et al 1997). FβhCG medians are given in Table 3.2.1. In unaffected pregnancies FβhCG levels decrease from 15 to 20 weeks gestation.

3.2.3.2 Down's Syndrome.

FβhCG levels, expressed as MOM, in maternal serum from 58 Down's syndrome pregnancies at 15 to 20 weeks gestation are shown in Figure 3.2.3. Twenty-two of of these samples have been investigated as part of another study (Berry et al 1997). In the majority of the Down's syndrome pregnancies FβhCG levels were above 1.00 MOM. The median serum FβhCG level in the Down's syndrome pregnancies was 3.06 MOM.

Table 3.2.1 Median levels of FβhCG (ng/ml) in maternal serum from unaffected control pregnancies at each week of gestation from 15 to 20 weeks (from Berry et al 1997).

Gestation	Median FβhCG level (ng/ml)
15	11.5
16	9.6
17	8.0
18	6.7
19	5.5
20	4.6

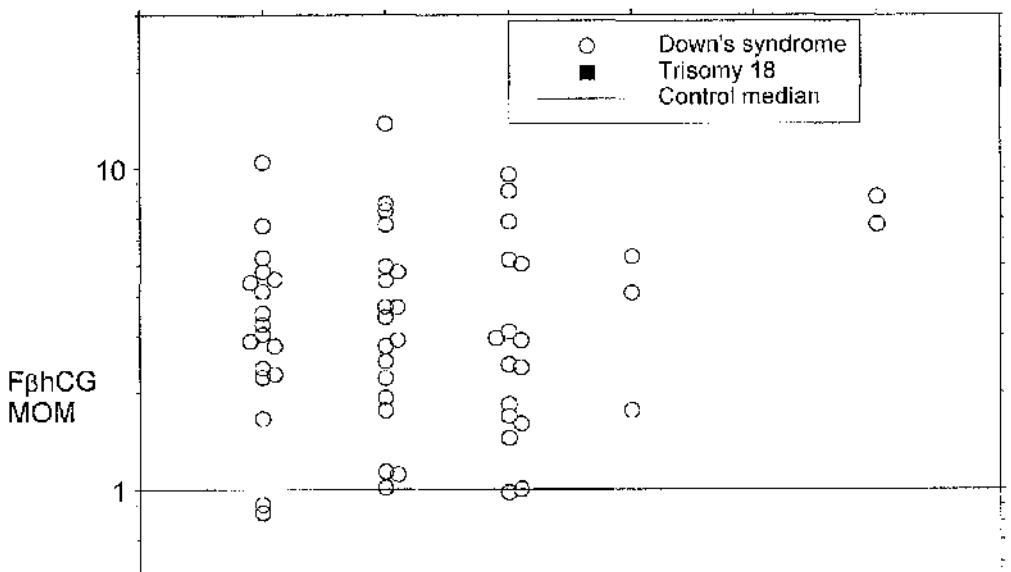

Figure 3.2.3 Levels of FβhCG (MOM) in maternal serum from 58 Down's syndrome pregnancies at 15 to 20 weeks gestation, and in two Trisomy 18 pregnancies.

3.2.3.3 Trisomy 18.

The concentrations of FβhCG in maternal serum from two Trisomy 18 pregnancies (cases T18/3 and T18/4) were 0.13 MOM at 16 weeks gestation (T18/3) and 0.12 MOM at 15 weeks gestation (T18/4) (Figure 3.2.3).

3.2.4 PREGNANCY ASSOCIATED PLASMA PROTEIN A.

3.2.4.1 Normal Ranges.

Median PAPP-A levels in maternal serum from unaffected pregnancies from 15 to 20 weeks gestation were established as part of another study in this Department (Berry et al 1997) and are presented in Table 3.2.2. In unaffected pregnancies, PAPP-A levels increase with advancing gestation from 15 to 20 weeks gestation.

3.2.4.2 Down's Syndrome.

PAPP-A levels, expressed as MOM, in maternal serum from 58 Down's syndrome pregnancied at 15 to 20 weeks gestation are shown in Figure 3.2.4. Twenty-three of these samples were also investigated as part of another study (Berry et al 1997). Serum PAPP-A levels in the Down's syndrome pregnancies were fairly evenly distributed above and below 1.00 MOM with an overall median PAPP-A level of 0.92 MOM.

Table 3.2.2 Median levels of PAPP-A (IU/l) in maternal serum from unaffected control pregnancies at each week of gestation from 15 to 20 weeks (from Berry et al 1997).

Gestation	Median PAPP-A level (IU/l)
15	6.66
16	8.62
17	10.9
18	13.4
19	16.1
20	19.2

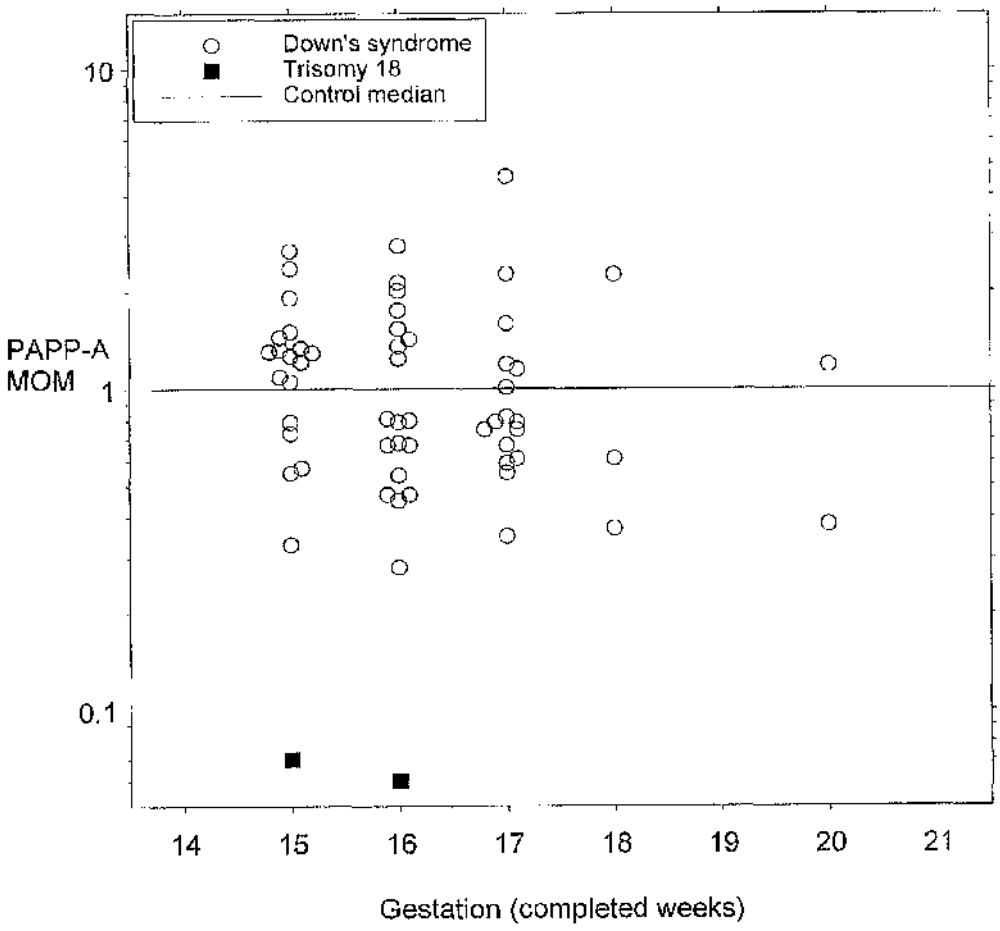

Figure 3.2.4 Levels of PAPP-A (MOM) in maternal serum from 58 Down's syndrome pregnancies at 15 to 20 weeks gestation, and in two Trisomy 18 pregnancies.

3.2.4.3 Trisomy 18.

The concentrations of PAPP-A in maternal serum from two Trisomy 18 pregnancies (cases T18/3 and T18/4) were 0.06 MOM at 16 weeks gestation (T18/3) and 0.07 MOM at 15 weeks gestation (T18/4) (Figure 3.2.4).

3.2.5 PREGNANCY SPECIFIC BETA 1 GLYCOPROTEIN.

3.2.5.1 Unaffected Controls.

The concentration of SP1 in maternal serum from 114 unaffected pregnancies at 15 to 20 weeks gestation is shown in Figure 3.2.5. Median and regressed median SP1 levels at each week of gestation are presented in Table 3.2.3. Regressed medians were calculated using the equation: SP1 median = $10^{(0.867+0.0344 \times \text{gestation})}$. In the unaffected pregnancies median SP1 levels increased with advancing gestation from 15 to 20 weeks. The overall median serum SP1 level in the unaffected control pregnancies was 1.00 MOM.

3.2.5.2 Down's Syndrome.

SP-1 levels, expressed as MOM, in maternal serum from 58 Down's syndrome pregnancies at 15 to 20 weeks gestation are illustrated in Figure 3.2.6. Maternal serum SP-1 levels in the Down's syndrome pregnancies were significantly higher than in the unaffected pregnancies (Mann-Whitney, $p<0.001$), with an overall median level of 1.33 MOM in the Down's syndrome pregnancies.

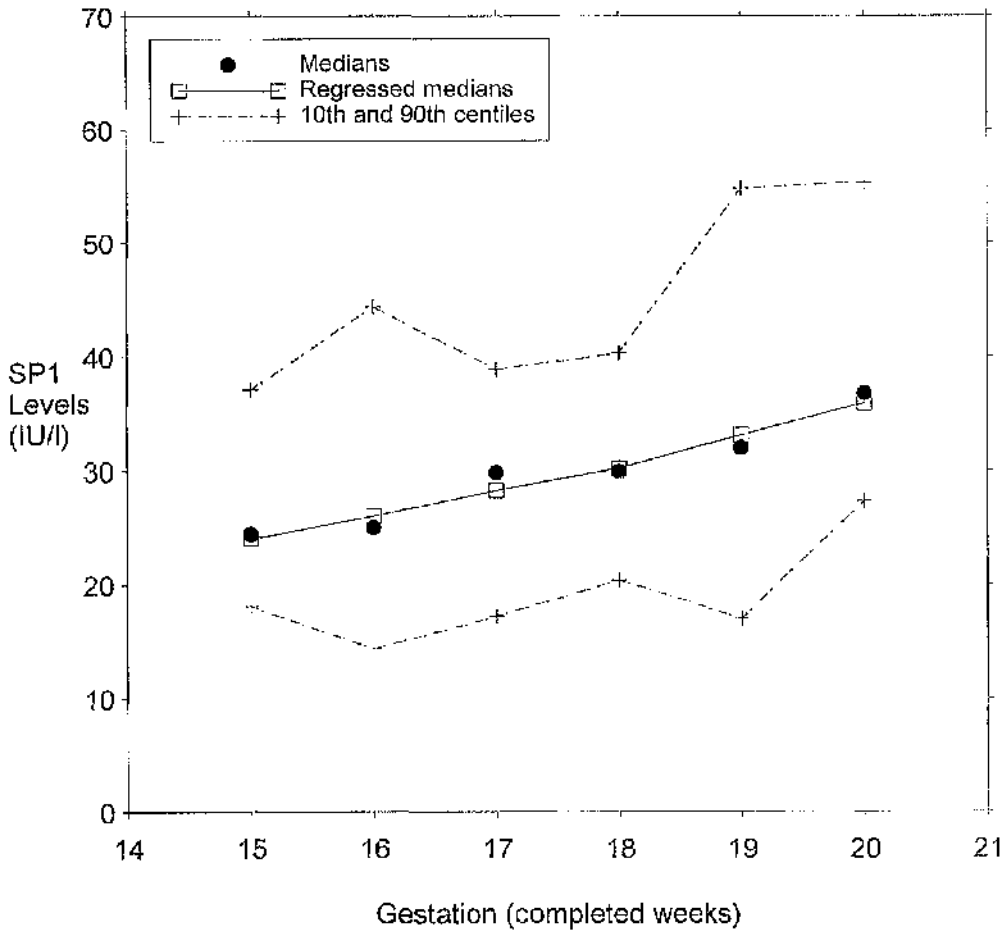

Figure 3.2.5 Median and regressed median levels of SP1 (IU/l) in maternal serum from 114 unaffected pregnancies at 15 to 20 weeks gestation.

Table 3.2.3 Median and regressed median levels of SP1 (IU/l) in maternal serum from 114 unaffected pregnancies at each week of gestation from 15 to 20 weeks.

Gestation	No. of samples	Median	Regressed median
15	20	24.5	24.1
16	20	25.1	26.1
17	20	29.8	28.3
18	20	29.9	30.6
19	16	32.0	33.1
20	18	36.8	35.9

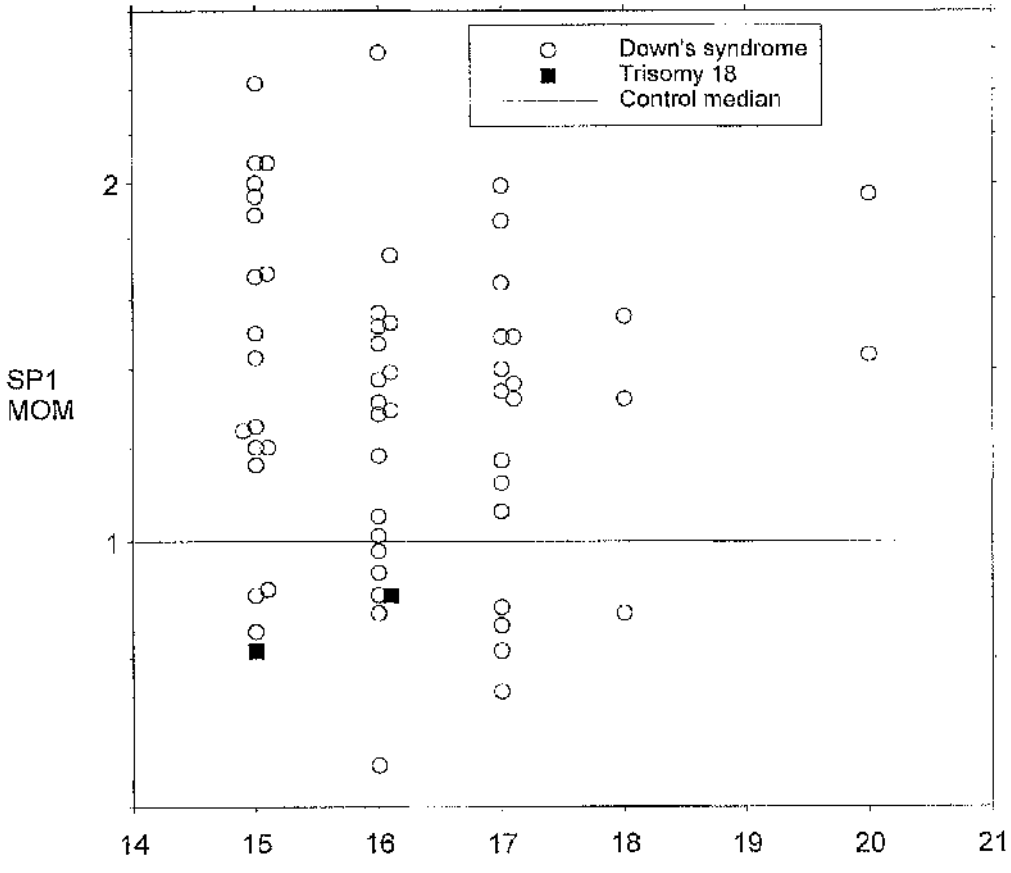

Figure 3.2.6 Levels of SP1 (MOM) in maternal serum from 58 Down's syndrome pregnancies at 15 to 20 weeks gestation, and in two Trisomy 18 pregnancies.

3.2.5.3 Trisomy 18.

The concentrations of SP1 in maternal serum from two Trisomy 18 pregnancies (cases T18/3 and T18/4) were 0.90 MOM at 16 weeks gestation (T18/3) and 0.81 MOM at 15 weeks gestation (T18/4) (Figure 3.2.6).

3.2.6 ALKALINE PHOSPHATASE.

3.2.6.1 Unaffected Controls.

Total and placental ALP activity in maternal serum from 114 unaffected pregnancies at 15 to 20 weeks gestation are shown in Figure 3.2.7 and Figure 3.2.8 respectively. Median total and placental ALP levels at each week of gestation are presented in Table 3.2.4, along with the percentage contribution of the placental isoenzyme to the total ALP activity at each week of gestation. Total and placental ALP regressed medians were calculated using the following equations: total ALP median = $10^{(2.82+0.0073 \times \text{gestation})}$, placental ALP median = $10^{(-0.106+0.0859 \times \text{gestation})}$. Regressed medians are presented in Table 3.2.5. Both total and placental ALP activity was found to increase with advancing gestation. The contribution of the placental isoenzyme to the total ALP activity was less than 5%, increasing gradually as gestation advanced. The median levels of total and placental ALP activity in the unaffected pregnancies were 0.97 MOM and 0.99 MOM respectively.

3.2.6.2 Down's Syndrome.

Total and placental ALP activity, expressed as MOM, in maternal serum from 54 Down's syndrome pregnancies at 15 to 20 weeks gestation are shown in Figure 3.2.9

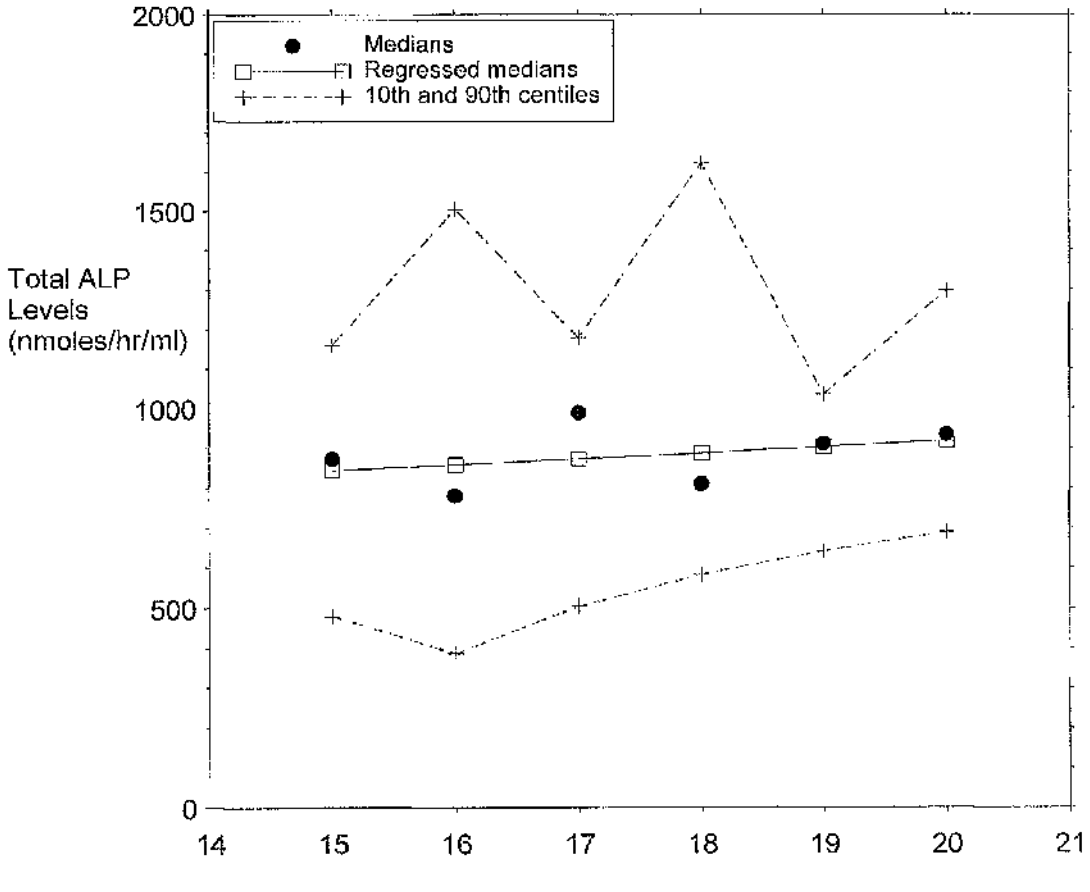

Figure 3.2.7 Median and regressed median levels of total ALP (nmoles/hr/ml) in maternal serum from 114 unaffected pregnancies at 15 to 20 weeks gestation.

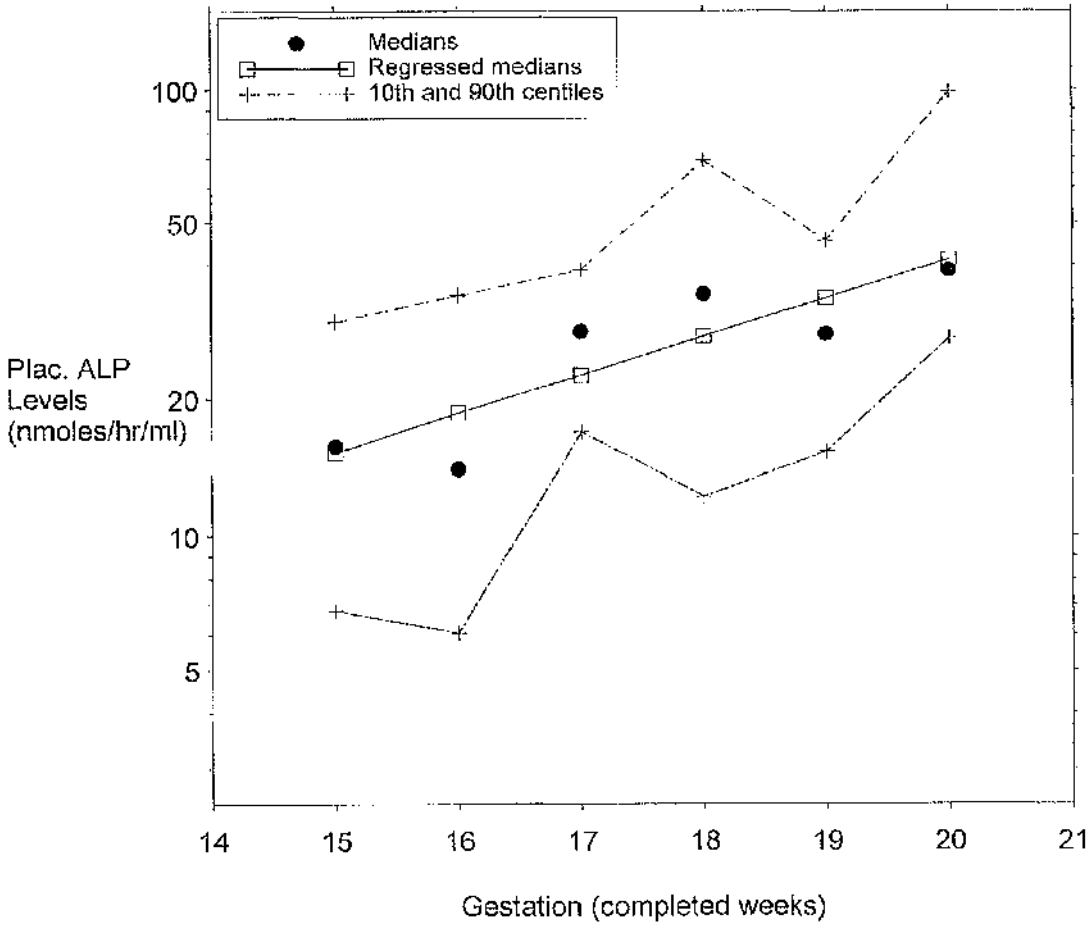

Figure 3.2.8 Median and regressed median levels of placental ALP (nmoles/hr/ml) in maternal serum from 114 unaffected pregnancies at 15 to 20 weeks gestation.

Table 3.2.4 Median total and placental ALP levels (nmoles/hr/ml) in maternal serum from 114 unaffected pregnancies at each week of gestation from 15 to 20 weeks, along with the percentage contribution of the placental isoenzyme to the total ALP activity.

Gestation	No. of samples	Medians		% placental
		Total ALP	Plac ALP	ALP
15	20	873	15.7	1.80
16	20	781	14.0	1.79
17	20	988	28.3	2.86
18	20	810	34.3	4.23
19	16	911	27.9	3.06
20	18	934	38.8	4.15

Table 3.2.5 Regressed median total and placental ALP levels (nmoles/hr/ml) in maternal serum from 114 unaffected pregnancies at each week of gestation from 15 to 20 weeks, along with the percentage contribution of the placental isoenzyme to the total ALP activity.

Gestation	No. of samples	Regressed medians		% placental
		Total ALP	Plac ALP	ALP
15	20	844	15.2	1.8
16	20	858	18.6	2.17
17	20	873	22.6	2.59
18	20	887	27.6	3.11
19	16	903	33.6	3.72
20	18	918	41.0	4.47

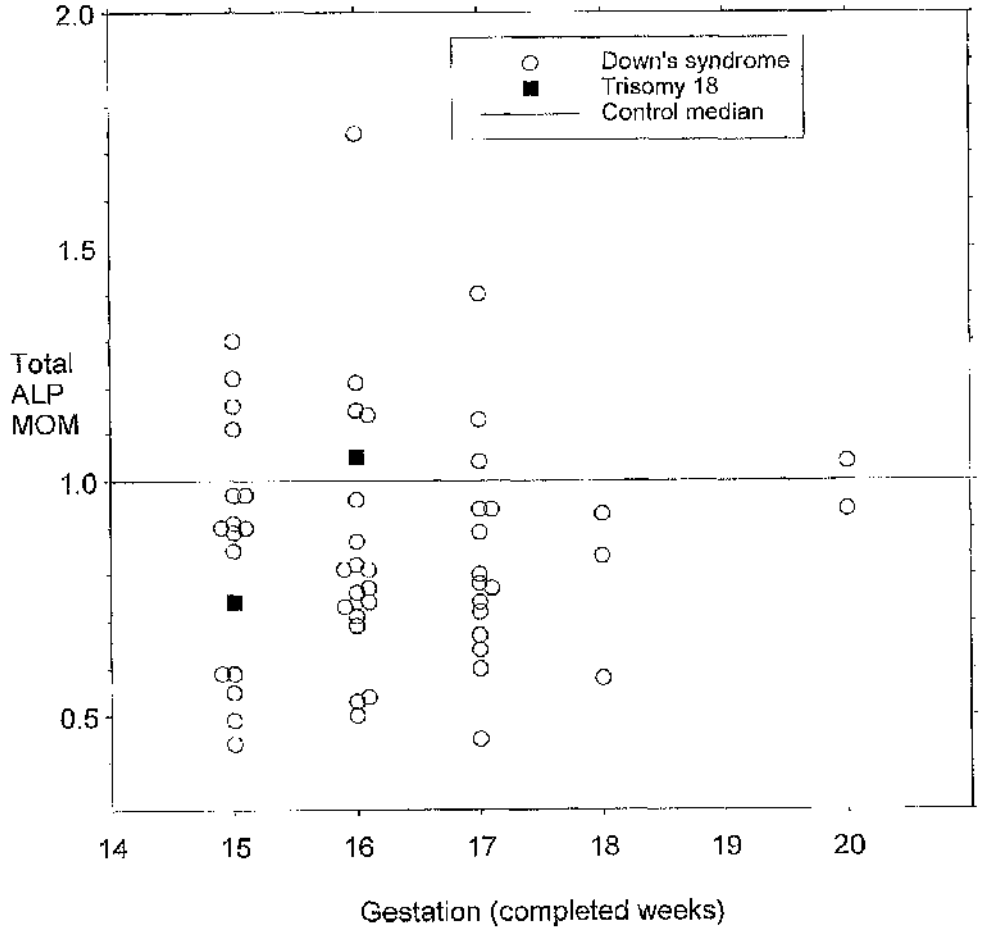

Figure 3.2.9 Levels of total ALP (MOM) in maternal serum from 54 Down's syndrome pregnancies at 15 to 20 weeks gestation, and in two Trisomy 18 pregnancies.

and Figure 3.2.10 respectively. Total ALP activity was lower in maternal serum from Down's syndrome pregnancies with a median level of 0.83 MOM. The reduction was found to be statistically significant using the Mann-Whitney Test (p=0.002). Placental ALP activity in maternal serum from the Down's syndrome pregnancies was not significantly different from normal (Mann-Whitney, p=0.50), with a median level of 1.04 MOM.

3.2.6.3 Trisomy 18.

Total and placental ALP activity in maternal serum from two Trisomy 18 pregnancies (cases T18/3 and T18/4) were 1.05 MOM and 0.64 MOM respectively at 16 weeks gestation (T18/3) and 0.74 MOM and 0.34 MOM respectively at 15 weeks gestation (T18/4) (Figure 3.2.9 and Figure 3.2.10).

3.2.7 SUMMARY OF MARKER LEVELS IN MATERNAL SERUM.

A summary of marker levels in maternal serum from Down's syndrome and unaffected pregnancies is presented in Table 3.2.6. Levels of AFP and total ALP were found to be lower in Down's syndrome pregnancies than in normal pregnanies. Intact hCG, FβhCG and SP-1 levels were elevated in maternal serum from Down's syndrome pregnancies, while PAPP-A and placental ALP levels were unchanged (Figure 3.2.11).

Marker levels in maternal serum from two Trisomy 18 pregnancies are summarised in Table 3.2.7.

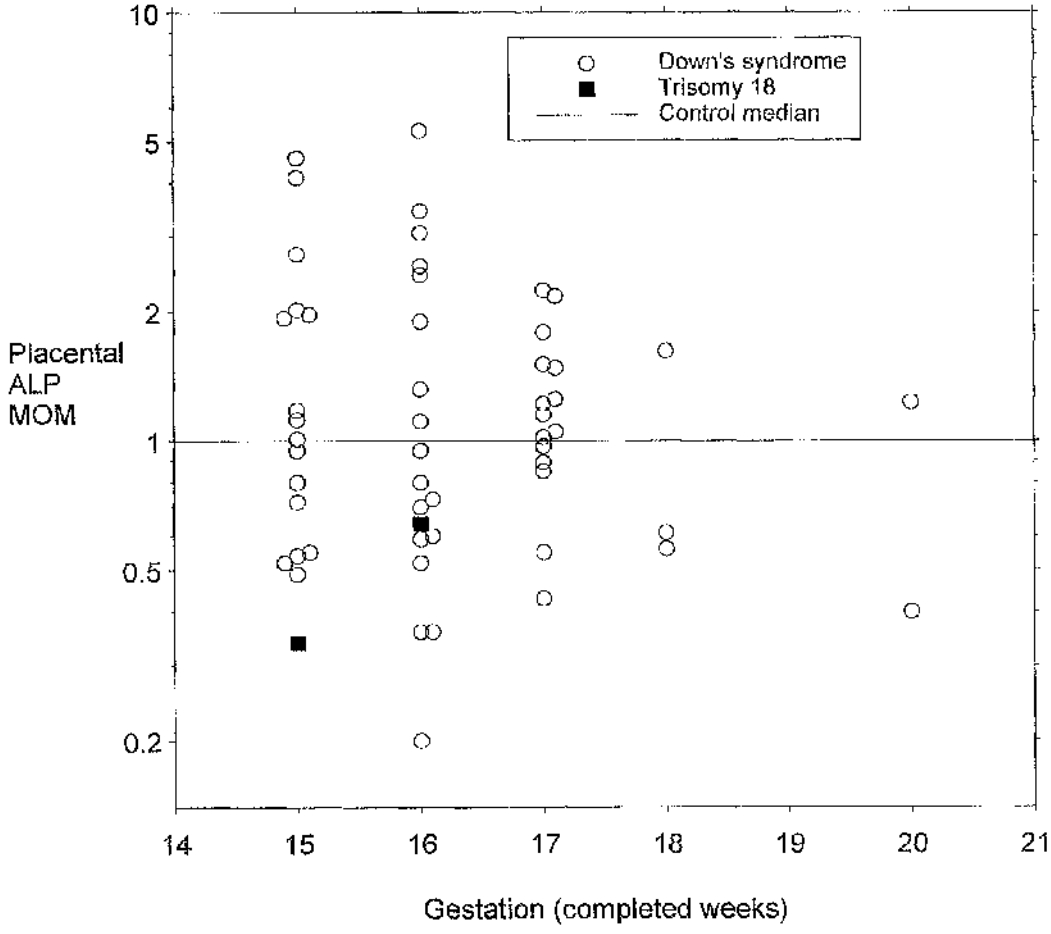

Figure 3.2.10 Levels of placental ALP (MOM) in maternal serum from 54 Down's syndrome pregnancies at 15 to 20 weeks gestation, and in two Trisomy 18 pregnancies.

Table 3.2.6 Summary of marker levels (median MOM) in maternal serum from Down's syndrome and unaffected pregnancies.

Marker	Median MOM		Mann-Whitney
	Unaffected controls	Down's syndrome	p-value
AFP	-	0.69	-
intact hCG	-	2.72	-
FβhCG	-	3.06	-
PAPP-A	-	0.92	-
SP-1	1.00	1.33	<0.001
Total ALP	0.97	0.83	0.002
Plac ALP	0.99	1.04	0.505

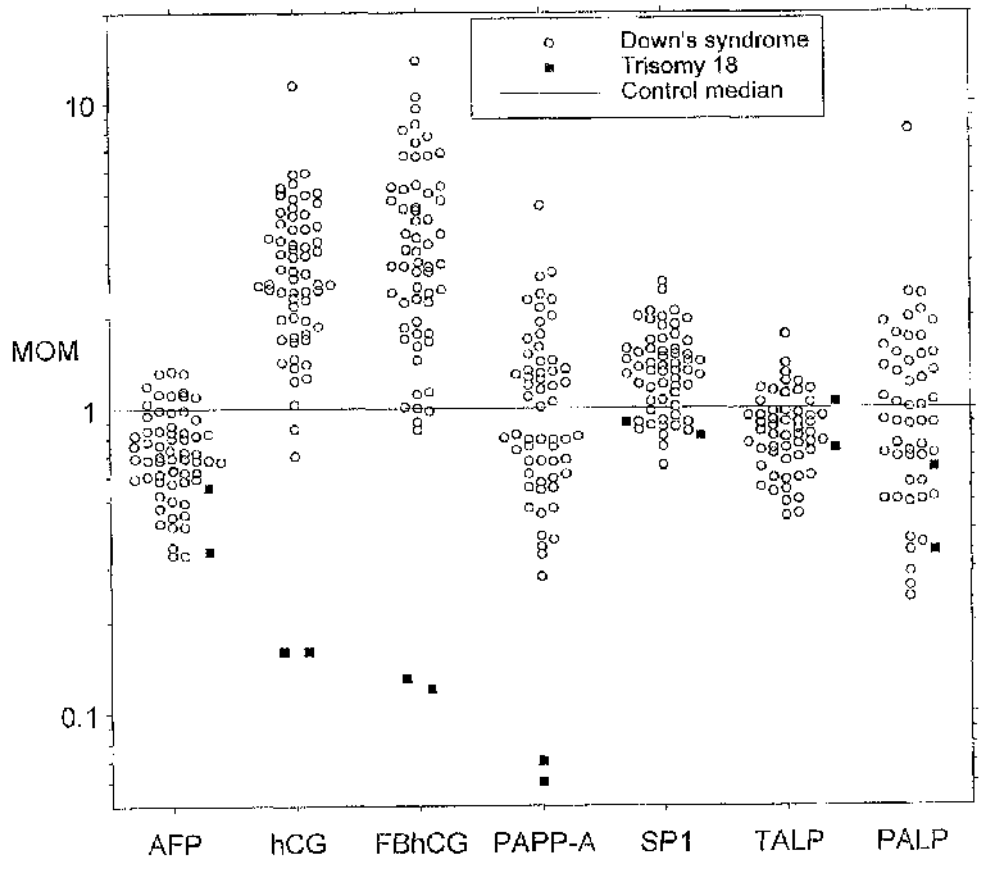

Figure 3.2.11 Levels of AFP, intact hCG, FβhCG, PAPP-A, SP1, total ALP and placental ALP (MOM) in maternal serum from Down's syndrome and Trisomy 18 pregancies.

Table 3.2.7 Summary of marker levels (MOM) in maternal serum from two Trisomy 18 pregnancies.

Case no.	Gest.	AFP	intact hCG	FβhCG	PAPP-A	SP1	Total ALP	Placental ALP
T18/3	16	0.55	0.16	0.13	0.06	0.90	1.05	0.64
T18/4	15	0.34	0.17	0.12	0.07	0.81	0.74	0.34

3.3 MARKER LEVELS IN AMNIOTIC FLUID.

3.3.1 ALPHAFETOPROTEIN.

3.3.1.1 Normal Ranges.

Median AFP levels in amniotic fluid from unaffected pregnancies established as part of the routine analysis of several hundred amniotic fluid specimens are presented in Table 3.3.1. Median amniotic fluid AFP levels decrease from 15 to 21 weeks gestation.

3.3.1.2 Down's Syndrome.

Amniotic fluid AFP levels were measured prospectively in 34 of the Down's syndrome pregnancies as part of the routine analysis of AFP and acetylcholinesterase in all amniotic fluids. AFP levels, expressed as MOM, in amniotic fluid from 34 Down's syndrome pregnancies at 15 to 21 weeks gestation are shown in Figure 3.3.1. The overall median level of AFP in amniotic fluid Down's syndrome pregnancies was 0.87 MOM.

3.3.1.3 Trisomy 18.

The level of AFP in amniotic fluid from one Trisomy 18 pregnancy (case T18/4) at 17 weeks gestation was 1.07 MOM (Figure 3.3.1).

Table 3.3.1 Median levels of AFP (MU/l) in amniotic fluid from unaffected pregnancies.

Gestation	Median (MU/l)
15	13.0
16	11.2
17	9.36
18	7.62
19	5.82
20	4.08
21	2.40

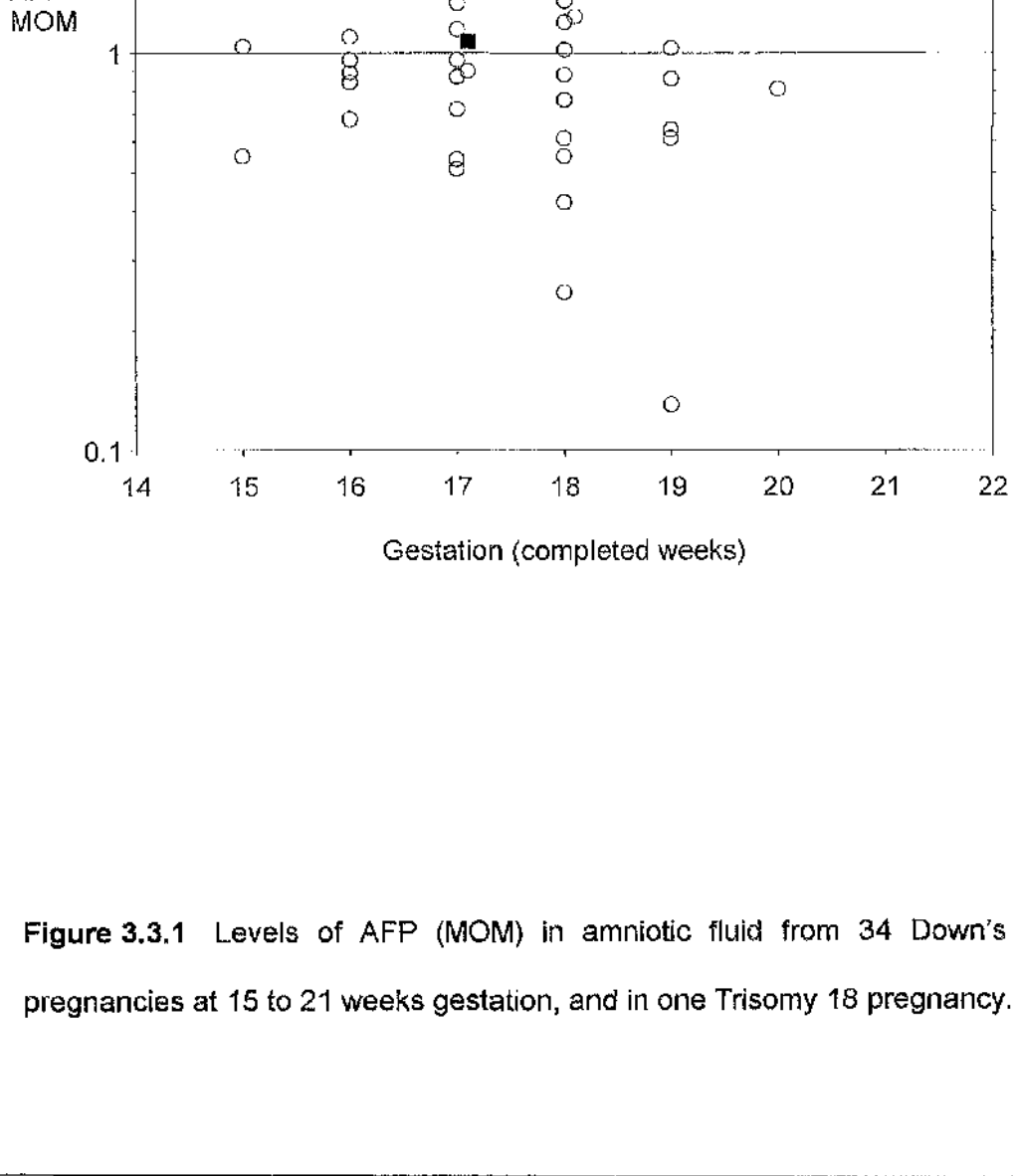

Figure 3.3.1 Levels of AFP (MOM) in amniotic fluid from 34 Down's syndrome pregnancies at 15 to 21 weeks gestation, and in one Trisomy 18 pregnancy.

3.3.2 HUMAN CHORIONIC GONADOTROPIN.

3.3.2.1 Unaffected Controls.

The concentration profile of intact hCG in amniotic fluid from 132 unaffected pregnancies at 15 to 21 weeks gestation is shown in Figure 3.3.2. Median and regressed median hCG levels at each week of gestation are presented in Table 3.3.2. Regressed intact hCG medians were calculated using the equation: Intact hCG median = $10^{(2.13-0.0897 \times \text{gestation})}$. Amniotic fluid hCG levels decreased with advancing gestation. The overall median level of intact hCG in amniotic fluid from the unaffected control pregnancies was 0.92 MOM.

3.3.2.2 Down's Syndrome.

Intact hCG levels, expressed as MOM, in amniotic fluid from 33 Down's syndrome pregnancies at 15 to 21 weeks gestation are shown in Figure 3.3.3. Amniotic fluid hCG levels were elevated in the Down's syndrome pregnancies with a median level of 1.38 MOM. This elevation was found to be statistically significant (p=0.028) using the Mann-Whitney Test.

3.3.2.3 Trisomy 18.

The level of intact hCG in amniotic fluid from one Trisomy 18 pregnancy (case T18/4) at 17 weeks gestation was 0.58 MOM (Figure 3.3.3).

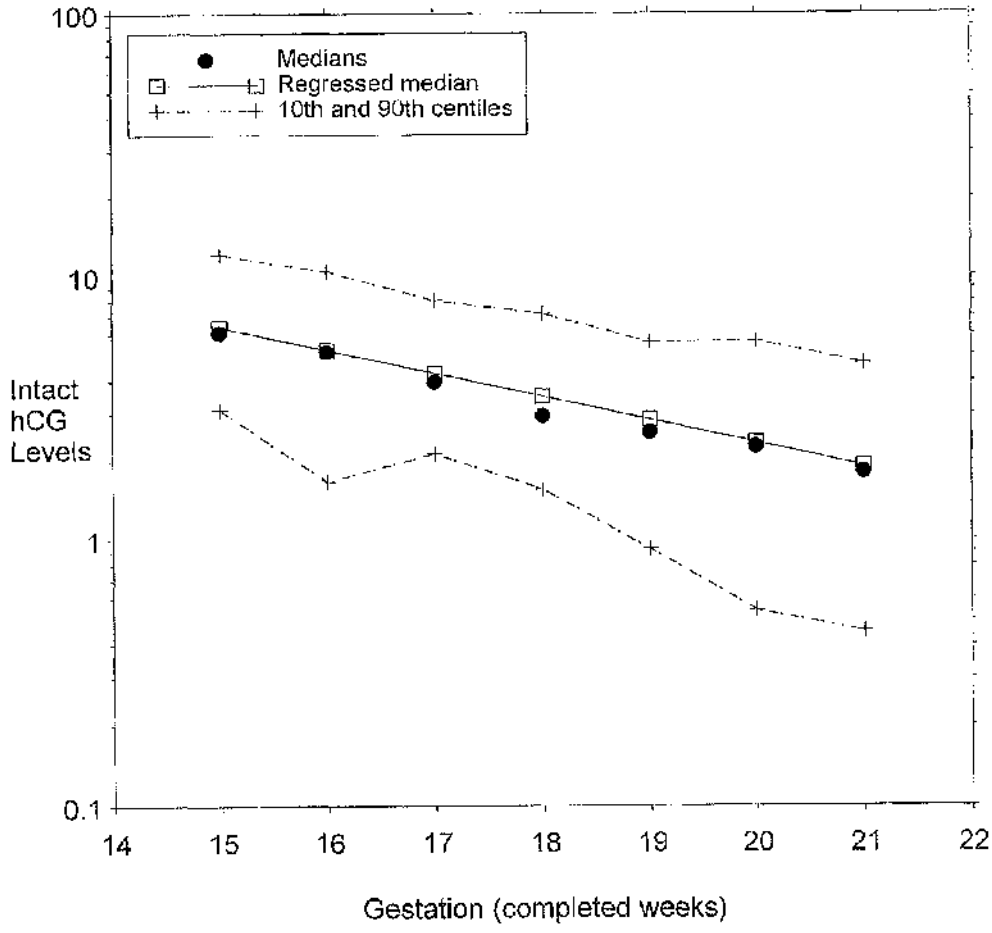

Figure 3.3.2 Median and regressed median levels of intact hCG (IU/ml) in amniotic fluid from 132 unaffected pregnancies at 15 to 21 weeks gestation.

Table 3.3.2 Median and regressed median levels of intact hCG (IU/ml) in amniotic fluid from 132 unaffected pregnancies at each week of gestation from 15 to 21 weeks.

Gestation	No. of samples	Median	Regressed median
15	20	6.10	6.41
16	20	5.15	5.24
17	20	3.95	4.28
18	18	2.95	3.49
19	20	2.55	2.85
20	20	2.25	2.33
21	14	1.80	1.90

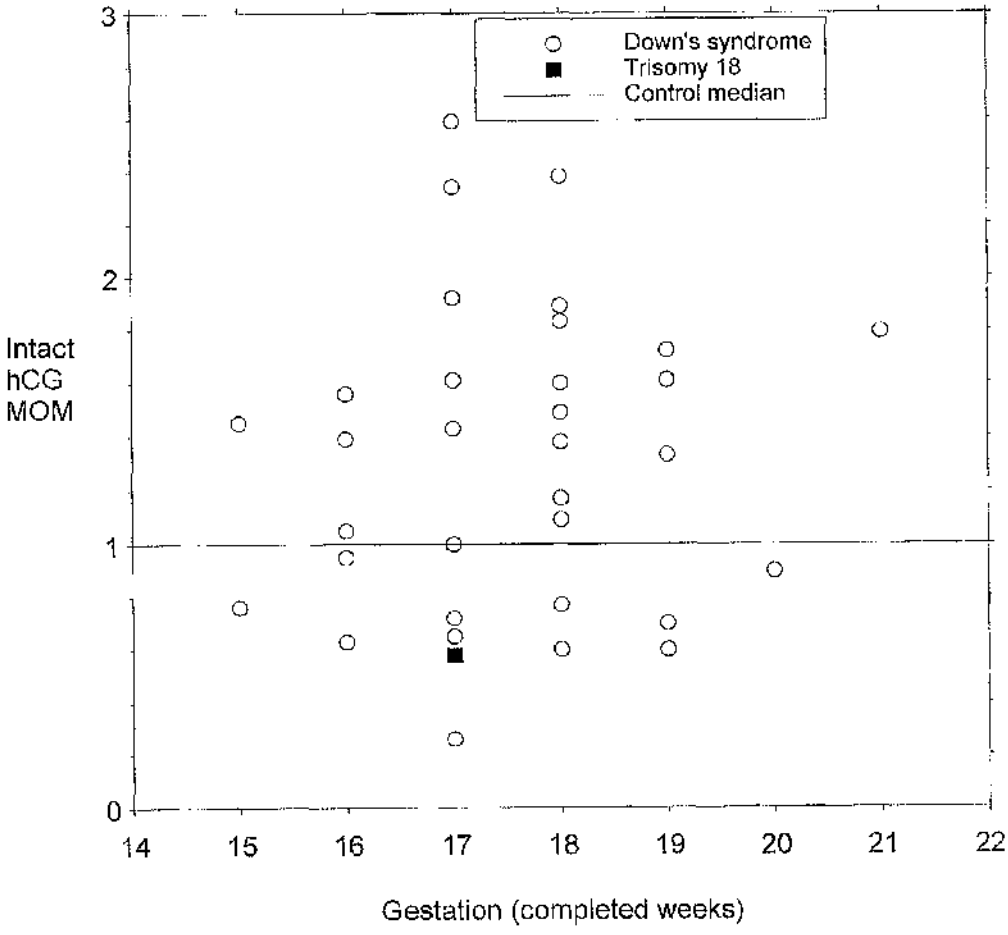

Figure 3.3.3 Levels of intact hCG (MOM) in amniotic fluid from 33 Down's syndrome pregnancies at 15 to 21 weeks gestation, and in one Trisomy 18 pregnancy.

3.3.3 FREE BETA HUMAN CHORIONIC GONADOTROPIN.

3.3.3.1 Unaffected Controls.

The concentration profile of FβhCG in amniotic fluid from 132 unaffected pregnancies at 15 to 21 weeks gestation is shown in Figure 3.3.4 Median and regressed median FβhCG levels at each week of gestation are presented in Table 3.3.3. Regressed amniotic fluid FβhCG medians were calculated using the equation: FβhCG median = $10^{(3.70-0.102 \times \text{gestation})}$. Amniotic fluid FβhCG levels follow a pattern similar to that in maternal serum, decreasing with advancing gestation from 15 to 21 weeks but are approximately 10 times higher than in maternal serum at equivalent gestations. The median level of FβhCG in amniotic fluid from the unaffected control pregnancies was 1.00 MOM.

3.3.3.2 Down's Syndrome.

FβhCG levels, expressed as MOM, in amniotic fluid from 33 Down's syndrome pregnancies at 15 to 21 weeks gestation are shown in Figure 3.3.5. Amniotic fluid FβhCG levels were elevated in the Down's syndrome pregnancies with a median level of 1.33 MOM. This elevation was found to be statistically significant ($p=0.002$) using the Mann-Whitney Test.

3.3.3.3 Trisomy 18.

The level of FβhCG in amniotic fluid from one Trisomy 18 pregnancy (case T18/4) at 17 weeks gestation was 0.97 MOM (Figure 3.3.5).

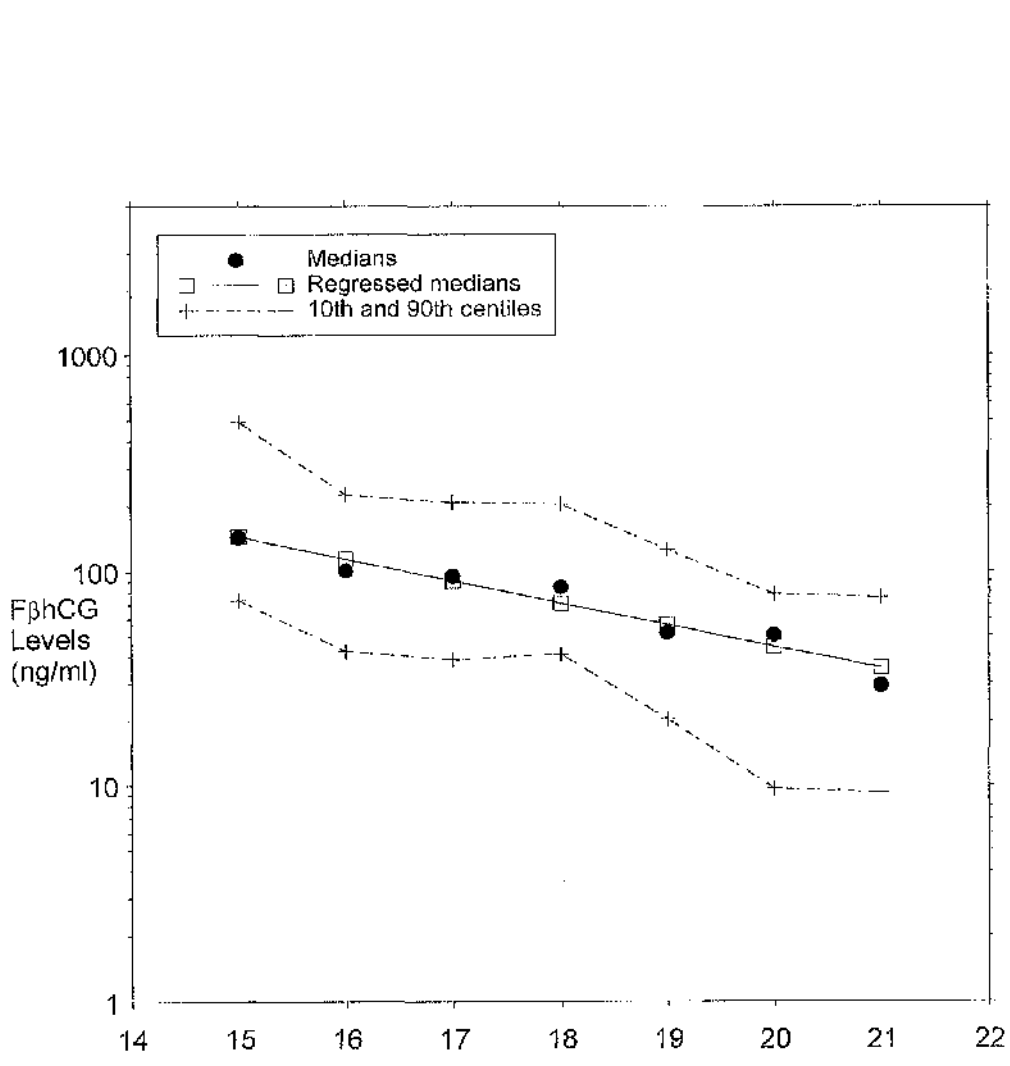

Figure 3.3.4 Median and regressed median levels of FβhCG (ng/ml) in amniotic fluid from 132 unaffected pregnancies at 15 to 21 weeks gestation.

Table 3.3.3 Median and regressed median levels of FβhCG (ng/ml) in amniotic fluid from 132 unaffected pregnancies at each week of gestation from 15 to 21 weeks.

Gestation	No. of samples	Median	Regressed median
15	20	144	145
16	20	101	114
17	20	95.1	90.3
18	18	84.5	71.3
19	20	51.6	56.4
20	20	50.5	44.5
21	14	29.4	35.2

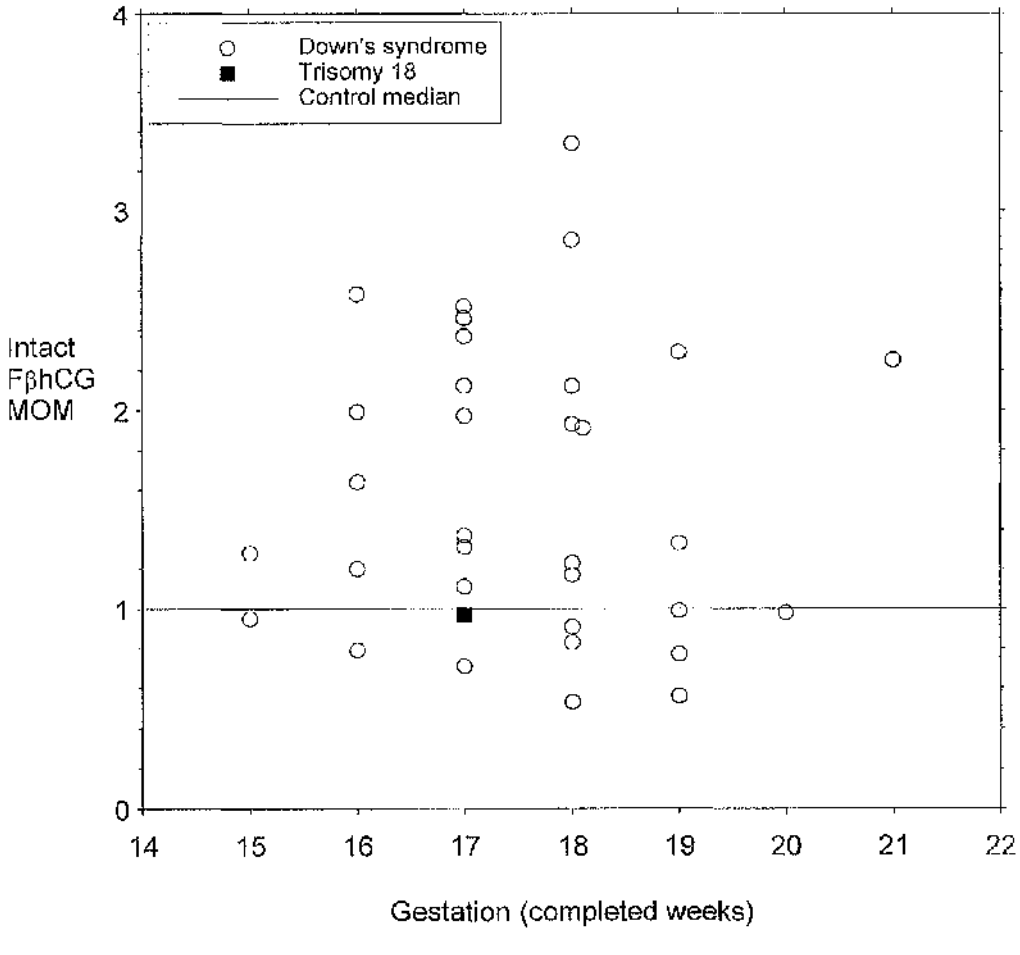

Figure 3.3.5 Levels of FβhCG (MOM) in amniotic fluid from 33 Down's syndrome pregnancies at 15 to 21 weeks gestation, and in one Trisomy 18 pregnancy.

3.3.4 PREGNANCY ASSOCIATED PLASMA A.

3.3.4.1 Unaffected Controls.

The concentration profile of PAPP-A in amniotic fluid from 132 unaffected pregnancies at 15 to 21 weeks gestation is shown in Figure 3.3.6. Median and regressed median PAPP-A levels at each week of gestation are presented in Table 3.3.4. Regressed median PAPP-A levels were calculated using the equation: PAPP-A median = $10^{(-0.662+0.0032 \times \text{gestation})}$. Amniotic fluid PAPP-A levels increased gradually with advancing gestation, similar to the rate of increase noted in placental tissues but more slowly than the rise observed in maternal serum at 15 to 21 weeks. The overall median level of PAPP-A in amniotic fluid from the unaffected control pregnancies was 1.04 MOM.

3.3.4.2 Down's Syndrome.

PAPP-A levels, expressed as MOM, in amniotic fluid from 33 Down's syndrome pregnancies at 15 to 21 weeks gestation are shown in Figure 3.3.7. PAPP-A levels in amniotic fluid from Down's syndrome pregnancies was not significantly different from normal (Mann-Whitney, p=0.126), with a median level of 0.95 MOM in the Down's syndrome pregnancies.

3.3.4.3 Trisomy 18.

The level of PAPP-A in amniotic fluid from one Trisomy 18 pregnancy (case T18/4) at 17 weeks gestation was 0.52 MOM (Figure 3.3.7).

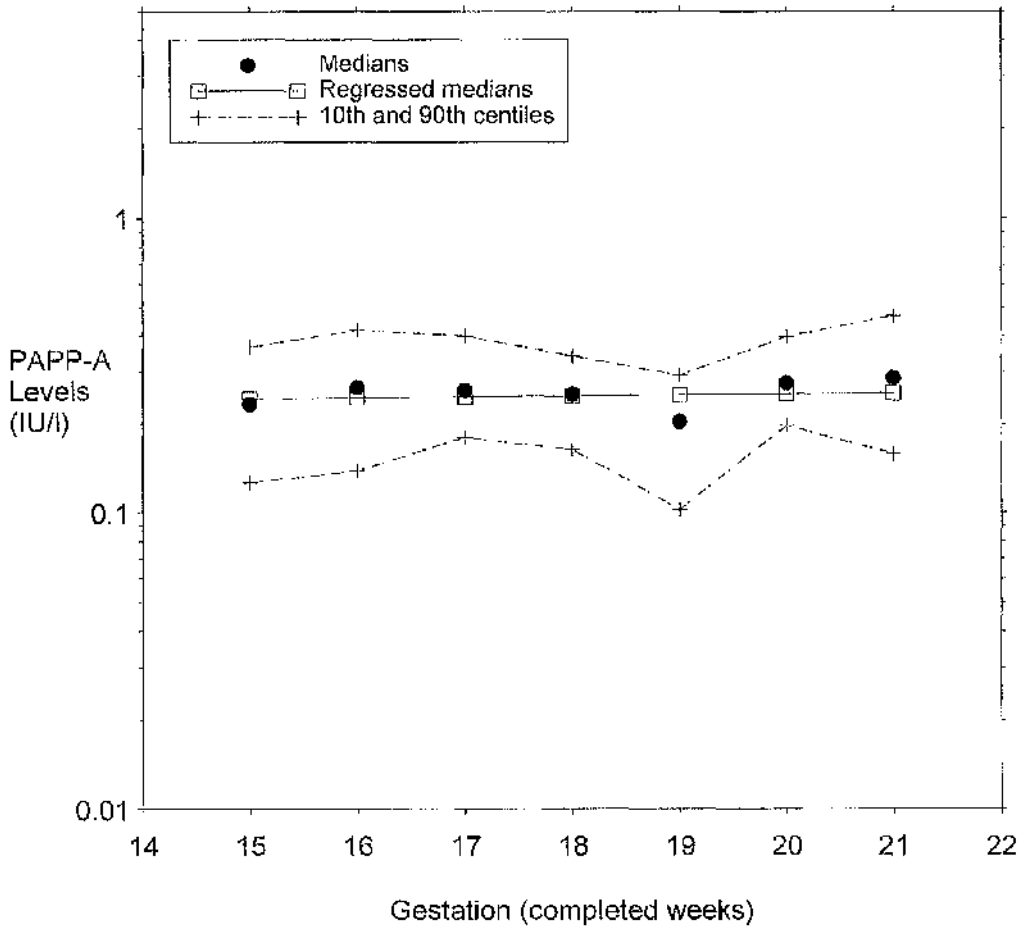

Figure 3.3.6 Median and regressed median levels of PAPP-A (IU/l) in amniotic fluid from 132 unaffected pregnancies at 15 to 21 weeks gestation.

Table 3.3.4 Median and regressed median levels of PAPP-A (IU/l) in amniotic fluid from 132 unaffected pregnancies at each week of gestation from 15 to 21 weeks.

Gestation	No. of samples	Median	Regressed median
15	20	0.233	0.243
16	20	0.265	0.245
17	20	0.260	0.247
18	18	0.252	0.249
19	20	0.204	0.251
20	20	0.275	0.253
21	14	0.286	0.254

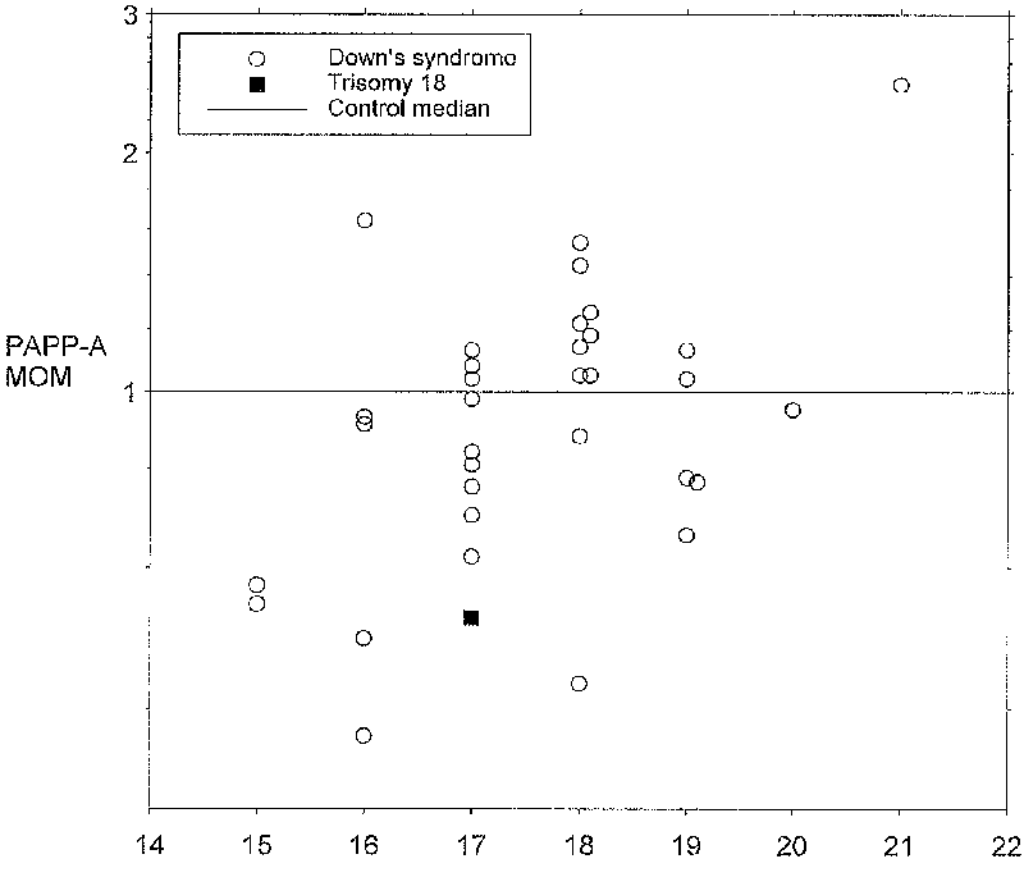

Figure 3.3.7 Levels of PAPP-A (MOM) in amniotic fluid from 33 Down's syndrome pregnancies at 15 to 21 weeks gestation, and in one Trisomy 18 pregnancy.

3.3.5 PREGNANCY SPECIFIC BETA 1 GLYCOPROTEIN.

3.3.5.1 Unaffected Controls.

The concentration profile of SP1 in amniotic fluid from 132 unaffected pregnancies at 15 to 21 weeks gestation is shown in Figure 3.3.8. Median and regressed median SP1 levels at each week of gestation are presented in Table 3.3.5. Regressed amniotic fluid SP1 medians were calculated using the equation: SP1 median = $10^{(2.34+0.0280 \times \text{gestation})}$. Amniotic fluid SP1 levels were found to increase with advancing gestation between 15 and 21 weeks. The overall median level of SP1 in amniotic fluid from the unaffected control pregnancies was 0.97 MOM.

3.3.5.2 Down's Syndrome.

SP1 levels, expressed as MOM, in amniotic fluid from 32 Down's syndrome pregnancies at 15 to 21 weeks gestation are shown in Figure 3.3.9. Amniotic fluid SP1 levels in the Down's syndrome pregnancies were not significantly different from the controls Mann-Whitney, p=0.749). The overall median level of SP1 in amniotic fluid from the Down's syndrome pregnancies was 0.91 MOM.

3.3.5.3 Trisomy 18.

The level of SP1 in amniotic fluid from one Trisomy 18 pregnancy (case T18/4) at 17 weeks gestation was 0.80 MOM (Figure 3.3.9).

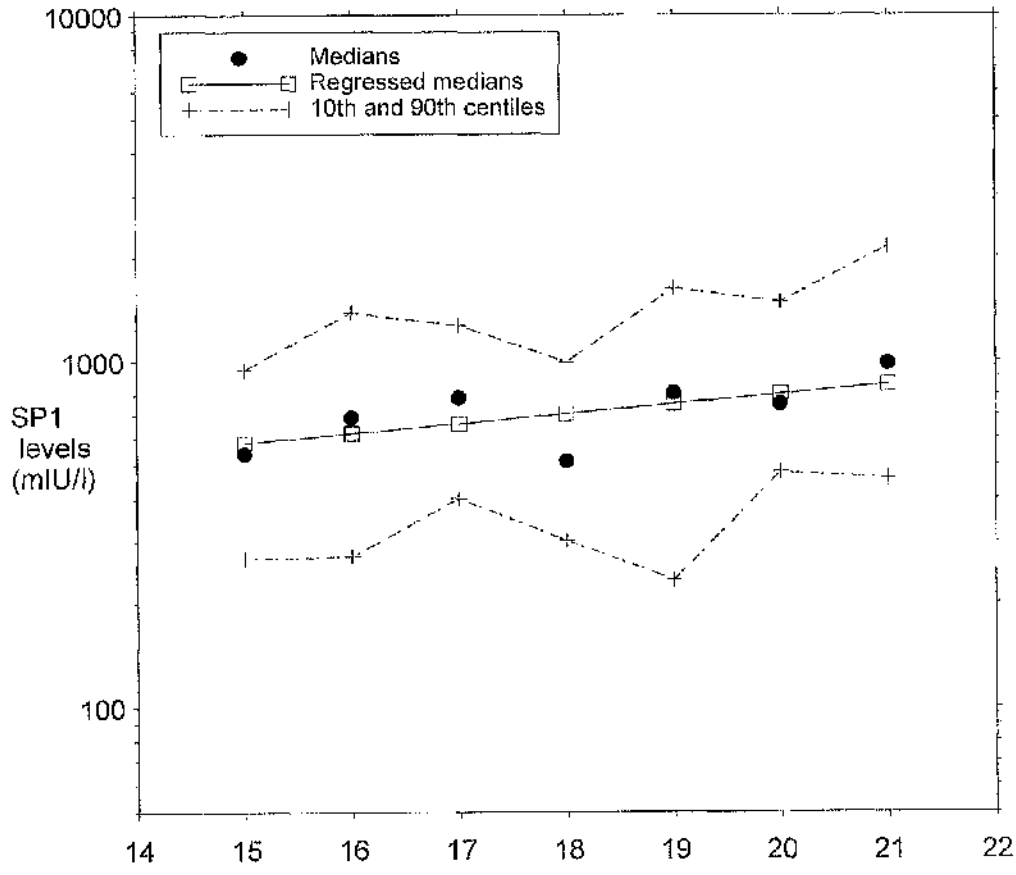

Figure 3.3.8 Median and regressed median levels of SP1 (mIU/l) in amniotic fluid from 132 unaffected pregnancies at 15 to 21 weeks gestation.

Table 3.3.5 Median and regressed median levels of SP1 (mIU/l) in amniotic fluid from 132 unaffected pregnancies at each week of gestation from 15 to 21 weeks.

Gestation	No. of samples	Median	Regressed median
15	20	539	581
16	20	689	620
17	20	784	661
18	18	513	705
19	20	809	752
20	20	751	802
21	14	948	855

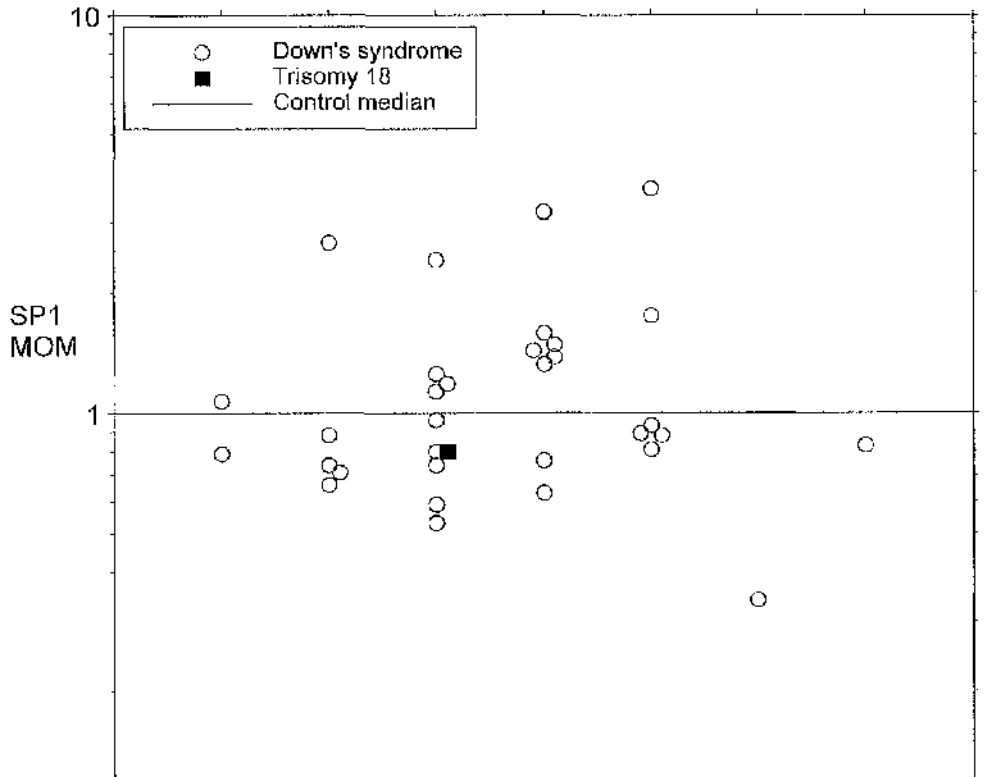

Figure 3.3.9 Levels of SP-1 (MOM) in amniotic fluid from 32 Down's syndrome pregnancies at 15 to 21 weeks gestation, and in one Trisomy 18 pregnancy.

3.3.6 ALKALINE PHOSPHATASE.

3.3.6.1 Unaffected Controls.

The activity profiles of total and placental ALP in amniotic fluid from 132 unaffected pregnancies at 15 to 21 weeks gestation are illustrated in Figure 3.3.10 and Figure 3.3.11 respectively. Median levels of total and placental ALP activity are presented in Table 3.3.6, along with the percentage contributions of the placental isoenzyme to the total ALP activity at each week of gestation. Regressed medians are presented in Table 3.3.7. Median total and placental ALP activities were calculated using the following equations: Total ALP median = $10^{(2.72-0.0215 \times \text{gestation})}$, placental ALP median = $10^{(0.863+0.0072 \times \text{gestation})}$. While total ALP activity was found to decrease with advancing gestation, placental ALP activity increased from 15 to 21 weeks gestation. Median levels of total and placental ALP in amniotic fluid from unaffected pregnancies were 0.92 MOM and 1.00 MOM respectively.

3.3.6.2 Down's Syndrome.

Total and placental ALP activity, expressed as MOM, in amniotic fluid from 33 Down's syndrome pregnancies at 15 to 21 weeks gestation are shown in Figure 3.3.12 and Figure 3.3.13 respectively. Total ALP levels were significantly reduced in Down's syndrome pregnancies (Mann-Whitney, $p<0.001$), with a median level of 0.39 MOM. There was also a significant reduction in placental ALP levels in amniotic fluid from Down's syndrome pregnancies (Mann-Whitney, $p=0.008$), but to a lesser extent than for total ALP (placental ALP median MOM = 0.75).

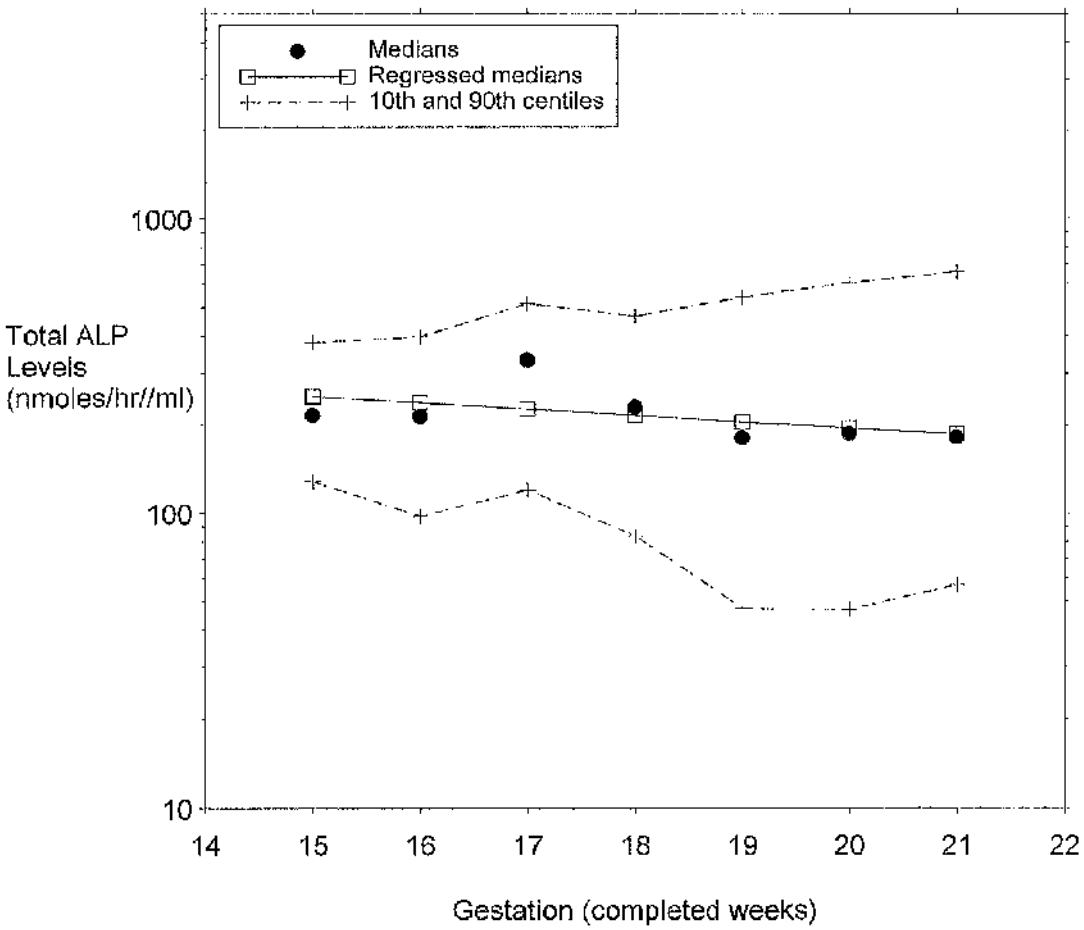

Figure 3.3.10 Median and regressed median levels of total ALP (nmoles/hr/ml) in amniotic fluid from 132 unaffected pregnancies at 15 to 21 weeks gestation.

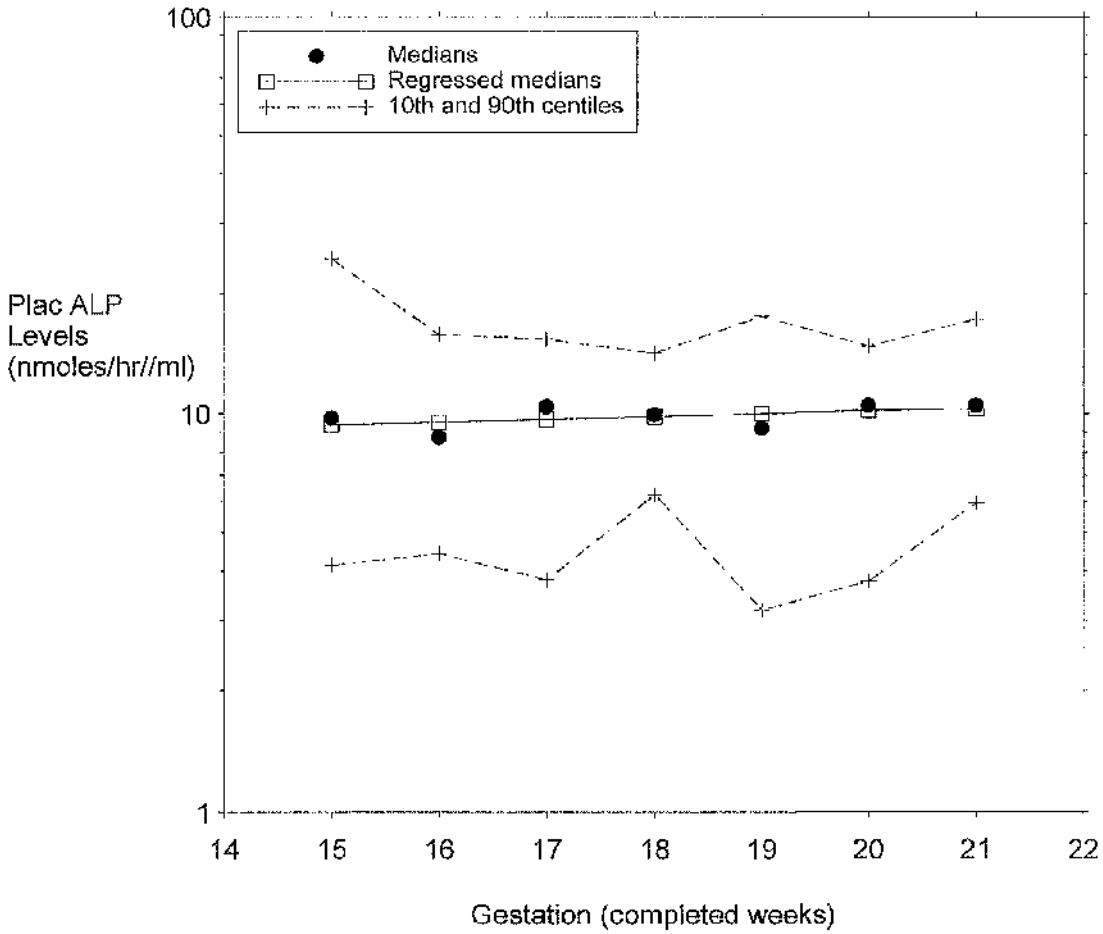

Figure 3.3.11 Median and regressed median levels of placental ALP (nmoles/hr/ml) in amniotic fluid from 132 unaffected pregnancies at 15 to 21 weeks gestation.

Table 3.3.6 Median total and placental ALP levels (nmoles/hr/ml) in amniotic fluid from 132 unaffected pregnancies at each week of gestation from 15 to 21 weeks, along with the percentage contribution of the placental isoenzyme to the total ALP activity.

Gestation	No of samples	Medians		% placental
		Total ALP	Plac ALP	ALP
15	20	216	9.73	4.5
16	20	214	8.72	4.07
17	20	333	10.4	3.12
18	18	230	9.92	4.31
19	20	181	9.21	5.09
20	20	187	10.5	5.61
21	14	181	10.5	5.80

Table 3.3.7 Regressed median total and placental ALP levels (nmoles/hr/ml) in amniotic fluid from 132 unaffected pregnancies at each week of gestation from 15 to 21 weeks, along with the percentage contribution of the placental isoenzyme to the total ALP activity.

Gestation	No.of samples	Regressed medians		% placental
		Total ALP	Plac ALP	ALP
15	20	250	9.35	3.80
16	20	238	9.51	4.00
17	20	227	9.67	4.26
18	18	216	9.83	4.55
19	20	205	10.0	4.88
20	20	195	10.2	5.23
21	14	186	10.3	5.54

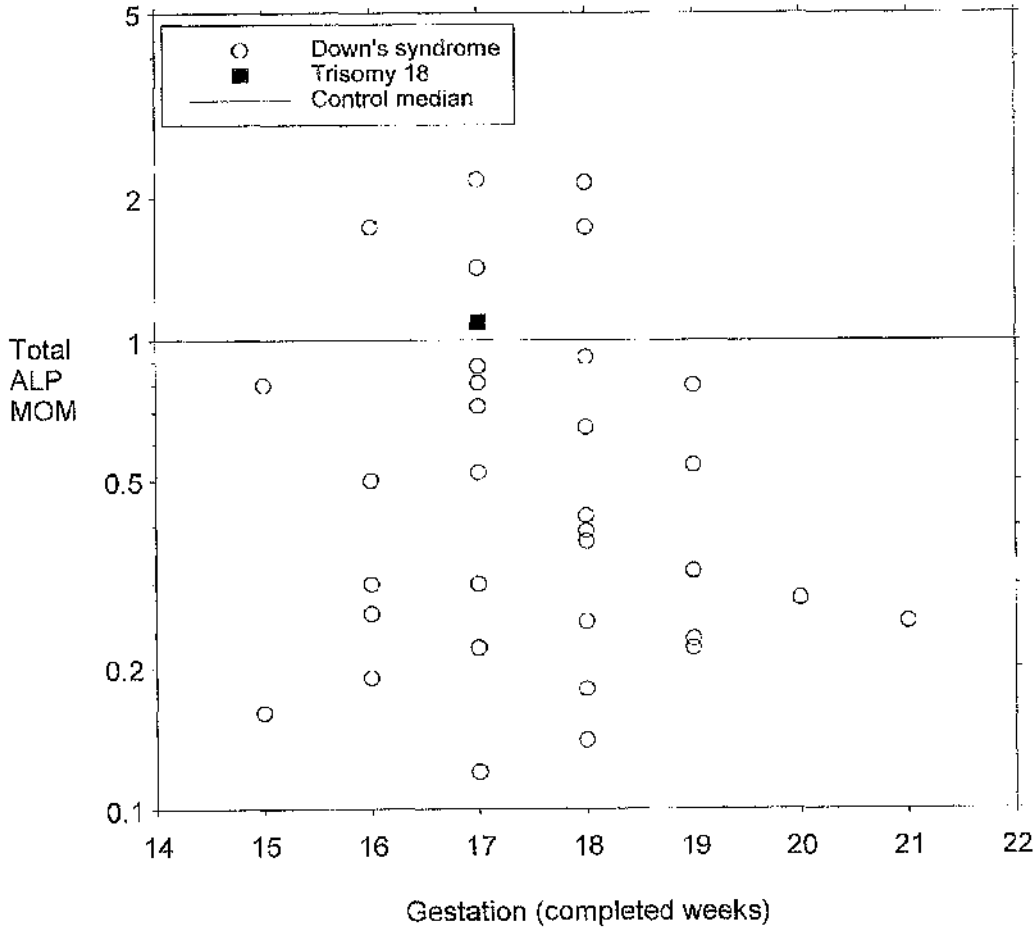

Figure 3.3.12 Levels of total ALP (MOM) in amniotic fluid from 33 Down's syndrome pregnancies at 15 to 21 weeks gestation, and in one Trisomy 18 pregnancy.

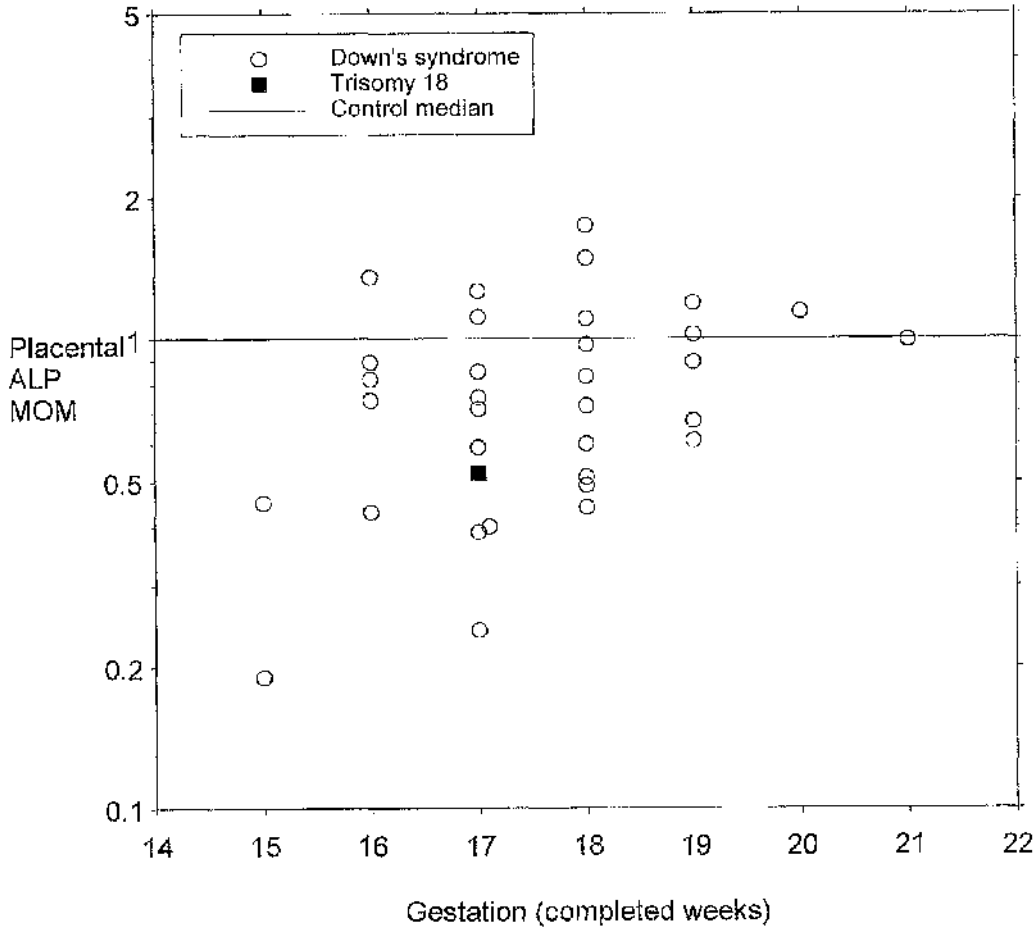

Figure 3.3.13 Levels of placental ALP (MOM) in amniotic fluid from 33 Down's syndrome pregnancies at 15 to 21 weeks gestation, and in one Trisomy 18 pregnancy.

3.3.6.3 Trisomy 18.

The levels of total and placental ALP in amniotic fluid from one Trisomy 18 (case T18/4) pregnancy at 17 weeks gestation were 1.09 MOM and 0.52 MOM respectively (Figure 3.3.12 and Figure 3.3.13 respectively).

3.3.7 GAMMA GLUTAMYL TRANSFERASE.

3.3.7.1 Unaffected Controls.

The activity profile of GGT in amniotic fluid from 132 unaffected pregnancies at 15 to 21 weeks gestation is shown in Figure 3.3.14. Median and regressed median GGT levels at each week of gestation are presented in Table 3.3.8. Regressed GGT medians were calculated using the equation: GGT median = $10^{(3.89-0.0732 \times \text{gestation})}$. Amniotic fluid GGT activity decreased with advancing gestation. The overall median level of GGT activity in the unaffected pregnancies was 0.99 MOM.

3.3.7.2 Down's Syndrome.

GGT activity, expressed as MOM, in amniotic fluid from 33 Down's syndrome pregnancies at 15 to 21 weeks gestation are shown in Figure 3.3.15. Amniotic fluid GGT activity was significantly lower in Down's syndrome pregnancies than in unaffected pregnancies (Mann-Whitney, $p<0.001$). The overall median level of GGT activity in amniotic fluid from the affected pregnanies was 0.48 MOM.

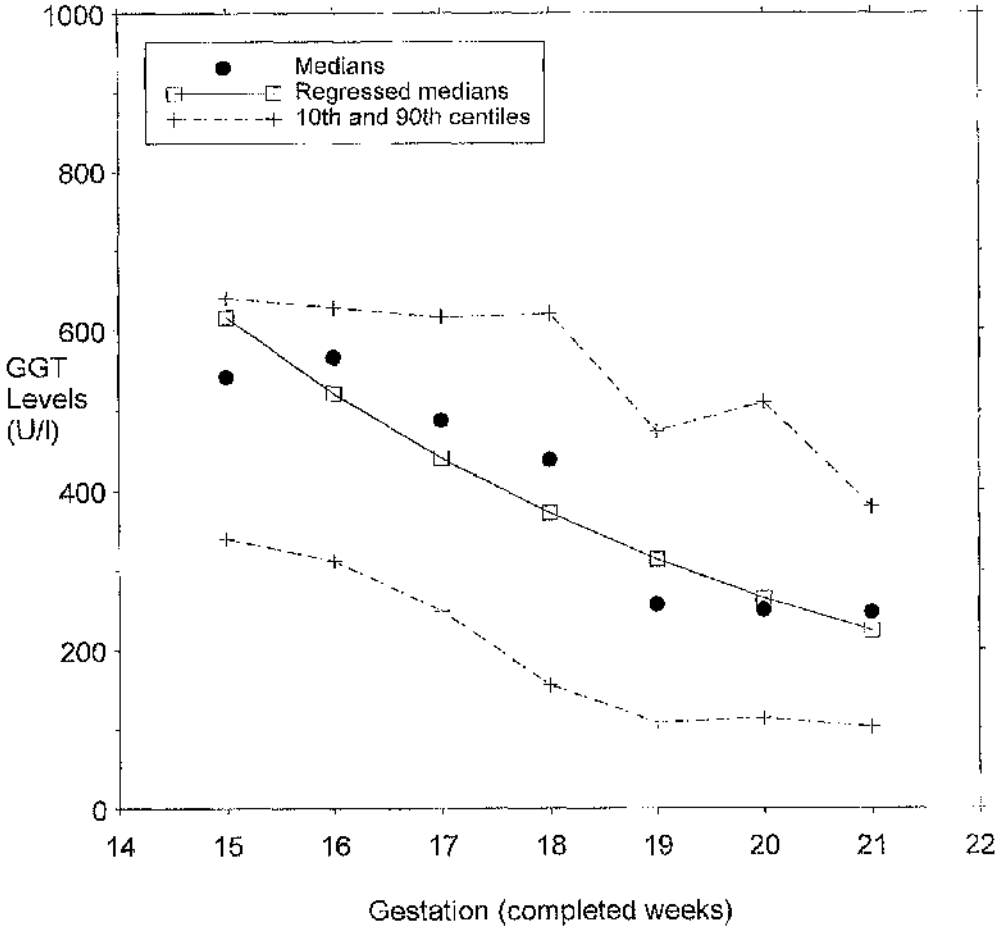

Figure 3.3.14 Median and regressed median levels of GGT (U/l) in amniotic fluid from 132 unaffected pregnancies at 15 to 21 weeks gestation.

Table 3.3.6 Median and regressed median levels of GGT (U/l) in amniotic fluid from 132 unaffected pregnancies at each week of gestation from 15 to 21 weeks.

Gestation	No. of samples	Median	Regressed median
15	20	542	616
16	20	566	521
17	20	487	440
18	18	438	372
19	20	257	314
20	20	250	265
21	14	247	224

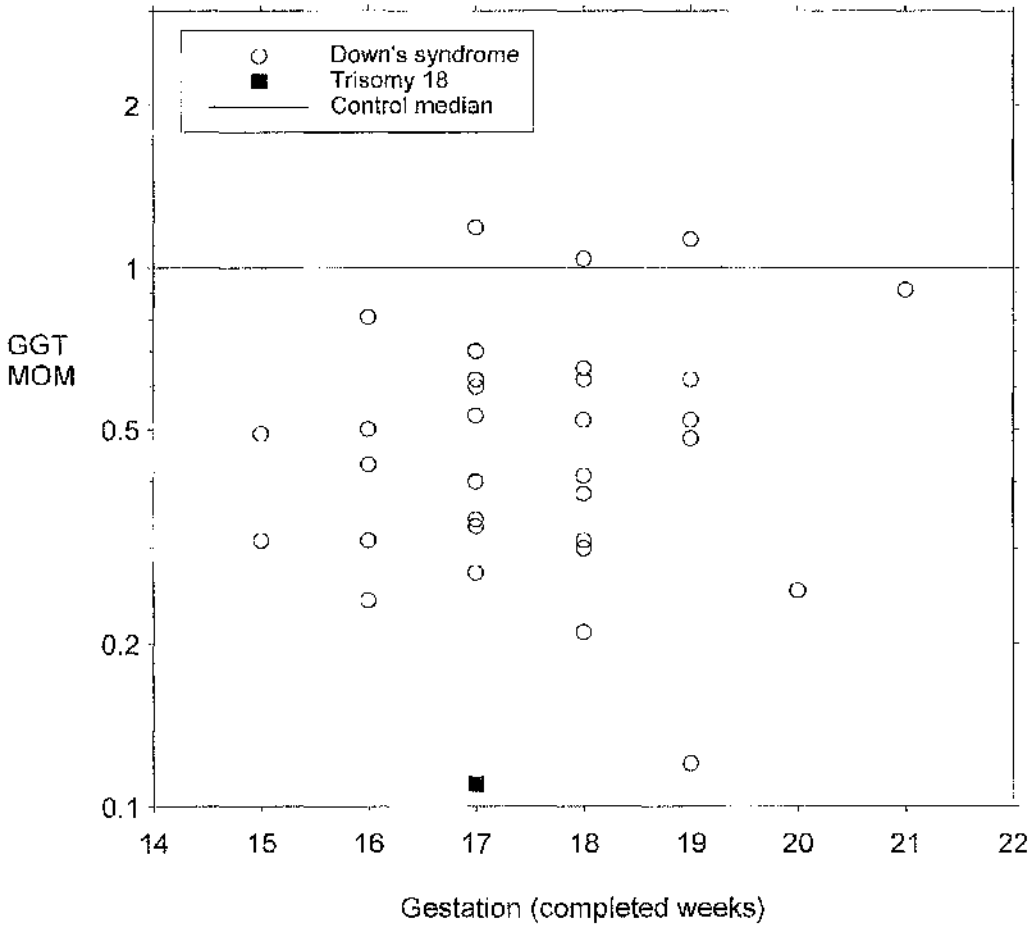

Figure 3.3.15 Levels of GGT (MOM) in amniotic fluid from 33 Down's syndrome pregnancies at 15 to 21 weeks gestation, and in one Trisomy 18 pregnancy.

3.3.7.3 Trisomy 18.

The level of GGT in amniotic fluid from one Trisomy 18 pregnancy (case T18/4) at 17 weeks gestation was 0.11 MOM (Figure 3.3.15).

3.3.8 SUMMARY OF MARKER LEVELS IN AMNIOTIC FLUID.

AFP, intact hCG, FβhCG, PAPP-A, SP1, total ALP, placental ALP and GGT levels (MOM) in amniotic fluid from Down's syndrome pregnancies are shown in Figure 3.3.16. A summary of marker levels in amniotic fluid from Down's syndrome and unaffected pregnancies is presented in Table 3.3.9. Levels of AFP, total ALP, placental ALP and GGT in amniotic fluid from Down's syndrome pregnancies were lower than in unaffected pregnancies, while intact hCG and FβhCG levels were elevated. PAPP-A and SP1 levels in amniotic fluid from Down's syndrome pregnancies were not significantly different from normal.

Marker levels in amniotic fluid from one Trisomy 18 pregnancy are summarised in Table 3.3.10.

3.4 CORRELATION ANALYSIS.

A summary of marker levels (median MOM) in fetal tissues and in corresponding maternal serum and amniotic fluid samples from Down's syndrome pregnancies is presented in Table 3.4.1. The relationships between marker levels in fetal tissue, maternal serum and amniotic fluid from the Down's syndrome pregnancies was examined using correlation analysis.

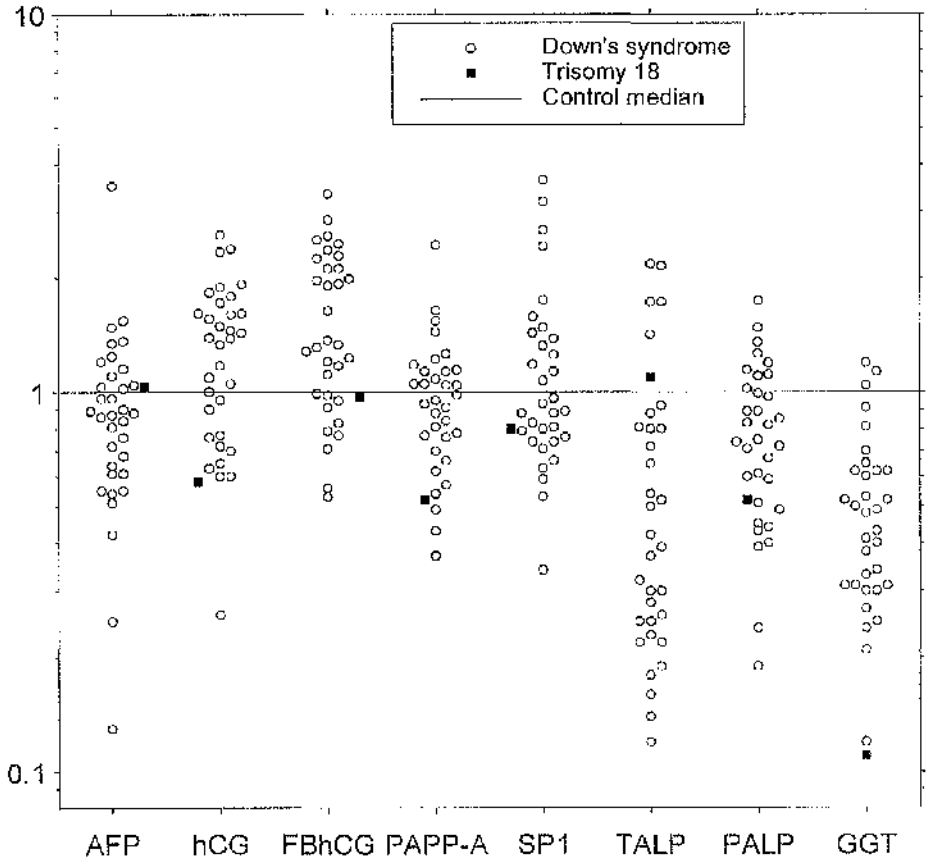

Figure 3.3.16 Levels of AFP, intact hCG, FβhCG. PAPP-A SP1, total ALP, placental ALP and GGT levels (MOM) in amniotic fluid from Down's syndrome and Trisomy 18 pregnancies.

Table 3.3.9 Summary of marker levels (median MOM) in amniotic fluid from Down's syndrome and unaffected pregnancies.

Marker	Median MOM		Mann-Whitney
	Unaffected controls	Down's syndrome	p-value
AFP	-	0.87	-
intact hCG	0.92	1.39	0.017
FβhCG	1.00	1.33	0.002
PAPP-A	1.04	0.95	0.126
SP-1	0.97	0.91	0.749
Total ALP	0.92	0.39	<0.001
Plac ALP	1.01	0.75	0.005
GGT	0.99	0.48	<0.001

Table 3.3.10 Summary of marker levels (MOM) in amniotic fluid from one Trisomy 18 pregnancy.

Case no.	AFP	intact hCG	FβhCG	PAPP-A	SP1	Total ALP	Placental ALP	GGT
T18/4	1.07	0.58	0.97	0.52	0.80	1.09	0.52	0.11

Table 3.4.1 Summary of marker levels (median MOM) in fetal tissues and in corresponding maternal serum and amniotic fluid samples from Down's syndrome pregnancies.

Marker	Median MOM				
	Ileum	Liver	Placenta	Maternal serum	Amniotic fluid
AFP	-	0.97	2.43	0.69	0.87
intact hCG	-	-	4.06	2.72	1.39
FβhCG	-	-	3.40	3.06	1.33
PAPP-A	-	-	0.96	0.92	0.95
SP-1	-	-	1.79	1.33	0.91
Total ALP	-	-	-	0.83	0.39
Plac ALP	-	-	0.91	1.04	0.75
GGT	0.43	2.01	1.29	-	0.48

The results of correlation analysis on AFP levels (logarithmically transformed MOMs) in the Down's syndrome pregnancies are presented in Table 3.4.2. There was no significant correlation between AFP levels in fetal tissues and in maternal serum or amniotic fluid from Down's syndrome pregnancies. No significant correlation was found between AFP levels in amniotic fluid and maternal serum from Down's syndrome pregnancies.

The results of correlation analysis on each of the placental markers (intact hCG, FβhCG, PAPP-A, SP-1 and placental ALP) in placental tissue and in corresponding maternal serum and amniotic fluid samples from Down's syndrome pregnancies are presented in Table 3.4.3. There were significant correlations between the levels of intact hCG in placental tissue and maternal serum (Figure 3.4.1), placental tissue and amniotic fluid (Figure 3.4.2) and in amniotic fluid and maternal serum (Figure 3.4.3) from the Down's syndrome pregnancies. A significant correlation was also found between the levels of FβhCG in placental tissue and maternal serum (Figure 3.4.4), and in maternal serum and amniotic fluid (Figure 3.4.5) from the Down's syndrome pregnancies. However, no such association was found between placental and amniotic fluid levels of FβhCG.

Despite the similarities between the median MOM levels of PAPP-A, SP-1 and placental ALP in placental tissue and maternal serum from the Down's syndrome pregnancies, no significant correlation was found. Neither was there a correlation between the levels PAPP-A or placental ALP in amniotic fluid and maternal serum. There was a significant correlation between maternal serum and amniotic fluid levels of SP1 in the Down's syndrome pregnancies (Table 3.4.3).

Table 3.4.2 Correlation between levels of AFP in fetal liver, placenta (plac), maternal serum (ms) and amniotic fluid (af) from corresponding Down's syndrome pregnancies.

		n	Correlation co-efficient	p-value
AFP	plac. AFP/ ms AFP	48	0.02	0.903
	plac. AFP/ af AFP	28	-0.09	0.650
	af AFP/ ms AFP	34	-0.002	0.993
	liver AFP/ ms AFP	50	0.08	0.583
	liver AFP/ af AFP	25	-0.08	0.698
	plac. AFP/ liver AFP	38	0.07	0.680

Table 3.4.3 Correlation between levels of intact hCG, FβhCG, PAPP-A, SP-1 and placental ALP in placental tissue (plac), maternal serum (ms) and amniotic fluid (af) from corresponding Down's syndrome pregnancies.

		n	Correlation co-efficient	p-value
Intact hCG	plac. hCG/ ms hCG	48	0.44	0.002
	plac. hCG/ af hCG	27	0.46	0.016
	af hCG/ ms hCG	33	0.49	0.004
FβhCG	plac. FβhCG/ ms FβhCG	44	0.63	<0.001
	plac. FβhCG/ af FβhCG	27	0.12	0.581
	af FβhCG/ ms FβhCG	32	0.53	0.002
PAPPA	plac. PAPPA/ ms PAPPA	44	0.27	0.079
	plac. PAPPA/ af PAPPA	27	-0.19	0.337
	af PAPPA/ ms PAPPA	32	-0.02	0.936
SP-1	plac. SP-1/ ms SP-1	44	0.06	0.677
	plac. SP-1/ af SP-1	26	0.08	0.687
	af SP-1/ ms SP-1	31	0.66	<0.001
Plac ALP	plac. PALP/ ms PALP	42	0.30	0.056
	plac. PALP/ af PALP	27	0.38	0.053
	af PALP/ ms PALP	29	0.22	0.251

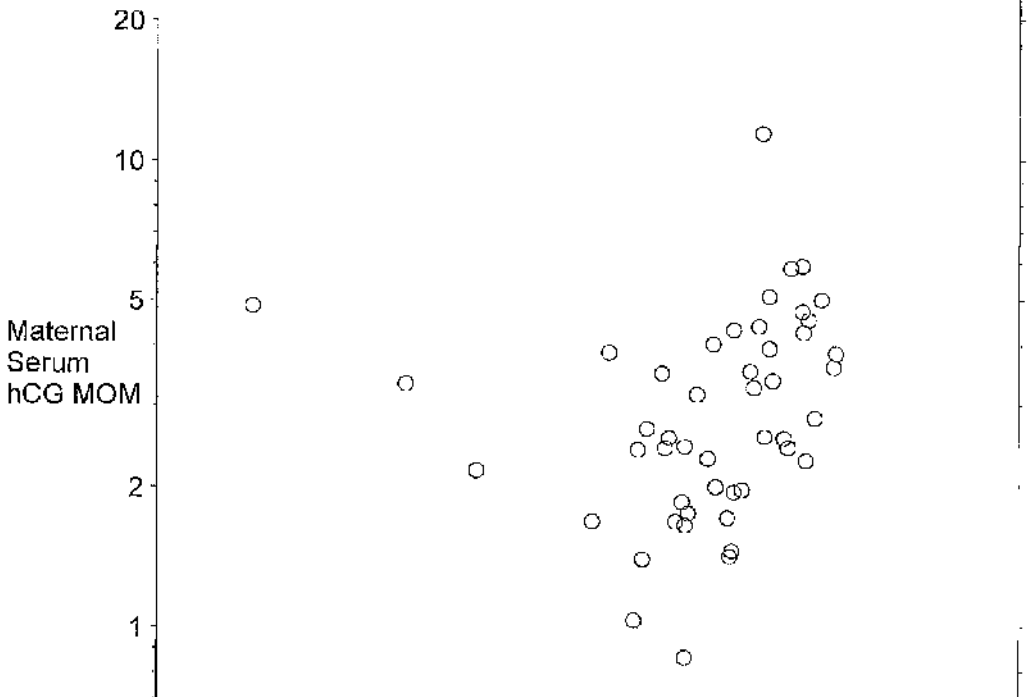

Figure 3.4.1 Correlation between the levels of intact hCG in placental tissue and maternal serum from 48 Down's syndrome pregnancies.

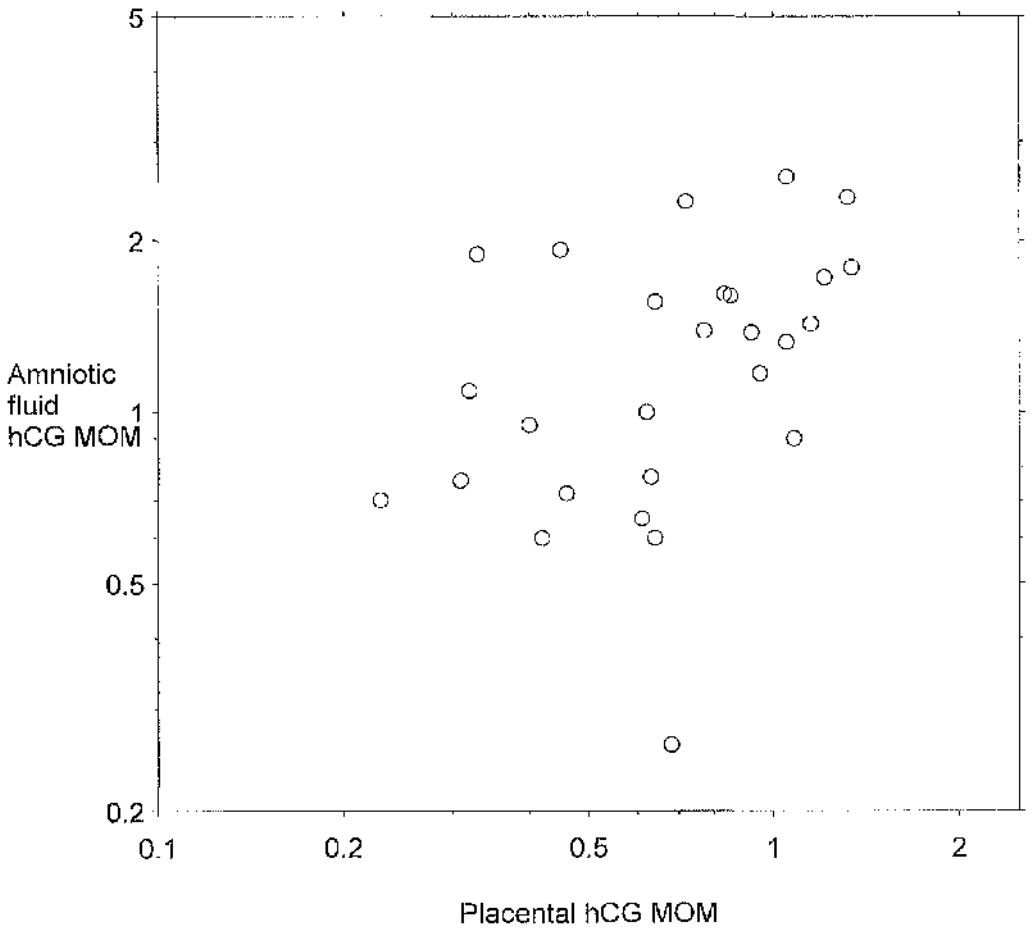

Figure 3.4.2 Correlation between the levels of intact hCG in placental tissue and amniotic fluid from 27 Down's syndrome pregnancies.

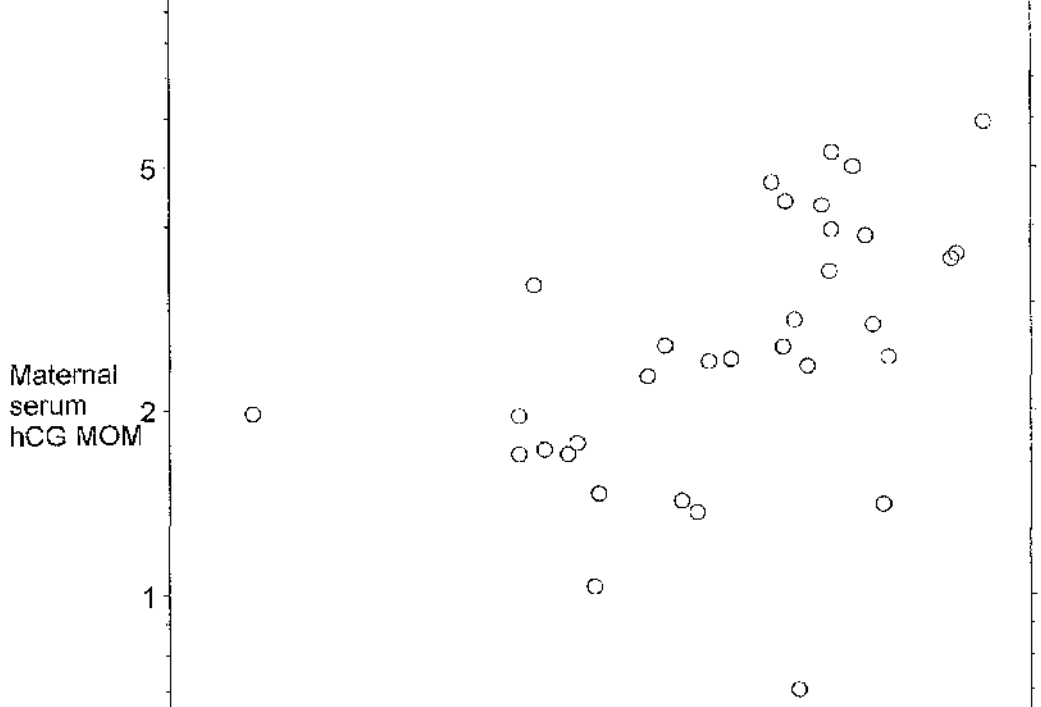

Figure 3.4.3 Correlation between the levels of intact hCG in amniotic fluid and maternal serum from 33 Down's syndrome pregnancies.

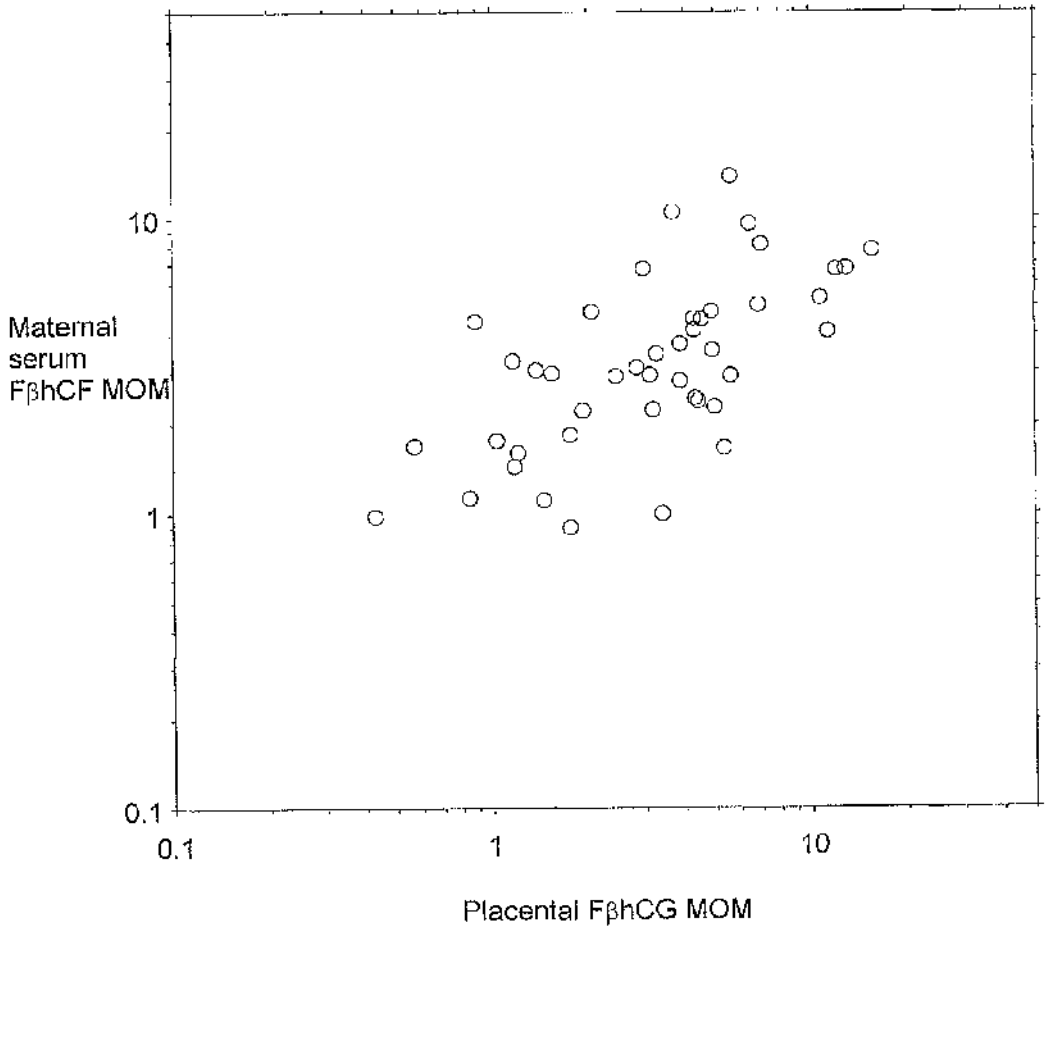

Figure 3.4.4 Correlation between the levels of FβhCG in placental tissue and maternal serum from 48 Down's syndrome pregnancies.

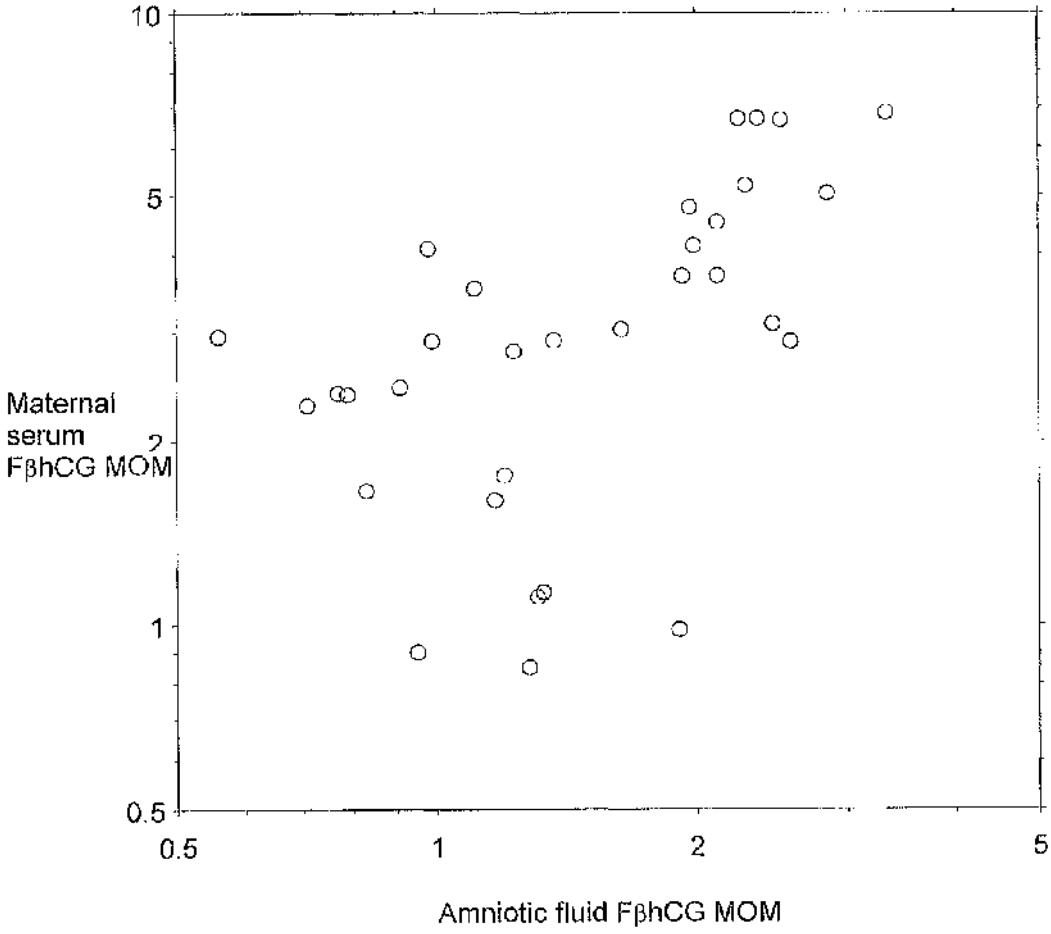

Figure 3.4.5 Correlation between the levels of FβhCG in amniotic fluid and maternal serum from 33 Down's syndrome pregnancies.

No significant correlation was found between the levels of GGT in amniotic fluid from Down's symdrome pregnancies and the levels of GGT in placenta, fetal liver or fetal ileum from corresponding Down's syndrome pregnancies (Table 3.4.4).

3.5 EFFECT OF FETAL SEX ON HCG LEVELS.

It has been reported that the level of intact hCG in the maternal circulation is influenced by the sex of the fetus. Maternal serum levels of hCG are reported to be significantly lower in pregnancies with male fetuses after 18 weeks gestation (Brody and Carlstrom 1965, Boroditsky et al 1975, Obiekwe and Chard 1982, Leporrier et al 1992, Muller et al 1993a).

The effect of fetal sex on the concentration of intact hCG and FβhCG in placental homogenates, maternal serum and amniotic fluid from Down's symdrome pregnancies in this study was examined. Median MOM levels of intact hCG and FβhCG in placental homogenates, maternal serum and amniotic fluid from Down's syndrome pregnancies with respect to fetal sex are presented in Table 3.5.1. There was no significant difference in the levels of intact hCG or FβhCG in either placental tissue, maternal serum or amniotic fluid from Down's syndrome pregnancies with a male fetus compared to Down's syndrome pregannncies with a female fetus. In unaffected pregnancies, placental intact hCG levels were higher in pregnancies with a male fetus (1.34 MOM) than in pregnancies with a female fetus (0.94 MOM), although the difference was not statistically significant (Mann-Whitney, p=0.110). Likewise, placental levels of FβhCG in unaffected pregnancies with a female fetus (median MOM = 1.01) were not significantly different (P = 0.222) from those with a male fetus (median MOM = 1.04).

Table 3.4.4 Correlation between levels of GGT in fetal liver, fetal ileum, placental tissue (plac) and amniotic fluid (af) from corresponding Down's syndrome pregnancies.

		n	Correlation co-efficient	p-value
GGT	plac. GGT/ af GGT	26	-0.02	0.940
	liver GGT/ af GGT	23	-0.21	0.329
	ileum GGT/ af GGT	19	-0.07	0.766
	plac. GGT/ liver GGT	36	-0.02	0.909
	plac. GGT/ ileum GGT	29	0.09	0.630
	liver GGT/ ileum GGT	45	0.24	0.112

Table 3.5.1 Intact hCG and FβhCG levels (MOM) in placental tissue, maternal serum and amniotic fluid from Down's syndrome pregnancies with respect to fetal sex.

		Fetal sex		Mann-Whitney
		Male	Female	p-value
Intact hCG	Placenta	4.60 (n=30)	3.38 (n=21)	0.193
	Maternal serum	2.81 (n=37)	2.67 (n=25)	0.736
	Amniotic fluid	1.36 (n=22)	1.45 (n=11)	0.909
FβhCG	Placenta	4.19 (n=30)	2.95 (n=21)	0.553
	Maternal serum	2.97 (n=34)	3.17 (n=24)	0.912
	Amniotic fluid	1.27 (n=22)	2.12 (n=11)	0.244

3.6 IMMUNOHISTOCHEMISTRY.

3.6.1 Placental Localisation of Markers.

The localisations of AFP, hCG, PAPP-A, SP-1 and PALP in placental tissue from unaffected control and Down's syndrome pregnancies are shown in Figures 3.6.1-3.6.5 respectively. For each of the markers staining was localised to the syncytiotrophoblast in both unaffected and Down's syndrome placental sections. The syncytiotrophoblast is a continuous layer around the circumference of the villi, beneath which is a barely visible discontinuous layer of cytotrophoblast cells. This is characteristic of placental morphology at this stage of pregnancy when the cytotrophoblast layer is less prominent than at earlier gestations. Within the trophoblast of the villi is the connective tissue core (stroma) which is derived from the extraembryonic mesoderm. Fetal blood vessels are visible in only a few of the placental sections. A detailed investigation of the morphology of the placental sections was not carried out due to poor structural integrity which is an inherent disadvantage of using frozen sections.

3.6.2 Semiquantitative Analysis of Stained Placental Sections.

After an initial examination of the placental sections, it appeared that the degree of staining intensity for each of the markers in placental sections from Down's syndrome and unaffected pregnancies may reflect the biochemical results e.g. stronger staining for hCG in the Down's syndrome sections than in the unaffected sections corresponding to the increased levels of hCG levels in the placental extracts from Down's syndrome pregnancies. To investigate this further the slides were scored as described in Chapter 2.4.4 and the scores analysed using the Sign test. The results of

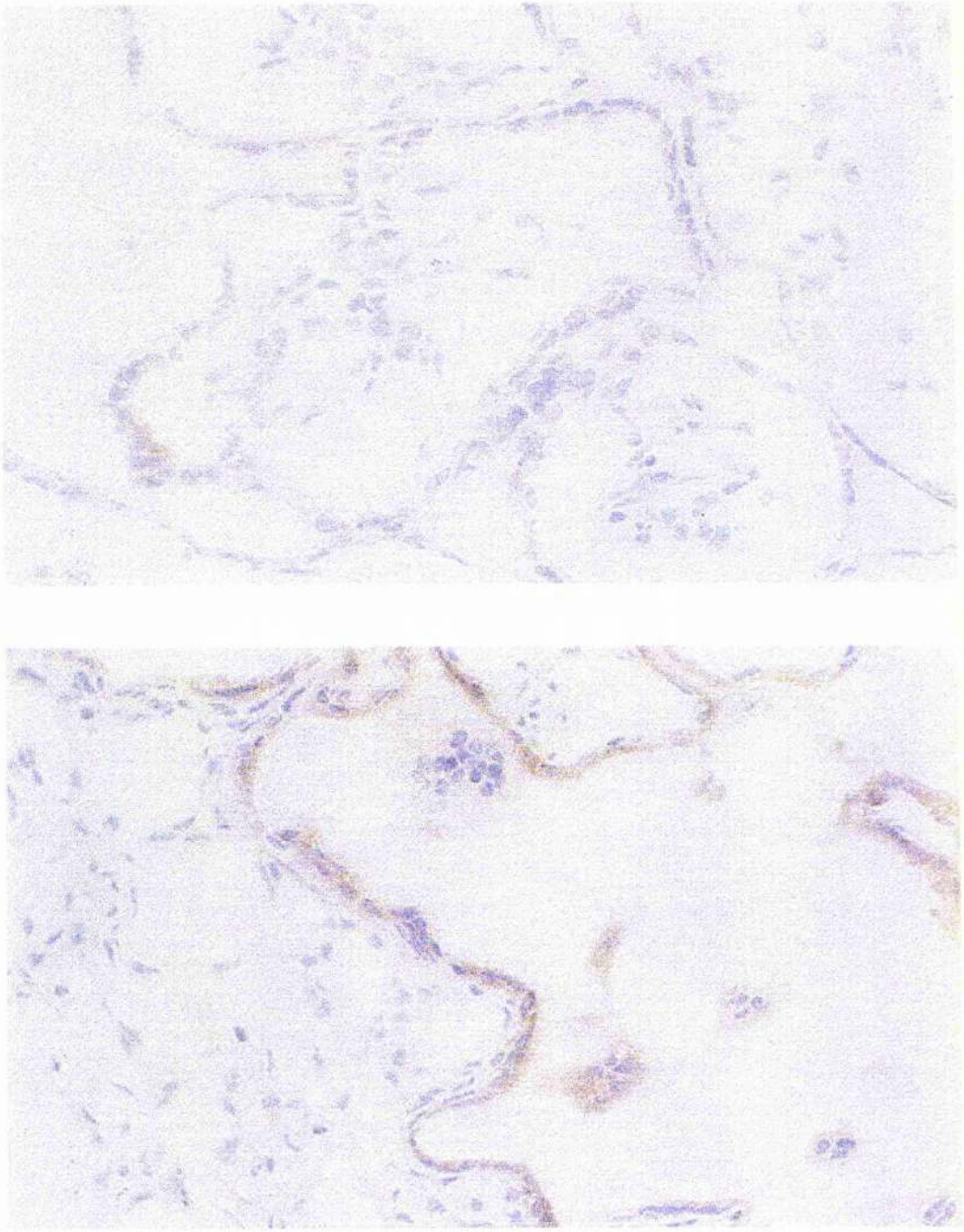

Figure 3.6.1. Immunohistochemical localisation of AFP in placental tissue from unaffected control (top) and Down's syndrome (bottom) pregnancies at 19 weeks gestation.

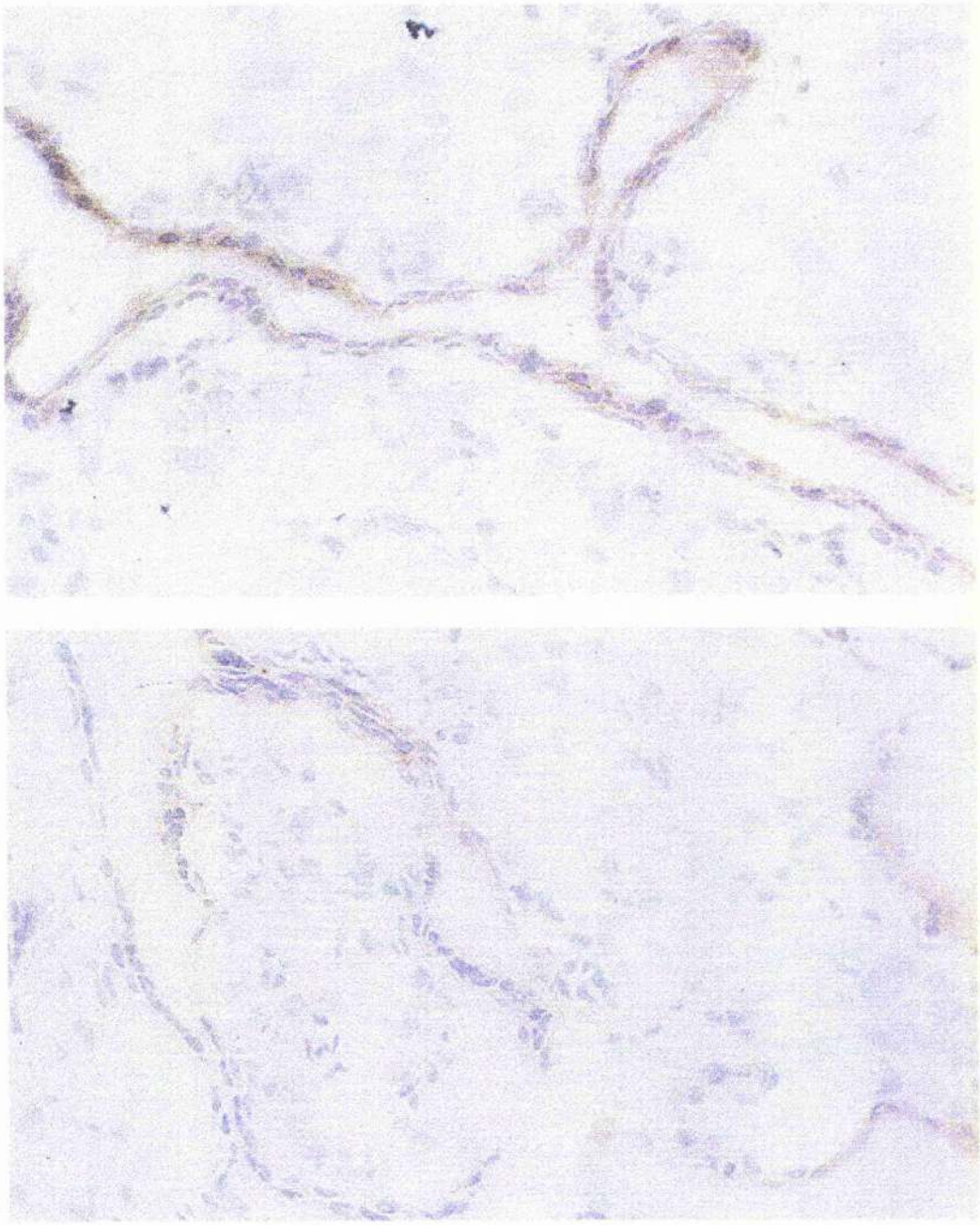

Figure 3.6.2. Immunohistochemical localisation of hCG in placental tissue from unaffected control (top) and Down's syndrome (bottom) pregnancies at 17 weeks gestation.

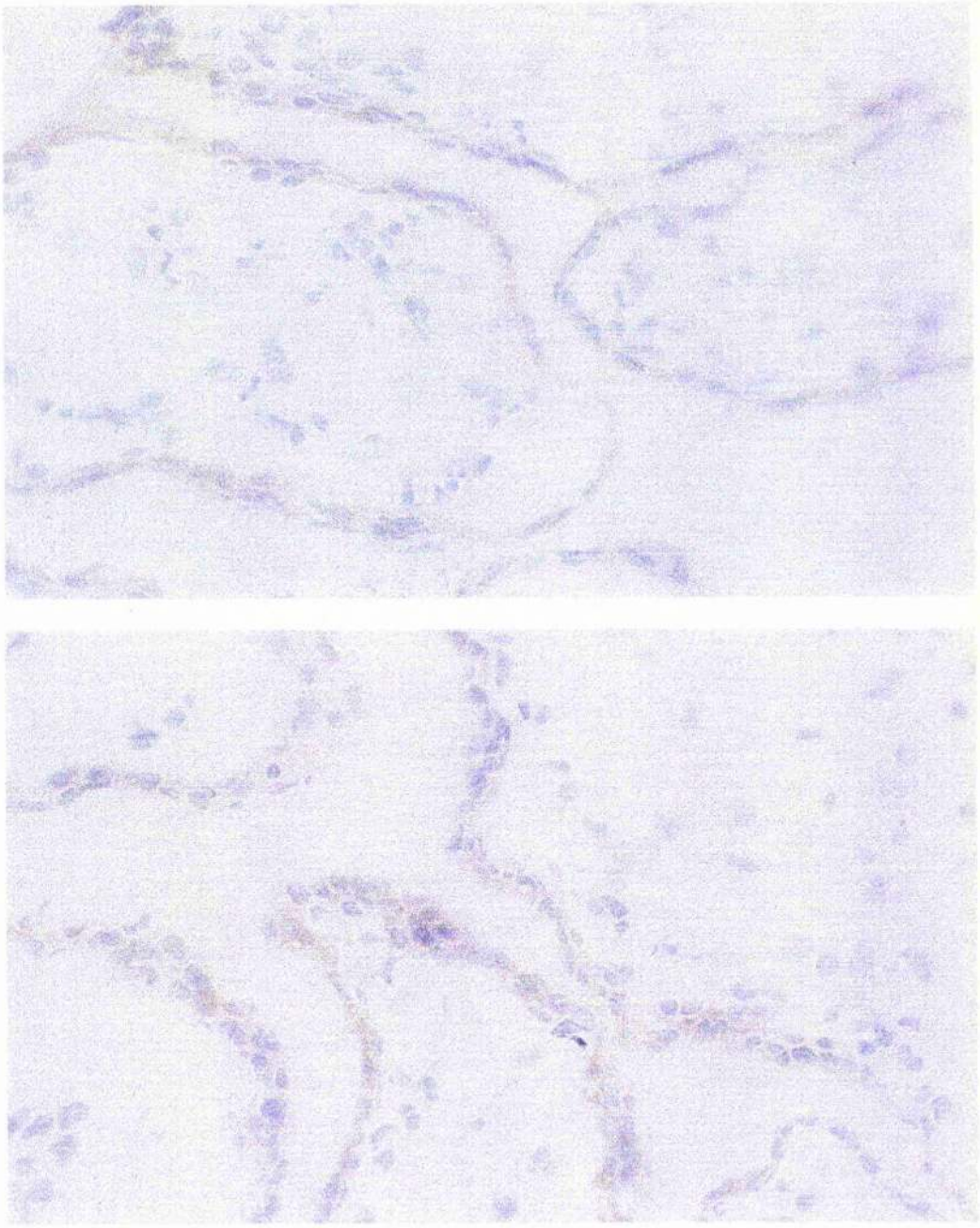

Figure 3.6.3. Immunohistochemical localisation of PAPP-A in placental tissue from unaffected control (top) and Down's syndrome (bottom) pregnancies at 14 weeks gestation.

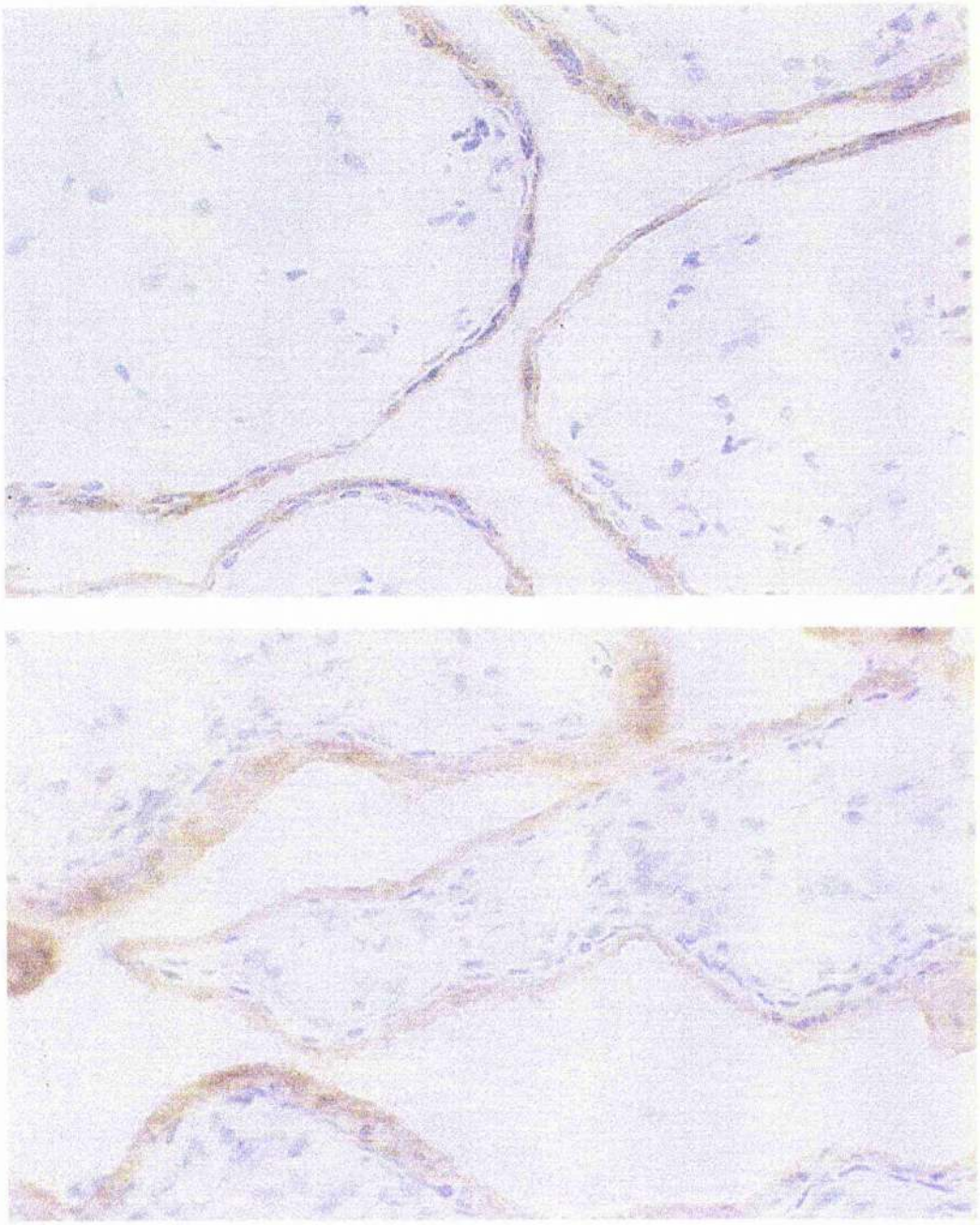

Figure 3.6.4. Immunohistochemical localisation of SP1 in placental tissue from unaffected control (top) and Down's syndrome (bottom) pregnancies at 14 weeks gestation.

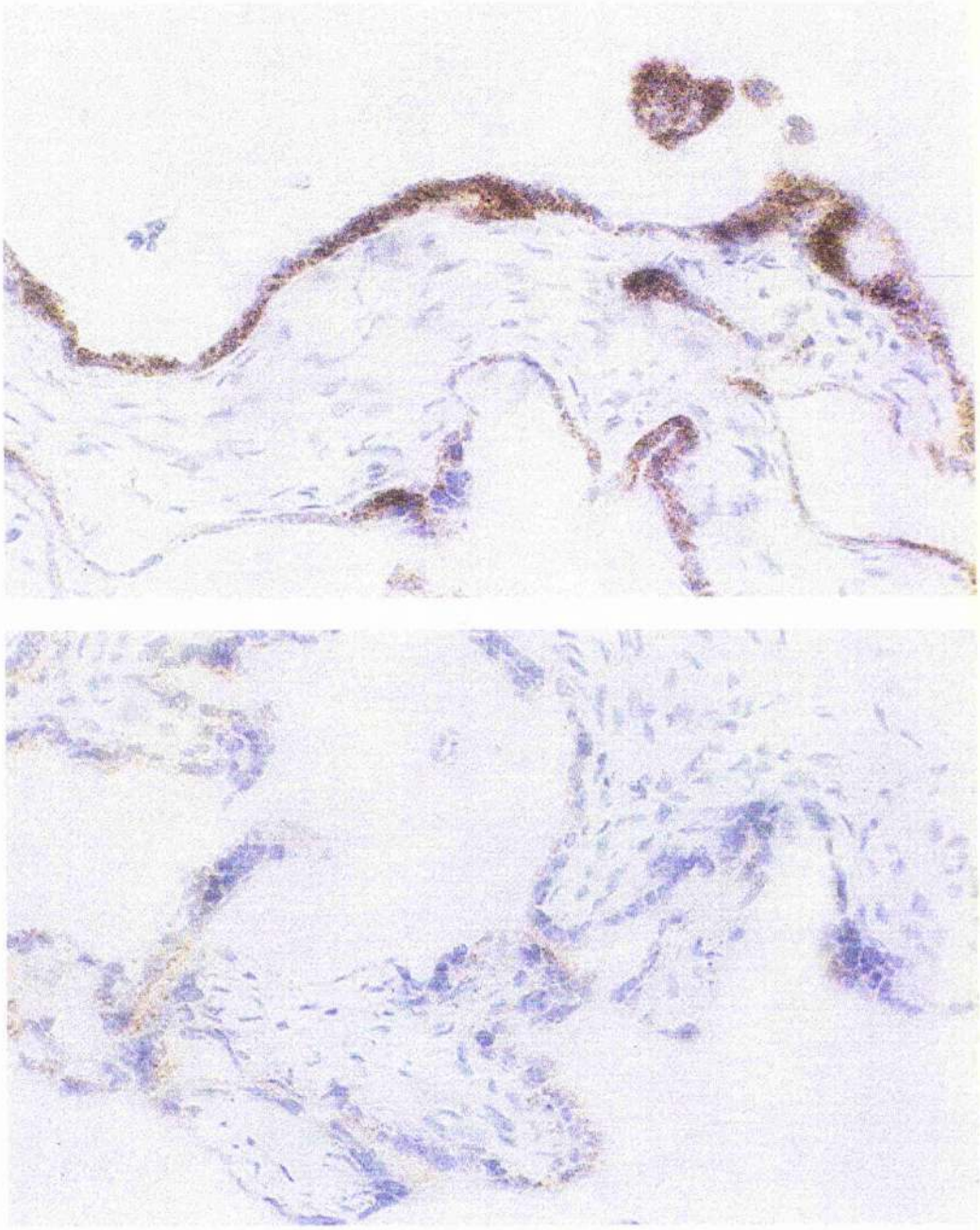

Figure 3.6.5 Immunohistochemical localisation of placental ALP in placental tissue from unaffected control (top) and Down's syndrome (bottom) pregnancies at 16 weeks gestation.

the Sign test for each of the markers are presented in Table 3.6.1. No significant difference was found in the staining intensities for any of the markers in unaffected control and Down's syndrome placental sections.

3.7 SUMMARY OF POST MORTEM REPORTS.

3.7.1 Down's Syndrome.

Post mortem reports were available for 55 of the 67 Down's syndrome fetuses from which fetal tissues were collected. The majority of the fetuses exhibited external pathology characteristic of Down's syndrome (e.g rounded head, protruding tongue, flat features, broad nasal bridge, small low-set ears). Nuchal thickening was observed in 36 of the Down's syndrome fetuses and a cystic hygroma was observed on one case. Internal developmental abnormalities were found in 25 of the Down's syndrome fetuses and included 22 cases with heart defects, 6 cases with Meckel's diverticulum, one with duodenal stenosis/atresia, one with lung abnormalities and one hydropic fetus. Placental pathology was recorded for 31 of the Down's syndrome cases and included varying degrees of fibrinoid degeneration, villous oedema, calcification and villous haemorrhage.

3.7.2 Other Abnormalities.

Post mortem reports were available for 2 of the Trisomy 18 fetuses. Both cases had developmental heart defects and widespread chorionic haemorrhage was evident on examination of the placenta.

Table 3.6.1 The number of positive and negative difference in the staining intensities of AFP, hCG, PAPP-A, SP-1 and placental ALP in placental sections from Down's syndrome and unaffected pregnancies, and the results of the significance test (Sign test).

Marker	No. of +ve differences	No. of -ve differences	Ties	p-value
AFP	6	4	6	0.754
hCG	6	2	8	0.289
PAPP-A	7	2	7	0.179
SP-1	3	8	5	0.227
PALP	5	7	4	0.774

CHAPTER 4.

DISCUSSION.

4.1 MARKER ANALYSIS IN FETAL TISSUES.

4.1.1 THE USE OF REGRESSION ANALYSIS TO ESTIMATE WEEKLY MEDIANS.

During pregnancy, the maternal serum concentration of most feto-placental markers changes with advancing gestation. Conversion of marker levels into MOM eliminates the gestational effect and facilitates comparison of marker levels between pregnancies. To convert marker levels in individual pregnancies to MOM, median marker levels in unaffected pregnancies must be estimated, usually by analysis of several hundred samples distributed across appropriate gestations. In the absence of sufficient samples at each gestation, reasonable estimates of gestational medians can be obtained by regression. The aim of the present study was to compare the levels of certain biochemical markers in fetal tissues from Down's syndrome and apparently unaffected control pregnancies. Fetal tissues were obtained from Down's syndrome pregnancies following therapeutic termination at 10 to 24 weeks gestation, the majority of cases being of 20 weeks gestation and over. Control fetal tissues were obtained from 75 putatively normal pregnancies terminated for psycho-social reasons at 12 to 22 weeks gestation. Because of the nature of the control samples, most were terminated before 20 weeks gestation and few tissues were available at later gestations. An alternative source of fetal material from spontaneous abortions was not used in this study due to the possibility of pathophysiological differences in fetal tissues between spontaneous and induced abortion. In the present study all fetal tissues were obtained following induction of labour by administration of Gemeprost (Cervigem) vaginal pessaries either alone or in combination with Mifepristone (RU 486). Chard et al (1990) reported an increase in maternal serum AFP levels following administration of Mifepristone for termination of pregnancy in the first trimester. RU

486 has also been shown to decrease hCG synthesis and secretion by placental cultures (Das and Catt 1987), although Olajide et al (1989) found that circulating levels if hCG *in vivo* were unchanged until after abortion was complete. Furthermore, spontaneous abortion itself may be associated with pathological changes in the levels of biochemical markers.

In view of the small number of control samples available at each gestation and the incomplete overlap of the gestational range of the affected and unaffected pregnancies, regression analysis was used to estimate weekly medians. Median values obtained by regression analysis will be most accurate within the gestational range of the pregnancies from which the regression equations are derived. However, in this study a significant proportion of the Down's syndrome tissue samples were obtained at gestations beyond the upper limit of the gestational range of the controls. Marker medians for gestational weeks outside the control range were therefore obtained by extrapolation of the regression curve. The validity of this approach was suggested from two pieces of evidence: (1) it was observed that the trends in the regression curves in placental tissues for the markers intact hCG, FβhCG, PAPP-A and placental ALP were similar to the changes in median levels of these markers in maternal serum and (2) from analysis of the tissue data using only the subset of cases (placenta - n = 34, liver - n = 51, ileum - n = 45) which were within the gestational range of the controls. The results of this analysis are presented in Table 4.1.1. Although there are some differences in the median MOM levels of the markers in the subset of Down's syndrome pregnancies compared to the entire series, the trends in marker variation are identical to those described in Section 3.1.11. One exception is that the increase in placental levels of GGT in Down's syndrome pregnancies is not statistically significant based on the analysis of only the Down's syndrome cases within the gestational range of the controls.

Table 4.1.1 Analysis of marker levels in fetal tissues using the subset of Down's syndrome cases which were within the gestational range of the controls.

Tissue	Marker	Controls		Down's syndrome		Mann-Whitney p-value
		No. of cases	Median MOM	No. of cases	Median MOM	
Placenta	Intact hCG	52	1.19	34	3.98	<0.001
	FβhCG	52	1.05	34	3.32	<0.001
	PAPP-A	52	0.84	34	1.16	0.069
	SP1	52	0.91	34	1.50	<0.001
	Placental ALP	52	1.10	34	1.13	0.474
	AFP	52	0.99	34	2.40	<0.001
	GGT	52	0.93	34	1.16	0.118
Liver	AFP	68	1.01	51	0.92	0.155
	GGT	65	0.90	50	2.03	<0.001
Ileum	GGT	63	1.02	45	0.48	0.001

By expressing marker levels in Down's syndrome pregnancies as an overall median MOM it is being assumed that the change in marker MOM in Down's syndrome pregnancies is of a constant magnitude at all gestations. However, this may not be the case as some studies have shown that MOM levels of certain markers in maternal serum (e.g. inhibin, PAPP-A, SP1) change with gestation in Down's syndrome pregnancies (Aitken et al 1996b, Qin et al 1996, Berry et al 1997, Qin et al 1997). The change with gestation of marker levels in fetal tissues from the Down's syndrome pregnancies was not investigated in this study due to insufficient numbers of cases, particularly at the earlier and later gestations.

4.2 ALPHAFETOPROTEIN.

4.2.1 AFP IN NORMAL PREGNANCY.

During pregnancy AFP is synthesised primarily by the yolk sac and by the fetal liver (Gitlin et al 1972). As pregnancy advances the fetal liver grows and becomes the principle source of AFP. In the fetal circulation, AFP levels increase to a peak of approximately 3mg/ml at 10 to 13 weeks gestation, after which levels decrease until term (Gitlin and Boesman 1966). The increase in the level of AFP in the fetal circulation is probably due to an increase in the synthesis of AFP by the fetal liver (Gitlin 1975). The yolk sac begins to decrease in size towards the end of the first trimester and has usually disappeared by 20 weeks gestation (Jauniaux and Moscoso 1992). Wathen et al (1992) found no correlation between yolk sac size and AFP concentration in maternal serum, amniotic fluid or extra-embryonic coelomic fluid (EECF) at 8 to 12 weeks gestation. AFP synthesis by the yolk sac has decreased substantially by approximately 9 to 11 weeks gestation (Gitlin et al 1972, Gitlin 1975) so that by the second trimester of pregnancy, AFP in the feto-maternal compartment is

almost exclusively of fetal hepatic origin. Gitlin and Boesman (1966) estimated the relative amount of AFP produced by the fetus at different stages of development by multiplying the plasma concentration of AFP by the weight of the fetus at a given gestation. They reported that while the fetal plasma concentration of AFP decreased from 14 to 20 weeks gestation, the relative amount of AFP synthesised by the fetus increased rapidly during this period and then remained constant until 30 to 32 weeks of gestation. Thus it was proposed that the decrease in the plasma concentration of AFP from 14 to 32 weeks was due to a greater increase in the weight of the fetus compared to the relative increase in the total AFP produced by the fetus.

In the present study, AFP was measured in extracts of fetal liver from 68 control pregnancies at 12 to 22 weeks gestation. Fetal hepatic AFP levels were found to decrease with advancing gestation. This is consistent with the results of Kronquist et al (1990a) who also found a reduction in hepatic AFP levels with advancing gestation. Brizot et al (1996b) reported that in normal fetuses at 12 to 15 weeks gestation the highest level of hepatic AFP mRNA expression was at 13 weeks, corresponding to the peak in fetal serum AFP levels at 13 weeks of gestation. These results suggest that the decrease in the concentration of AFP after 13 weeks of gestation is due to a decrease in synthesis of AFP by the fetal liver.

Amniotic fluid AFP is derived from the fetal circulation through the unkeratinised skin of the fetus in early pregnancy and from fetal urine once the fetal kidney starts to function around the end of the first trimester (Gitlin and Boesman 1966, Seppala and Rouslahti 1972, Aitken and Crossley 1995). In the present study, the concentration of AFP in the amniotic fluid was found to decrease from 15 weeks to 21 weeks of gestation. This is consistent with previous studies which show that the concentration of AFP in the amniotic fluid increases from 11 weeks gestation to a peak at 13 weeks gestation, followed by a gradual decrease to term (Seppala and Rouslahti 1972,

Crandall et al 1989, Palomaki et al 1993). Wathen et al (1991, 1993) found that high levels of AFP in amniotic fluid at 8 weeks gestation decreased rapidly until 11 weeks followed by a increase to a peak at 13 weeks and subsequent decline thereafter. The authors propose that high amniotic fluid AFP levels in early pregnancy may be due to excessive leakage of AFP into the amniotic fluid and reduced clearance by fetal swallowing prior to the development of the fetal renal and digestive systems. The concentration of the Con-A non-reactive form of AFP (yolk sac) decreases with advancing gestation corresponding to the decline of the yolk sac towards the end of the first trimester (Roushlati et al 1979, Toftager-Larsen et al 1980, Machiewicz et al 1984, Jauniaux and Moscoso 1992).

In the maternal circulation AFP levels increase to a peak of approximately 450ng/l at 32 weeks of gestation and then decline to term (Gitlin 1975, Rouslahti and Hirai 1978). Using data from the present study it is estimated that the concentration of AFP in the maternal circulation at 16 weeks gestation is approximately 0.3% of that in the amniotic fluid. Thus, a concentration gradient exists between AFP levels in the amniotic fluid and the maternal circulation. However, there is little correlation between amniotic fluid and maternal serum AFP levels in the second trimester and it is thought that only a small percentage of AFP in the maternal circulation is derived from the amniotic fluid across the fetal membranes (Gitlin 1975, UK Collaborative Study II 1979, Wald and Cuckle 1980, Los et al 1985). In the first trimester, before the fusion of the decidua capsularis and the decidua parietalis at 12 to 16 weeks gestation, AFP transfer across the fetal membranes is limited. Maternal serum AFP is derived primarily from the fetal circulation by means of diffusion through the placenta (Gitlin 1975, Los et al 1985, Brumfield et al 1990). The concentration of AFP in the maternal circulation at any stage of gestation is related to the concentration of AFP in the fetal circulation and the size of the placenta (Gitlin 1975, Rouslahti and Hirai 1978). In the present study, it was found that AFP levels in homogenates of placental tissue from 52

unaffected control pregnancies decreased from 12 to 20 weeks gestation. This corresponds to the decrease in AFP levels in the fetal circulation during this period. The concentration of AFP in placental homogenates was approximately 10% of that in the fetal liver and is presumed to be endogenous as the result of transfer from the fetal serum.

The mechanisms involved in the transfer of AFP from the fetal circulation to the maternal circulation are not known. There are regions on the surface of placental villi where the syncytiotrophoblast layer is very thin and is in direct contact with the underlying fetal capillaries. It is at these regions where feto-maternal transport presumably occurs (Chard 1986, Jauniaux et al 1992). In the present study, AFP was found to be localised to the syncytiotrophoblast layer of placental villi. No specific staining for AFP was found in the villous core. It is possible that AFP is transferred from the fetal circulation into the maternal circulation across the syncytiotrophoblast layer at sites where the syncytiotrophoblast is in direct contact with the fetal capillaries.

Brownbill et al (1995) investigated the mechanisms of AFP transport in perfused term placental cotyledons. Immunohistochemical analysis showed that AFP was localised to cytotrophoblast cells associated with fibrinoid deposits at regions where there was a break in the syncytiotrophoblast and the authors proposed that AFP transport from the fetal circulation to the maternal circulation occurs at these sites. AFP was also found to be present in the syncytiotrophoblast and in trophoblast cells in the decidua. A second route of AFP transport was proposed by which AFP in the decidua enters the maternal circulation via vessels which cross the basal plate.

Brownbill et al (1995) also reported that in perfused placental cotyledons the rate of transfer of AFP from the fetal circulation to the maternal circulation was faster than from the maternal circulation to the fetal circulation. This suggests that the mechanism

of AFP transfer across the placenta is more complex than passive diffusion. Cellular uptake of AFP by various tumour and embryonic tissues occurs via specific AFP cell-surface receptors (Moro et al 1993, Mizejewski 1995). Thus, if AFP receptors are present in the placenta, cellular uptake and transport of AFP may occur via a receptor-mediated mechanism.

The presence of AFP in the syncytiotrophoblast and cytotrophoblast of the placenta and in trophoblast cells in the decidua may simply reflect uptake by these cells but may also be an indication of AFP synthesis. Trace amounts of AFP have been detected in culture media from placental cultures (Van Firth and Adinolf 1969, Gitlin et al 1972, Hustin et al 1980). However, this was not common to all cultures studied and may reflect assay non-specificity or release of endogenous AFP from the cultures rather than synthesis of AFP. Preliminary results of a study on placental AFP mRNA levels suggest that AFP gene expression in the placenta is of little functional significance (Kronquist et al 1990b).

4.2.2 AFP IN DOWN'S SYNDROME PREGNANCIES.

Numerous studies have shown that second trimester maternal serum AFP levels are significantly lower in Down's syndrome pregnancies than in unaffected pregnancies (Table 4.2.1). Maternal serum AFP levels are also reduced in the first trimester of Down's syndrome pregnancies (Table 4.2.2). In the present study, maternal serum samples were available from 62 of the Down's syndrome pregnancies from which fetal tissues were obtained and serum AFP levels were reduced to a median of 0.69 MOM.

In this study, it was also found that AFP levels were reduced to 0.87 MOM in amniotic fluid obtained from 34 of the Down's syndrome pregnancies prior to termination. This is consistent with, although less markedly reduced than the levels reported in previous

Table 4.2.1 Summary of 33 published studies on maternal serum levels of AFP in the second trimester of Down's syndrome pregnancies.

Reference	No. of cases	Gestation	AFP median MOM
Seller (1984)	8	15-20	0.80
Guibald et al (1984)	13	14-20	0.76
Fuhrmann et al (1984)	43	16-20	0.80
Cowchock & Ruch (1984)	40	14-20	1.00
Hershey et al (1985)	28	16-24	0.87
Spencer & Carpenter (1985)	27	16-20	0.82
Murday & Slack (1985)	45	15-19	0.63
Voigtlander & Vogel (1985)	29	NS	0.78
Doran et al (1986)	46	15-19	0.79
Ashwood et al (1987)	26	14-20	0.69
Cuckle et al (1987)	68[a]	14-20	0.72
Tabor et al (1987)	86[b]	14-20	0.64
Di Maio et al (1987)	18	14-22	0.76
Del Junco et al (1989)	22	NS	0.64
Osanthanondh et al (1989)	26	16-22	0.84
Heyl et al (1990)	16	15-19	0.82
Kronquist et al (1990a)	18	18-22	0.72
Norgaard-Pedersen et al (1990)	42	14-18	0.70
Suchy & Yeager (1990)	16	15-22	0.70
Waller et al (1990)	113	15-20	0.77
MacDonald et al (1991)	54	14-21	0.90
Mancini et al (1991)	9	15-18	0.62
Miller et al (1991)	8	14-20	0.75
Spencer (1991a)	29	16-18	0.73
Zeitune et al (1991)	114	16-20	0.72
Haddow et al (1992)	20	NS	0.77
Phillips et al (1992)	7	15-19	0.89
Ryall et al (1992)	57	15-21	0.74
Spencer and Macri (1992)	23	16-17	0.76
Spencer et al (1992a)	90	14-21	0.72
Stone et al (1993)	21	15-17	0.68
Norgaard-Pedersen et al (1994)	72	14-18	0.70
Berry et al (1997)	47	15-21	0.72
Total	1281		0.75*

* weighted geometric mean.

NS not stated.

[a] includes cases previously reported by Cuckle et al (1984).

[b] includes cases previously reported by Tabor et al (1984).

Table 4.2.2 Summary of 15 published studies on maternal serum levels of AFP in Down's syndrome pregnancies in the first trimester.

Reference	No. of cases	Gestation	AFP median MOM
Scioscia et al (1987)	8	6-14	1.06
Cuckle et al (1988)	22[a]	7-12	0.72
Brock et al (1990)	21	7-14	0.71
Crandall et al (1991)	10	9-12	0.59
Johnson et al (1991)	11	8-12	0.70
Aitken et al (1993a)	16	6-14	0.65
Crandall et al (1993)	11	11-15	0.74
Fuhrmann et al (1993)	19[b]	8-12	0.97
Kellner et al (1994)	5	5-14	0.90
Biagiotti et al (1995)	41	8-12	0.74
Brizot et al (1995c)	35	10-13	0.84
Casals et al (1996)	19	10-13	0.78
Wald et al (1996b)	77	8-14	0.87
Zimmerman et al (1996)	4	10-13	0.62
Berry et al (1997)	53	7-14	0.80
Total	352		0.80*

* weighted geometric mean.

[a] includes cases reported by Brambati et al (1986), Barkai et al (1987), Milunsky et al (1988) and Neibolo et al (1990).

[b] includes cases previously reported by Wenger et al (1990) and some cases included Cuckle et al (1984).

studies (Table 4.2.3). There was no correlation between the levels of AFP in corresponding amniotic fluid and maternal serum samples from the Down's syndrome pregnancies.

Although the association between low AFP levels and Down's syndrome is well established, the mechanisms behind this reduction are not known. It was proposed initially that immaturity of the Down's syndrome fetus was the reason behind reduced maternal serum AFP levels in the second trimester of Down's syndrome pregnancies, with AFP levels being characteristic of those at an earlier gestation (Canick et al 1988, Wald et al 1988). Waller et al (1992) investigated the association between reduced maternal serum AFP levels and fetal growth retardation in 54 pregnancies which resulted in the birth of a Down's syndrome child. There was no association between maternal serum AFP levels and birth weight. In a study of the prenatal growth of 563 Down's syndrome fetuses at 29 weeks gestation to term, Kucera (1971) found no evidence of abnormal growth of the Down's syndrome fetuses compared to normal between 29 weeks gestation and term. Librach et al (1988) reported that there was no significant difference in the weights of Down's syndrome fetuses (n=50) compared with the weights of unaffected control fetuses during the second trimester. Thus, there is no evidence to support slow fetal growth as the underlying cause of reduced AFP levels in Down's syndrome pregnancies.

Several authors have proposed that low maternal serum and amniotic fluid levels in Down's syndrome pregnancies may be caused by reduced synthesis of AFP by the fetal liver (Cuckle et al 1984, Davis et al 1985, Cuckle and Wald 1986, Crandall et al 1988). Cuckle and Wald (1986) investigated this possibility by measuring AFP levels in cord blood from 22 Down's syndrome babies and from 66 unaffected control babies. AFP levels in cord blood from the Down's syndrome babies were significantly lower (0.45 MOM) than in cord blood from the unaffected babies. These results provide

Table 4.2.3 Summary of 19 published studies on amniotic fluid levels of AFP in Down's syndrome pregnancies.

Reference	No. of cases	Gestation	AFP median
Cowchock and Ruch (1984)	52	14-20	0.80
Tabor et al (1984)	25	NS	0.64
Baumgarten et al (1985)	27	NS	0.71
Cuckle et al (1985)	48	13-20	0.64
Davis et al (1985)	18	15-18	0.70[a]
Hershey et al (1985)	28	16-24	0.72
Hullin et al (1985)	37	15-21	0.58
Voitlander & Vogel (1985)	44	16-21	0.78
Doran et al (1986)	46	15-19	0.62[a]
Jones et al (1986)	78	15-20	0.73
Ashwood et al (1987)	6	14-20	0.71
Crandall et al (1988)	111	15-24	0.73
Jones et al (1988)	15	15-17	0.76[a]
Kaffe et al (1988)	90	15-21	0.72
Giddy et al (1989)	26	16	0.74
Zeitune et al (1989)	52	16-22	0.60
Kronquist et al (1990a)	12	17-22	0.72
Spencer et al (1993a)	14	16-20	0.77
Spencer et al (1997a)	91	17-22	0.72
Total	890		0.67*

* weighted geometric mean.

NS not stated.

[a] mean MOM.

indirect support for the theory that AFP synthesis by the fetal liver is reduced in Down's syndrome at term. However, Scioscia et al (1988) reported that AFP levels in fetal serum from 11 Down's syndrome fetuses at 17 to 25 weeks gestation were not significantly different from normal. Likewise, Nicolini et al (1988) found that the median multiple of the mean level of AFP in fetal serum from 6 Down's syndrome fetuses was 1.08. Seller (1990) measured fetal serum AFP levels in 21 fetuses with Down's syndrome at 19 to 27 weeks gestation. Fetal serum levels were not significantly different from normal at 19 and 20 weeks of gestation, but after 20 weeks, serum AFP levels in the Down's syndrome fetuses declined more rapidly than in the normal fetuses. Scioscia et al (1988) also found that the concentration of AFP in fetal serum from four Down's syndrome fetuses at 21 weeks of gestation and over were below normal median levels. These results and the findings of Cuckle and Wald (1986) suggest that Down's syndrome fetuses may synthesise lower amounts of AFP than unaffected fetuses during the second half of pregnancy. However, normal levels of AFP in fetal serum prior to 21 weeks gestation are not consistent with the theory that reduced synthesis of AFP is the cause of low maternal serum and amniotic fluid AFP levels in Down's syndrome pregnancies in the second trimester.

Jones et al (1988) reported that despite an overall reduction in the levels of AFP in amniotic fluid from 15 Down's syndrome pregnancies, there was no difference in the percentage contribution of Con-A non-reactive AFP (yolk sac origin) to total AFP levels between the Down's syndrome and unaffected pregnancies. This suggests that AFP derived from the fetal liver and the yolk sac are affected in a similar manner in Down's syndrome pregnancies. Los et al (1995) measured AFP levels in homogenised fetal material (fetal limbs, trunk, thorax and skull) from six first trimester Down's syndrome fetuses and from 12 fetuses with Mendalian disorders but with normal karyotypes. AFP levels were significantly lower in the Down's syndrome fetuses compared to the fetuses with the normal karyotypes but there was no difference in the percentage of

Con-A non-reactive AFP between the two groups. The authors propose that AFP synthesis by the fetal liver and yolk sac is reduced in Down's syndrome pregnancies. However, only a small number of cases was included in this study and the experimental design was far from ideal.

Kronquist et al (1990a) measured AFP levels in homogenates of fetal liver from 28 Down's syndrome fetuses at 17 to 22 weeks gestation. AFP levels were significantly reduced in fetal liver homogenates from the Down's syndrome pregnancies despite apparently normal levels of total protein and total mRNA production. The reduction of AFP levels was most evident at 17 to 19 weeks of gestation. Kronquist et al (1994) later confirmed these findings in a larger series of 50 Down's syndrome cases, including some at 12 to 15 weeks gestation. The reduction in AFP levels was found to be significant only at 17 to 19 weeks gestation. In the present study, AFP was measured in homogenates of fetal liver from 54 Down's syndrome fetuses and 68 unaffected control fetuses at 12 to 24 weeks gestation. AFP levels in homogenates of fetal liver from the Down's syndrome fetuses (0.97 MOM) were not significantly different from those of the unaffected control fetuses (1.01 MOM). There was no correlation between fetal hepatic levels of AFP and AFP levels in the maternal serum or amniotic fluid. In a recent study, Brizot et al (1996b) examined fetal hepatic AFP mRNA levels in 13 Down's syndrome and 24 unaffected control pregnancies at 12 to 15 weeks gestation. Hepatic AFP mRNA levels in the Down's syndrome pregnancies were not significantly different from normal and the authors proposed that reduced AFP levels in Down's syndrome pregnancies may be caused by post-transcriptional alterations such as increased degradation of AFP mRNA, modified stability of AFP or increased degradation of AFP in the maternal circulation or amniotic fluid. However, Kronquist et al (1990a) found no evidence of alteration in AFP mRNA processing or in the post-translational modification of the AFP polypeptide in Down's syndrome pregnancies.

Thus, considering the results of the present study and of published studies of AFP levels in fetal serum (Scioscia et al 1988, Nicolini et al 1988, Seller 1990) and in fetal liver (Kronquist et al 1990a, Brizot et al 1996b) from Down's syndrome fetuses, it seems that reduced synthesis of AFP by the fetal liver is not the reason for low maternal serum and amniotic fluid AFP levels in Down's syndrome pregnancies in the second trimester.

As noted above (Section 4.2.1), the principle source of AFP in second trimester amniotic fluid is fetal urine, while maternal serum AFP is derived primarily directly from the fetal circulation across the placenta and indirectly from the amniotic fluid across the maternal decidua. In the absence of reduced hepatic synthesis in Down's syndrome fetuses, the reduction in maternal serum and amniotic fluid AFP levels in Down's syndrome pregnancies may be caused by disruption of these routes of transfer. It has been proposed that reduced AFP levels in Down's syndrome pregnancies are caused by impaired or delayed fetal kidney function and/ or impaired membrane or placental passage of AFP (Scioscia et al 1988, Seller 1990, van Lith et al 1991a). In the present study, placental AFP levels were significantly higher (2.43 MOM) in the Down's syndrome pregnancies than in the unaffected pregnancies. The median maternal serum level of AFP from 62 of the Down's syndrome pregnancies included in this study was 0.69 MOM. There was no correlation between the levels of AFP in placental tissue and in corresponding maternal serum from the Down's syndrome pregnancies. Immunohistochemical studies demonstrated that AFP was localised to the syncytiotrophoblast in placental tissue from both the Down's syndrome and unaffected control pregnancies. In normal pregnancy, the concentration gradient of AFP from high levels in fetal blood to low levels in maternal blood promotes transfer of AFP from the fetal circulation to the maternal circulation. The endogenous level of AFP in the placenta is presumably a balance between the rate of uptake of AFP by the syncytiotrophoblast from the fetal circulation and the rate of its secretion into the

maternal circulation. Any reduction in the rate of release of AFP at the feto-maternal interface will result in the accumulation of AFP in the syncytiotrophoblast. It is possible that in Down's syndrome pregnancies AFP is synthesised in normal amounts, at least during the first half of pregnancy, but that there is a defect in the placental transport of AFP resulting in elevated placental and reduced maternal serum levels of AFP.

The concentration of AFP in amniotic fluid from the Down's syndrome pregnancies at 15 to 21 weeks gestation was reduced to 0.87 MOM, despite apparently normal hepatic AFP levels. This may indicate that the excretion of AFP by the fetal kidneys is affected in Down's syndrome pregnancies. There was no evidence of renal abnormalities in any of the Down's syndrome cases for which port-mortem reports were available. Fetal urine does not become the major source of amniotic fluid until around 16-20 weeks gestation (Whittle 1995). If impaired fetal kidney function is the cause of reduced amniotic fluid AFP in the second trimester, it would be expected that AFP levels would be normal in the first trimester as AFP reaches the amniotic fluid by a different route, for example by exudation from fetal capillaries through unkeratinised fetal skin. However, due to the difficulty of obtaining amniotic fluid samples at these early gestations there are no published data on amniotic fluid AFP levels in the first trimester of Down's syndrome pregnancies.

4.2.3 AFP IN TRISOMY 13 AND TRISOMY 18 PREGNANCIES.

AFP levels in fetal liver, placenta, maternal serum and amniotic fluid from one Trisomy 13 and four Trisomy 18 pregnancies included in this study are summarised in Table 4.2.4.

Table 4.2.4 AFP levels (MOM) in fetal liver, placental tissue, maternal serum and amniotic fluid from Trisomy 13 and Trisomy 18 pregnancies.

Case No.	AFP MOM (gestation)			
	Fetal liver	Placenta	Maternal serum	Amniotic fluid
T13/1	1.24 (23)	3.25 (23)	-	-
T18/1	1.88 (23)	1.18 (23)	-	-
T18/2	1.22 (22)	3.18 (22)	-	-
T18/3	1.83 (21)	2.01 (21)	0.55 (16)	-
T18/4	0.92 (19)	-	0.34 (15)	1.07 (17)

A summary of published studies on maternal serum levels of AFP in Trisomy 18 pregnancies in the first and second trimesters is given in Table 4.2.5. A meta-analysis of the results shown in Table 4.2.5, weighted for the number of Trisomy 18 cases in each study, reveals an overall maternal serum AFP level of 0.90 MOM in the first trimester (total of 40 cases) and 0.64 MOM in the second trimester (total of 296 cases). In the present study, second trimester maternal serum was available from two Trisomy 18 pregnancies. Serum AFP levels were substantially reduced in both cases, consistent with previous studies. Median maternal serum AFP levels of 1.19 MOM (n=5), 0.5 MOM (n=5) and 0.74 MOM (n=9) have been reported in Trisomy 13 pregnancies in the second trimester (Doran et al 1986, Johnson et al 1991, Zeitune et al 1991).

Amniotic fluid from one Trisomy 18 pregnancy was found to have an AFP level of 1.07 MOM. This is comparable with published studies which show that amniotic fluid AFP levels appear to be normal in association with Trisomy 13 and Trisomy 18 (Davis et al 1985, Doran et al 1986, Ashwood et al 1987, Lindenbaum et al 1987, Kaffe et al 1988, Crandall et al 1988, Zeitune et al 1989).

Nicolini et al (1988) reported that the concentration of AFP in fetal serum from six Trisomy 18 pregnancies at 16 to 33 weeks gestation was reduced to 0.78 MOM. Therefore, reduced synthesis of AFP by the fetal liver may be the cause of low maternal serum AFP levels in the second trimester of Trisomy 18 pregnancies. Zeitune et al (1989) proposed that if AFP synthesis was reduced in Trisomy 18 pregnancies, amniotic fluid AFP levels may be restored to normal by the reduced turnover of AFP caused by lack of fetal swallowing. However, in the present study, AFP levels in fetal liver homogenates from the Trisomy 13 pregnancy and three of the four Trisomy 18 pregnancies were higher than in the normal pregnancies. Placental levels of AFP were also elevated in the trisomic pregnancies suggesting that reduced maternal serum

Table 4.2.5 Summary of published studies on maternal serum AFP levels in Trisomy 18 pregnancies in the first and second trimesters.

Reference	No. of cases	1st/2nd trimester	AFP median MOM
Crandall et al (1991)	7	1st	0.99
Johnson et al (1991)	7	1st	1.3
Aitken et al (1993a)	5	1st	0.71
Fuhrman et al (1993)	17	1st	0.80
Van Lith et al (1994)	8	1st	1.26
Zimmermann et al (1996)	3	1st	0.62
Doran et al (1986)	10	2nd	0.64
Ashwood et al (1987)	10	2nd	0.72
Lindenbaum et al (1987)	38	2nd	0.60
Canick et al (1990a)	10	2nd	0.57
Nebiolo et al (1990)	6	2nd	0.73
Norgaard-Pedersen et al (1990)	7	2nd	0.49
Miller et al (1991)	9	2nd	0.49
Zeitune et al (1991)	19	2nd	0.68
Barkai et al (1993)	14	2nd	0.66
Spencer et al (1993b)	52	2nd	0.71
Palomaki et al (1995)	89	2nd	0.65
Aitken et al (1996b)	32[a]	2nd	0.53

[a] includes four cases at 8-12 weeks which were previously reported by Aitken et al (1993a).

AFP levels may be caused by the abnormal placental transport of AFP. Reduced placental size associated with Trisomy 13 and Trisomy 18 pregnancies may also affect the transfer of AFP from the fetal circulation to the maternal circulation.

4.3 HUMAN CHORIONIC GONADOTROPHIN.

4.3.1 HCG LEVELS IN NORMAL PREGNANCIES.

It is generally accepted that the syncytiotrophoblast of the placenta is the principle source of dimeric hCG during pregnancy (Gosseye and Fox 1984, Kurmann et al 1984, Hay et al 1988). The syncytiotrophoblast is in direct contact with the maternal blood. Once formed, dimeric hCG is secreted continuously from the syncytiotrophoblast into the maternal circulation with very little being stored within the cells (Sideri et al 1980, Billingsley and Wooding 1990). The concentration of intact hCG in the maternal circulation at any stage of pregnancy should therefore reflect the rate of synthesis of hCG by the placenta. The concentration of intact hCG in the maternal circulation increases to a peak at 8 to 12 weeks gestation, followed by a decline to a plateau at around 18 weeks after which the concentration remains relatively constant to term (Braunstein et al 1976, Kletzy et al 1985, Ozturk et al 1987).

In the present study, intact hCG levels were measured in homogenates of placental tissue from 52 control pregnancies at 12 to 20 weeks gestation. Median hCG levels decreased with advancing gestation, in parallel with the changing concentration of hCG in the maternal circulation at this stage of pregnancy. This is consistent with the results of Vaitukaitis (1974) who found that hCG levels in placental tissue was highest in the first trimester and decreased thereafter. These results indicate that the changing

concentration of hCG in the maternal circulation with advancing gestation reflects the changing rate of synthesis and/ or secretion of hCG by the placenta.

Intact hCG was also measured in amniotic fluid from 132 control pregnancies at 15 to 21 weeks gestation. Amniotic fluid hCG levels were found to decrease with advancing gestation. This is consistent with previous studies which show that amniotic fluid hCG levels increase to a peak at 11 to 14 weeks gestation, followed by a marked decline to low levels which are maintained to term (Clements et al 1976, Belisle and Tulchinsky 1980, Ozturk et al 1988). It was also found that the concentration of intact hCG in the amniotic fluid was approximately 15% of the maternal serum concentration at 15 to 20 weeks gestation. This is consistent with previous studies which have shown that amniotic fluid hCG levels are less than 20% of maternal serum levels in both the first and second trimesters of pregnancy (Ozturk et al 1988, Iles et al 1992). HCG is present in the fetal circulation at a lower concentration than in amniotic fluid and maternal serum (Crosignani et al 1972, Clements et al 1976). Thus it appears that intact hCG is preferentially secreted into the maternal circulation with a slower transfer into the amniotic fluid through the chorionic and amniotic membranes.

HCG is also present in the maternal serum and amniotic fluid in the form of its free subunits. In the maternal circulation, free αhCG increases from very low levels in early pregnancy to 30-40% of total hCG levels at term (Cole et al 1984, Ozturk et al 1987, Ozturk et al 1988). A small proportion (<5%) of the total hCG concentration in the maternal circulation is accounted for by FβhCG, the concentration of which increases to a peak at around nine weeks gestation and declines thereafter (Cole et al 1984, Ozturk et al 1987, Berry et al 1995). In the present study maternal serum levels of FβhCG were found to decrease with advancing gestation from 15 to 20 weeks. In placental tissue samples obtained from 52 control pregnancies at 10 to 20 weeks

gestation in this study, FβhCG levels were found to decrease with advancing gestation. Thus, maternal serum FβhCG levels appear to reflect the change in placental concentrations of FβhCG at this stage of pregnancy.

In contrast to intact hCG, the levels of FαhCG and FβhCG in the amniotic fluid are greater than in the maternal circulation (Ozturk et al 1988, Iles et al 1992, Spencer et al 1997). In the present study, amniotic fluid levels of FβhCG were up to 10 times higher than in maternal serum at 15 to 20 weeks gestation. Amniotic fluid FβhCG levels were found to decrease with advancing gestation. The highest concentration of FαhCG in the amniotic fluid occurs around 15 to 16 weeks gestation with levels decreasing rapidly to 21 weeks when levels are lower than in maternal serum (Ozturk et al 1988). Amniotic fluid FβhCG levels are lower than FαhCG levels but the concentration profile is similar to that of FαhCG (Ozturk et al 1988).

The mechanisms leading to the varying proportions of hCG and its subunits in maternal serum and amniotic fluid are not yet understood. HCG is present in maternal urine in the form of the intact molecule, free hCG subunits and the β-core fragment. Maternal serum and urine also contain hCG molecules that are 'nicked' between residues 47 and 48 or 45 and 46 on the β-subunit (Cole et al 1993, Cole 1994). The β-core fragment may be of placental origin or may be a degradation product of hCG and FβhCG (Cole and Birkin 1984, Wehmann et al 1984, Wehmann et al 1990). Nicked hCG rapidly dissociates into FαhCG, and nicked FβhCG and Cole et al (1993) proposed a pathway by which there is increased nicking and dissociation of hCG as pregnancy advances, contributing to the decline in maternal serum hCG levels at the end of the first trimester and resulting in increased urinary hCG subunit and β-core levels. The metabolic clearance rate of free hCG subunits from the maternal circulation is more rapid than for intact hCG (Wehman and Nisula 1979). The different

concentrations of hCG and its subunits in maternal serum and amniotic fluid may be a reflection of different rates of dissociation and clearance from the two compartments. However, there is no evidence of increased dissociation of hCG in the amniotic fluid (Ozturk et al 1988, Iles et al 1992). Ozturk et al (1988) proposed that the high concentration of intact hCG in maternal serum compared to amniotic fluid is due to the preferential secretion of intact hCG into the maternal circulation and that lower hCG subunit levels are a result of a low rate of placental secretion and more rapid metabolic clearance of subunits from the circulation.

Another possible explanation is that there are alternative sites of hCG and hCG subunit synthesis. In the first trimester of pregnancy, the concentration of hCG and its subunits is higher in extraembryonic coelomic fluid (EECF) than in maternal serum or amniotic fluid indicating that the extra-placental chorionic trophoblast, as opposed to the placental trophoblast, may be the principle site of hCG synthesis in the first trimester (Iles et al 1992, Chard et al 1995). Furthermore, Chard et al (1995) proposed that the peak in maternal serum hCG concentrations at the end of the first trimester may be due to secretion of hCG by the extra-placental chorion which is reduced following fusion with the amnion.

Immunohistochemical studies have demonstrated the presence of hCG in the epithelial cells of the amniotic membrane from early pregnancy (six weeks) to term (de Ikonicoff and Cedard 1973, Ho et al 1982). Plouzek et al (1993) found that at term, βhCG mRNA is expressed at lower levels in the amnion and chorion than in the placenta, while αhCG mRNA was expressed in the amnion but not in the chorion. HCG synthesis has also been reported in the fetal liver and kidneys (McGregor et al 1981, McGregor et al 1983). Low levels of hCG have been detected in a number of adult

tissues (testis, ovary, liver, kidney, pituitary and lung) and in non-pregnant adult serum (Braunstein et al 1979, Odell and Griffin 1987).

Inhibins (inhibin-A and inhibin-B) are products of the placenta which may have a physiological role in the regulation of hCG synthesis. Inhibin suppresses in-vitro secretion of hCG by trophoblast cell cultures, while hCG itself stimulates inhibin secretion (Petraglia et al 1987, Petraglia et al 1989). The concentration profile of inhibin-A in the maternal circulation in early pregnancy is similar to that of intact hCG. Maternal serum inhibin-A levels increase to a peak at 8-10 weeks gestation, decline to a plateau at around 16 weeks with a subsequent rise in the third trimester so that highest levels are reached at term (Muttukrishna et al 1995, Aitken et al 1996b, Wallace et al 1996a). The trophoblast of the placenta is believed to be the principle source of circulating inhibin-A during pregnancy (Petraglia et al 1987, Wallace and Healy 1996, Petraglia 1997). Inhibin-A is also present in second trimester amniotic fluid and levels increase with advancing gestation (Wallace et al 1996a). In contrast to intact hCG but similar to FαhCG and FβhCG, the concentration of inhibin-A in amniotic fluid is significantly higher than in maternal serum indicating that there may be an alternative source of inhibin-A in amniotic fluid (Wallace et al 1996a). The decidua, fetal tissues, amnion and chorion are potential sites of synthesis of inhibin subunits (Petraglia et al 1990, Petraglia et al 1993, Jaffe et al 1993). Riley et al (1996) reported that in the first trimester, inhibin-A is present in maternal serum and EECF but is absent from amniotic fluid. This suggests that in the first trimester of pregnancy, the chorionic trophoblast synthesises inhibin-A as well as intact hCG and free hCG subunits.

4.3.2 HCG IN DOWN'S SYNDROME PREGNANCIES.

In the present study, intact hCG levels were measured in homogenates of placental tissue from 51 Down's syndrome pregnancies at 10 to 24 weeks gestation. The majority of the pregnancies were terminated during the second trimester. Intact hCG levels were significantly higher in placental tissue from the Down's syndrome pregnancies (4.06 MOM) than in placental tissue from the controls. The median level of intact hCG in second trimester maternal serum samples from 62 Down's syndrome pregnancies from which fetal tissues were collected was 2.72 MOM. This is slightly higher than the results of previous studies on maternal serum hCG levels in the second trimester of Down's syndrome pregnancies (Table 4.3.1) and may reflect the bias in selection of study cases through prenatal screening. Maternal serum intact hCG levels are also elevated in the first trimester of Down's syndrome pregnancies (Table 4.3.2), but the elevation is less than in the second trimester and appears to occur towards the end of the first trimester. Intact hCG levels in amniotic fluid from 33 of the Down's syndrome pregnancies in this study were also found to be significantly higher (1.39 MOM) than normal, but the magnitude of elevation was less than in maternal serum. This confirms previous studies which show that intact hCG levels are elevated in amniotic fluid from Down's syndrome pregnancies (Cuckle et al 1991, Wolf et al 1992, Spencer et al 1993a, Spencer et al 1997a).

In the present study, there was significant correlation between the levels of intact hCG in placental tissue and in corresponding maternal serum and amniotic fluid samples from the Down's syndrome pregnancies. Elevated amniotic fluid intact hCG levels in Down's syndrome pregnancies may therefore be a reflection of elevated maternal serum hCG levels. Furthermore, the increase in maternal serum intact hCG levels in

Table 4.3.1 Summary of 25 published studies on maternal serum levels of intact hCG in Down's syndrome pregnancies in the second trimester.

Reference	No. of cases	Gestation	hCG median MOM
Arab et al (1988)	77	NS	1.61
Wald et al (1988)	77	13-28	2.04
Del Junco et al (1989)	22	NS	2.10
Knight et al (1989)	24	NS	2.70
Osathanondh et al (1989)	26	16-22	2.59
Petrocik et al (1989)	38	15-19	2.50
Bartels et al (1990)	43[a]	14-24	2.18
Heyl et al (1990)	16	15-19	1.89
Muller & Boue (1990)	50	14-19	2.39
Norgaard Pedersen et al (1990)	42	14-18	1.57
Suchy & Yeager (1990)	16	15-22	2.40
Bogart et al (1991)	6	15-20	2.14
Crossley et al (1991b)	49	15-20	2.18
MacDonald et al (1991)	54	14-21	1.79
Mancini et al (1991)	9	15-18	2.33
Miller et al (1991)	8	14-20	2.11
Spencer (1991a)	29	16-18	1.88
Haddow et al (1992)	35	NS	2.50
Herrou et al (1992)	24	15-19	2.96
Phillips et al (1992)	7	15-19	2.77
Ryall et al (1992)	57	15-21	2.12
Spencer et al (1992a)	90	14-21	2.03
Stone et al (1993)	21	15-17	2.00
Bartels et al (1994)	50	14-21	2.19
Norgaard-Pedersen et al (1994)	72	15-17	2.11
Total	942		2.12*

* weighted geometric mean.

NS not stated.

[a] includes cases previously reported by Bogart et al (1987,1989).

Table 4.3.2 Summary of 13 published studies on maternal serum levels of intact hCG in Down's syndrome pregnancies in the first trimester.

Reference	No. of cases	Gestation	hCG median MOM
Cuckle et al (1988)	22	7-12	1.10
Bogart et al (1989)	6	9-11	1.14[a]
Brock et al (1990)	21	7-14	1.43
Johnson et al (1991)	11	8-12	0.91
Kratzer et al (1991)	17	9-12	1.23
Van Lith et al (1992)	24	9-11	1.19
Aitken et al (1993a)	16	6-14	0.97
Crandall et al (1993)	11	11-14	1.73
Kellner et al (1994)	5	5-14	0.90
MacIntosh et al (1994)	23	8-14	1.40
Biagiotti et al (1995)	41	8-12	1.50
Brizot et al (1995c)	41	10-13	1.50
Wald et al (1996b)	77	8-14	1.23
Total	315		1.25*

* weighted geometric mean.

[a] estimated from publication.

the second trimester of Down's syndrome pregnancies appears to be caused by an increase in the synthesis of hCG by the placenta.

The concentration of FβhCG was also higher in placental tissue from Down's syndrome pregnancies (3.40 MOM) compared to normal. The median level of FβhCG in second trimester maternal serum from 62 Down's syndrome pregnancies was 3.06 MOM. This is consistent with, although slightly higher than, the results of previous studies (Table 4.3.3) and again reflects the selection of Down's syndrome pregnancies through abnormal maternal serum biochemistry. Maternal serum FβhCG levels are also elevated in the first trimester of Down's syndrome pregnancies (Table 4.3.4). There was a significant correlation between the levels of FβhCG in placental tissue and in corresponding maternal serum from the Down's syndrome pregnancies. This suggests that the secretion of FβhCG into the maternal circulation in Down's syndrome pregnancies is not altered and that elevated second trimester maternal serum levels may be caused by increased placental synthesis of the FβhCG subunit.

Abbas et al (1995) measured hCG concentrations in fetal and maternal serum from 41 aneuploid pregnancies at 17-32 weeks, including eight with Down's syndrome and 41 gestationally matched controls samples. Fetal serum and maternal serum hCG levels were not significantly different from the controls, although there was a slight increase in maternal serum hCG levels. There was a significant association between fetal serum and maternal serum hCG levels in the normal and aneuploid groups combined and the authors proposed that the route of secretion of hCG into the fetal and maternal circulation is the same in normal and aneuploid pregnancies. However, there were too few cases included in the study to give a clear picture of the changes in fetal serum and maternal serum hCG concentrations in Down's syndrome pregnancies.

Table 4.3.3 Summary of eight published studies on maternal serum FβhCG levels in Down's syndrome pregnancies in the second trimester.

Reference	No. of cases	Gestation	FβhCG median MOM
Crossley et al (1991a)	81	NS	2.30
Ryall et al (1992)	57	15-21	2.36
Spencer and Carpenter (1993)	11	15-19	2.62
Stone et al (1993)	21	15-17	2.50
Wald et al (1993)	75	14-24	2.22
Macri et al (1994)	480[a]	14-22	2.64
Norgaard Pederson et al (1994)	72	14-18	2.31
Berry et al (1997)	47	15-21	2.79
Total	844		2.53*

* weighted geometric mean.

NS not stated.

[a] includes cases previously reported by Macri et al (1990, 1992), Spencer (1991a), Larsen et al (1992), Spencer and Carpenter (1992), Spencer and Macri (1992) and Spencer et al (1992a).

Table 4.3.4 Summary of 14 published studies on maternal serum FβhCG levels in Down's syndrome pregnancies in the first trimester.

Reference	No. of cases	Gestation	FβhCG median MOM
Ozturk et al (1990)	9	8-12	1.62[a]
Macri et al (1993a)	38[b]	9-13	2.20
Macintosh et al (1994)	23[c]	8-14	2.10
Brambati et al (1994b)	13	8-12	1.13
Kellner et al (1994)	5	5-14	2.20
Biagiotti et al (1995)	41	8-12	2.00
Brizot et al (1995c)	41	10-13	2.00
Brizot et al (1995b)	9	12-15	1.43
Noble et al (1995)	61	10-14	2.13
Casals et al (1996)	19	10-13	1.35
Krantz et al (1996)	22	10-13	2.09
Wald et al (1996b)	77	8-14	1.79
Zimmermann et al (1996)	4	10-13	1.50
Berry et al (1997)	54	7-14	1.99
Total	416		1.93*

* weighted geometric mean.

[a] estimated from publication.

[b] includes cases previously reported by Spencer et al (1992b) and Aitken et al (1993a).

[c] seven cases also included in Macri et al (1993a).

Significantly elevated levels of FβhCG in the amniotic fluid from Down's syndrome pregnancies have previously been reported (Wolf et al 1992, Spencer et al 1997a). In the present study, FβhCG levels in amniotic fluid from Down's syndrome pregnancies were significantly higher (1.33 MOM) than in normal control pregnancies. There was no correlation between the levels of FβhCG in placental tissue and amniotic fluid from the Down's syndrome pregnancies and the magnitude of elevation of FβhCG levels was much greater in placental tissue than in the amniotic fluid. This suggests that the placenta may not be the main source of FβhCG in the amniotic fluid. Although the magnitude of elevation of FβhCG in the amniotic fluid is less than in the maternal circulation, the concentration of FβhCG in the amniotic fluid is still greater than in the maternal circulation. In amniotic fluid from Down's syndrome pregnancies, intact hCG and FβhCG levels are elevated to a similar extent. This indicates that increased dissociation of intact hCG into its subunits is unlikely to be the cause of increased FβhCG levels in amniotic fluid.

In the present study, intact hCG and FβhCG levels were found to be substantially elevated in placental tissue from Down's syndrome pregnancies. This suggests that increased maternal serum concentrations of intact hCG and FβhCG in Down's syndrome pregnancies may be caused by increased synthesis of hCG subunits by the placenta. Evidence to support this theory is provided in a study by Eldar-Geva et al (1995). These investigators found that trophoblast cultures from second trimester (17-23 weeks gestation) Down's syndrome pregnancies secreted 10 times more hCG than trophoblast cultures from control placentae at the same gestation. This increase appeared to be due to a marked increase in the levels of βhCG mRNA and a smaller increase in the levels of αhCG mRNA.

Brizot et al (1995b) measured αhCG and βhCG mRNA in homogenates of placental tissue from nine Down's syndrome and 30 unaffected control pregnancies at 11 to 15 weeks gestation. αhCG and βhCG mRNA levels were higher in the Down's syndrome cases than in the controls but the increase was not statistically significant. The concentration of FβhCG in corresponding maternal serum samples from the Down's syndrome pregnancies was significantly higher than in the controls. There was no correlation between the levels of βhCG mRNA in placental tissue levels from the control and Down's syndrome pregnancies. The authors propose that placental expression of αhCG and βhCG subunits is not altered in Down's syndrome pregnancies but that there may be alterations in the post-transcriptional processing of the hCG molecule. However, the number of cases included in this study is small and the samples were obtained mainly from first trimester pregnancies, at which stage the increase in maternal serum intact hCG levels is less marked than in the second trimester.

HCG is a glycoprotein which contains up to 30% carbohydrate. The biological activity of hCG, interaction of αhCG and βhCG subunits, receptor binding and metabolic clearance rate of hCG are all affected by alterations in the carbohydrate moiety. For example, removal of sialic acid residues results in decreased activity due to the increased metabolic clearance of asialo-hCG from the circulation (Merz 1994). Kratzer et al (1991a) reported that there was no difference in the bioactivity of hCG in maternal serum from Down's syndrome and control pregnancies. This suggests that the post-transcriptional processing of hCG is not altered in Down's syndrome pregnancies.

In second trimester maternal serum from Down's syndrome pregnancies, the proportion of FβhCG which is nicked is 5 times greater than in normal pregnancies (Cole 1994). The concentration of the β-core fragment in second trimester maternal

urine from Down's syndrome pregnancies has been reported to be approximately five times higher than normal (Cuckle et al 1994b, Cuckle et al 1995b, Canick et al 1995), although some studies found a less marked increase (Hayashi and Kozu 1995, Spencer et al 1996a). Cuckle et al (1996a) reported that maternal urinary β-core concentrations in 10 Down's syndrome pregnancies in the first trimester were not significantly different from normal (range: 0.36-3.57 MOM). However, in a larger series of 22 Down's syndrome pregnancies at 10 to 12 weeks gestation, Spencer et al (1997b) reported that the concentrations of FβhCG and β-core in maternal urine were significantly increased to 1.87 MOM (range: 0.15-27.64) and 2.91 MOM (range: 0.01-20.33) respectively. This suggests that there may be an increase in the production of nicked hCG molecules and/ or increased degradation of hCG molecules in Down's syndrome pregnancies.

4.3.2.1 Effect of fetal sex on hCG levels.

Previous studies have shown that the concentration of hCG in the maternal circulation, from approximately 18 weeks gestation to term, is significantly higher in women carrying a female fetus than in women carrying a male fetus (Crosignani et al 1972, Boroditsky et al 1975, Obiewick and Chard 1982, Leporrier et al 1992, Muller et al 1993a). In contrast, Crosignani et al (1972) found that the concentration of hCG in amniotic fluid at term was significantly higher in women carrying male fetuses. The concentration of hCG in cord blood from male and female fetuses at term was not significantly different. Clements et al (1976) reported that there was no difference in fetal serum and amniotic fluid hCG levels in relation to fetal sex.

Placental levels of hCG do not appear to be affected by fetal sex during the second trimester of pregnancy, however, at term placental hCG levels are higher in

pregnancies with female fetuses than with male fetuses (Hobson and Wide 1974, Wide and Hobson 1974). In the present study, there was no significant difference in placental levels of hCG between pregnancies with female fetuses and those with male fetuses.

Wolf et al (1992) investigated the effect of fetal sex on amniotic fluid concentrations of total hCG and FβhCG in Down's syndrome and unaffected pregnancies. In the unaffected control pregnancies there was no significant difference between the sexes but in Down's syndrome pregnancies, amniotic fluid total hCG and FβhCG concentrations were higher when the fetus was female. In contrast, Spencer et al (1997a) reported that amniotic fluid total hCG and FβhCG concentrations were higher in unaffected pregnancies with female fetuses than those with male fetuses but that there was no fetal sex difference in hCG concentration in amniotic fluid from Down's syndrome pregnancies.

In the present study, there was no significant difference in the concentration of total hCG and FβhCG in placental tissue, maternal serum and amniotic fluid from Down's syndrome pregnancies with female fetuses compared to those with male fetuses.

4.3.2.2 Factors Affecting the Regulation of hCG Synthesis in Normal and Down's syndrome Pregnancies.

Extensive studies on the regulation of hCG synthesis have shown that the mechanisms involved in the control of hCG synthesis and secretion throughout pregnancy are complex (Jameson and Hollenberg 1993, Cole 1994, Merz 1994).

The relative amounts of αhCG and βhCG subunits produced by the placenta varies at different stages of pregnancy, suggesting that there may be independent regulation of hCG subunits. Levels of both αhCG and βhCG mRNA decrease towards term with βhCG mRNA levels barely detectable in placenta at term. However the ratio of αhCG mRNA to βhCG mRNA increases from 2:1 in the first trimester to 12:1 in term placenta (Boothby et al 1983, Kelly et al 1991). The relative excess of the αhCG subunit throughout pregnancy implies that synthesis of the βhCG subunit is the rate limiting step in the formation of dimeric hCG. The mechanisms involved in the regulation of hCG subunit gene expression have been studied extensively and are reviewed by Jameson and Hollenberg (1993). Elevated levels of intact hCG and FβhCG in Down's syndrome pregnancies connot be the result of a gene dosage effect as the genes encoding the αhCG and βhCG subunits are not located on chromosome 21. The αhCG subunit is coded by a single gene on chromosome 6 (Fiddes and Goodman 1981) and the βhCG subunit by a cluster of six genes on chromosome 19 (Graham et al 1987). However, an increase in the level of chromosome 21 encoded regulatory proteins may indirectly affect the regulation of synthesis of other proteins, including hCG.

A number of substances have been shown to regulate the synthesis and/ or secretion of hCG by placental cultures or trophoblast tumour cell lines (Table 4.3.5). However, the mechanisms by which these modulators exert their effects, or whether they have a role in the *in vivo* production of hCG has still to be determined. Furthermore, many of these studies were carried out on term or on first trimester placentae, therefore the physiological effects of these substances on placental function throughout pregnancy are not known.

Table 4.3.5 Factors affecting the synthesis or secretion of hCG by placental cultures or trophoblast tumour cell lines.

Modulator	Effect	Reference
GnRH	I	Barneau and Kaplan (1988)
		Merz et al (1991)
Epidermal growth factor	I	Morrish et al (1987)
γ-amino butyric acid	I	Licht et al (1992)
Fibroblast growth factor	I	Oberbauer et al (1988)
Macrophage colony stimulating factor	I	Saito et al (1991)
Glucocortcoids	I	Wilson and Jawad (1982)
Retinoic acid	I	Kato and Braunstein (1991)
Insulin	I	Ren and Braunstein (1991)
Cyclic AMP	I	Jameson and Hollenberg (1993)
Activin	I	Petraglia et al (1989)
Norepinephrine	I	Shi and Zuang (1993)
Fetal neural tissue	D/I[a]	Shurtz-Swirski et al (1991)
Transforming growth factor β	D	Morrish et al (1991)
	NE	Petraglia et al (1989)
Tumour necrosis factor-α	D	Ohashi et al (1992)
Progesterone	D	Wilson et al (1980)
	I	Ahmed et al (1988)
Prolactin	D	Yuen et al (1986)
Inhibin	D	Petraglia et al (1989)
	D/NE[a]	Mersol-Barg et al (1990)
Testosterone	D/NE[a]	Ahmed et al (1988)
Estradiol	NE	Ahmed et al (1988)

I - increased, D - decreased, NE - no effect.

[a] - gestation dependent

Gonadotropin-releasing hormone (GnRH) stimulates the release of hCG from placental cultures in a dose-dependent manner (Barneau and Kaplan 1989, Merz et al 1991). GnRH gene expression has been demonstrated in the stroma of the placenta and in both the cytotrophoblast and syncytiotrophoblast (Kelly et al 1991) and receptors for GnRH have been identified in the placenta (Iwashita et al 1986). Thus GnRH may be a possible regulator of hCG synthesis in vivo, although its precise physiological role is not clear (Siler-Khodr et al 1991).

Activin and inhibin have also been shown to modulate the secretion of hCG by placental cells in vitro. Petraglia et al (1987) reported that hCG secretion by trophoblast cultures was increased in the presence of antibody to inhibin, while hCG itself increased trophoblast secretion of inhibin. Petraglia et al (1989) reported that activin stimulated the production of GnRH and potentiated the GnRH induced secretion of hCG. Steele et al (1993) reported that recombinant activin increased hCG secretion by trophoblast cells independently of GnRH. Both studies demonstrated that inhibin alone did not affect hCG secretion but was capable of reversing activin induced changes. Activin also increases the production of progesterone by cultured placental cells (Petraglia et al 1989), while progesterone itself has been shown to affect the production of hCG (Wilson et al 1980, Wilson and Jawad 1982, Ahmed et al 1988). The effects of inhibin and progesterone on hCG synthesis are of interest as both inhibin and progesterone have been shown to be elevated in the second trimester of Down's syndrome pregnancies (Knight et al 1989, Van Lith et al 1992, Spencer et al 1993c, Cuckle et al 1994a). Maternal serum inhibin levels are not significantly different from normal in the first trimester of Down's syndrome pregnancies (Van Lith et al 1994, Wallace et al 1994). Studies using assays specific for dimeric inhibin-A have shown that inhibin-A is elevated in both the first and second trimesters of Down's syndrome pregnancies (Cuckle et al 1995a, Wallace et al 1995, Aitken et al 1996b, Wald et al 1996a, Wallace et al 1996b). However, in contrast to intact hCG, inhibin-A

levels are significantly lower in amniotic fluid from Down's syndrome pregnancies than in normal pregnancies suggesting that there may be differential expression of inhibin-A by the chorionic trophoblast and the placental trophoblast in Down's syndrome pregnancies (Wallace et al 1997).

Interferons (α, β, γ) were originally identified for their ability to protect cells from viral infection but are now known to have a wide range of regulatory functions particularly in association with cell growth and differentiation and the immune response (Balkwill 1985, Taylor-Papadimitriou and Rozengurt 1985, Tamm et al 1987). Interferon-α (IFNα) is produced by the placenta and fetal tissues during pregnancy (Chard 1989, Loke and King 1990). Iles and Chard (1989) reported that IFNα increased the secretion of the βhCG subunit by bladder tumour cells in vitro. This is of particular interest as the gene encoding IFNα/β receptors are located on the pathological section of chromosome 21. The expression of IFN receptors has been shown to be increased in a dose-dependent manner in Down's syndrome and the biological response to IFNα is increased 3-14 fold (Cooper and Hall 1988). This may have important implications in the mechanisms which give rise to elevated hCG in Down's syndrome pregnancies.

Receptors for hCG (hCG/LH receptors) have been demonstrated in both the cytotrophoblast and syncytiotrophoblast of the mid-trimester and term placenta, indicating possible autocrine and paracrine roles in the placenta (Reshef et al 1990, Lei and Rao 1992). Licht et al (1993) proposed a mechanism by which hCG down-regulates its own synthesis in the term placenta via 80kDa hCG/LH receptors. HCG was unable to regulate its own synthesis in first trimester placental cultures and in human gestational trophoblastic neoplasms, both of which secrete high levels of hCG (Licht et al 1993, Licht et al 1994). A 50kDa receptor, which is a truncated form of the 80kDa receptor found in term placenta, was found to be expressed in first trimester

placenta and in trophoblastic neoplasms. The concentration profile of hCG in maternal serum may be a reflection of the differential expression of hCG/LH receptors and the ability of hCG to regulate its own synthesis in term but not first trimester placenta. If so, it may be that in Down's syndrome hCG lacks the ability to down regulate its own synthesis, possibly due to the abnormal expression or function of hCG/LH receptors.

The trophoblast of the placenta is made up of mononucleated cytotrophoblast cells, multinucleated syncytiotrophoblast cells and intermediate trophoblast cells. The syncytiotrophoblast is derived from the cytotrophoblast cells through a process of aggregation and differentiation. The intermediate cells may represent an intermediate stage of cytotrophoblast differentiation (Kurman et al 1984). Hoshina et al (1982,1983) demonstrated that αhCG and βhCG subunit mRNA was localised primarily to the syncytiotrophoblast, but was also present in some cytotrophoblast cells. Subsequent studies have localised the αhCG subunit primarily to the cytotrophoblast, while βhCG is localised almost exclusively to the syncytiotrophoblast (Hay 1988, Kelly et al 1991). Synthesis of dimeric hCG is linked predominantly to the syncytiotrophoblast and intermediate trophoblast cells (Gosseye and Fox 1984, Kurman et al 1984, Hay 1988). Marou et al (1992) reported that hCG was localised primarily to the cytotrophoblast in 4-5 week placentae and exclusively to the syncytiotrophoblast in placentae at 6-12 weeks. The morphology of the placenta and the ratio of cytotrophoblast to syncytiotrophoblast changes with advancing gestation. Although the concentration of hCG in the maternal circulation throughout pregnancy does not reflect the increase in trophoblastic mass (Braunstein et al 1980), synthesis and secretion of dimeric hCG may be directly related to the pattern of differentiation of the trophoblast (Hoshina et al 1985, Hay 1988, Boime 1991).

Hay (1988) reported that the increase in maternal serum levels of hCG between 3 and 9 weeks gestation coincided with the presence of an extensive layer of syncytiotrophoblast in the placenta. The decline in serum hCG levels after 10 weeks gestation coincided with declining numbers of cytotrophoblast cells resulting in a reduction of the syncytial layer and the reduced capacity of the placenta to synthesise hCG subunits and secrete dimeric hCG. Thus, the authors proposed that hCG synthesis depends on the rate of differentiation of cytotrophoblasts into syncytiotrophoblasts. Another possibility is that expression of αhCG and βhCG subunit genes depends on the stage of differentiation of the trophoblast (Hoshina et al 1985, Boime 1991). Expression of the αhCG and then βhCG subunit genes is initiated in the cytotrophoblast cells following commitment to differentiation into syncytiotrophoblast. The presence of numerous cytotrophoblast cells in the placenta in early pregnancy, when hCG levels are high, may serve to maintain a pool of intermediate trophoblast cells which are capable of expressing both αhCG and βhCG subunits and dimeric hCG. Declining levels of hCG are associated with the formation of fully differentiated syncytiotrophoblast cells and reduced numbers of cytotrophoblasts. Both of these theories are based on the reduction of the number of cytotrophoblast cells with advancing gestation. However, Mayhew and Simpson (1994) reported that the absolute number of cytotrophoblast cells does not decrease as gestation advances, but suggested that the cytotrophoblast cells become more thinly distributed as the villous surface expands. Shi et al (1993) proposed that hCG regulated its own synthesis by promoting the differentiation of cytotrophoblast into syncytiotrophoblast. Low to moderate concentrations of exogenous hCG were found to promote trophoblast differentiation in the term placenta, while high concentrations inhibited differentiation. It is possible that in Down's syndrome pregnancies there are morphological changes to the placenta, such as abnormal differentiation of

cytotrophoblast into syncytiotrophoblast, that affect the synthesis of hCG subunits and the formation of dimeric hCG.

4.3.3 HCG LEVELS IN TRISOMY 13 AND TRISOMY 18 PREGNANCIES.

Intact hCG levels in placental tissue, maternal serum and amniotic fluid from the Trisomy 13 and Trisomy 18 pregnancies included in this study are presented in Table 4.3.6. Intact hCG concentrations were extremely low in second trimester maternal serum samples from two of the Trisomy 18 pregnancies. These results are consistent with previous studies (Table 4.3.7). Combining data from six published studies, totaling 159 Trisomy 18 cases, gives an overall second trimester maternal serum intact hCG level of 0.33 MOM. Analysis of data from four published studies on first trimester maternal serum intact hCG levels in Trisomy 18 pregnancies, including a total of 37 affected cases, reveals an overall level of 0.43 MOM. The concentration of intact hCG in amniotic fluid from one Trisomy 18 pregnancy was also found to be reduced. Abbas et al (1995) reported that hCG levels in fetal serum and maternal serum from 18 Trisomy 18 pregnancies at 17-32 weeks gestation were significantly lower than in unaffected pregnancies.

FβhCG levels in placental tissue, maternal serum and amniotic fluid from the Trisomy 13 and Trisomy 18 pregnancies included in this study are presented in Table 4.3.8. As with intact hCG, FβhCG levels were also extremely low in second trimester maternal serum from two Trisomy 18 pregnancies. This is consistent with previous studies which show that maternal serum FβhCG levels are reduced in both the first and second trimesters of Trisomy 18 pregnancies (Ozturk et al 1990, Spencer et al 1993b, Aitken et al 1993a, Macri et al 1993a, Brizot et al 1995c, Aitken et al 1996b, Zimmerman et al 1996). The reduction in maternal serum FβhCG concentrations from one of the

Table 4.3.6 Intact hCG levels (MOM) in placental tissue, maternal serum and amniotic fluid from Trisomy 13 and Trisomy 18 pregnancies.

Case No.	Intact hCG MOM (gestation)		
	Placental	Maternal serum	Amniotic fluid
T13/1	4.20 (23)	-	-
T18/1	0.59 (23)	-	-
T18/2	2.97 (22)	-	-
T18/3	0.31 (21)	0.16 (16)	-
T18/4	-	0.17 (15)	0.58 (17)

Table 4.3.7 Summary of published studies on maternal serum intact hCG levels in Trisomy 18 pregnancies in the first and second trimesters.

Reference	No. of cases	1st/2nd trimester	Intact hCG median MOM
Johnson et al (1991)	7	1st	0.32
Van Lith et al (1992)	6	1st	0.80
Aitken et al (1993a)	5	1st	0.27
Brizot et al (1995c)	19	1st	0.40
Arab et al (1988)	5	2nd	0.34[a]
Canick et al (1990a)	10	2nd	0.27
Crossley et al (1991b)	4[b]	2nd	<0.40
Miller et al (1991)	9	2nd	0.36
Barkai et al (1993)	14	2nd	0.23
Palomaki et al (1995)	89	2nd	0.36
Aitken et al (1996b)	32[c]	2nd	0.30

[a] estimated from publication.

[b] not included in meta-analysis.

[c] includes four cases at 8-12 weeks which were previously reported by Aitken et al (1993a).

Table 4.3.8 FβhCG levels (MOM) in placental tissue, maternal serum and amniotic fluid from Trisomy 13 and Trisomy 18 pregnancies.

Case No.	FβhCG MOM (gestation)		
	Placenta	Maternal serum	Amniotic fluid
T13/1	0.95 (23)	-	-
T18/1	ND (23)	-	-
T18/2	1.66 (22)	-	-
T18/3	0.04 (21)	0.13 (16)	-
T18/4	-	0.12 (15)	0.97 (17)

ND not detectable.

Trisomy 18 pregnancies did not appear to be reflected in amniotic fluid from the same pregnancy.

Studies on small numbers of Trisomy 13 pregnancies suggest that maternal serum intact hCG and FβhCG levels are low in the first trimester (Kratzer et al 1991b, Brizot et al 1995b). In the second trimester of Trisomy 13 pregnancies, maternal serum intact hCG levels have been reported to be normal (Crossley et al 1991a), elevated (Muller et al 1993a) and reduced (Suchy and Yeager 1990, Johnson et al 1991)

Clearly, the underlying reasons for abnormal maternal serum hCG concentrations in Trisomy 18 and Down's syndrome pregnancies are different. Placental insufficiency may be a contributory factor in the reduction of intact hCG and FβhCG levels in Trisomy 18 pregnancies. Low maternal serum intact hCG and FβhCG levels may be attributed to reduced placental synthesis in one of the Trisomy 18 cases, however it is not possible to identify a trend in placental intact hCG and FβhCG levels in Trisomy 18 pregnancies as the results from the other cases are variable.

4.4 PREGNANCY ASSOCIATED PLASMA PROTEIN A.

4.4.1 PAPP A LEVELS IN NORMAL PREGNANCIES.

PAPP A was measured in homogenates of placental tissue from 52 unaffected control pregnancies at 12 to 20 weeks gestation. Logarithmic regression was used to provide the regression equation which best fitted the control data. However, the overall median PAPP-A level in the controls was slightly lower (0.86 MOM) than would be expected for an accurate regression model. Median placental PAPP-A levels were found to increase between 12 and 20 weeks gestation, corresponding to the increase in

maternal serum PAPP-A levels with advancing gestation in normal pregnancies (Folkersen et al 1981, Bischof et al 1982a, Berry et al 1997). Immunohistochemical studies demonstrated that PAPP-A was localised to the syncytiotrophoblast of the placenta. This is consistent with previous studies and confirms that the syncytiotrophoblast is the likely source of PAPP-A in the maternal circulation (Wahlstrom et al 1981, Gosseye and Fox 1984, Tornhave et al 1984). It has been proposed that the synthesis and secretion of placental proteins is directly related to the total mass of the placental trophoblast, the rate of blood flow into the intervillous space and the concentration of the product in the intervillous space (Gordon and Chard 1979, Chard 1986, Chard 1993). However, Sorensen et al (1995) reported that the production rate of PAPP-A, estimated from median maternal serum PAPP-A concentrations at 7-20 weeks gestation, was higher than the estimated growth rate of the placenta. This suggests that other factors may be involved in the regulation of PAPP-A synthesis and secretion.

PAPP-A levels were also measured in amniotic fluid from 132 control pregnancies at 15 to 21 weeks gestation. PAPP-A is present in the amniotic fluid at approximately 2% of maternal serum levels. Amniotic fluid PAPP-A levels increased gradually with advancing gestation, similar to the change in concentration of PAPP-A in the maternal circulation during the same gestational period (Bischof et al 1982a). This indicates that amniotic fluid PAPP-A may be derived directly from the maternal circulation. However, PAPP-A is not detectable in amniotic fluid until 13 weeks gestation (Bischof et al 1982a, Iles et al 1994). Iles et al (1994) reported that in corresponding samples of maternal serum, EECF and amniotic fluid from normal pregnancies at 8-14 weeks gestation, the concentration of PAPP-A was highest in maternal serum, low in EECF and absent from amniotic fluid prior to 13 weeks gestation. This is similar to the pattern seen for inhibin-A (Riley et al 1996), but is in contrast to hCG (total βhCG, intact hCG

and FαhCG), the concentration of which is highest in the EECF in the first trimester (Iles et al 1992). One possible explanation for the absence of PAPP-A from first trimester amniotic fluid is that PAPP-A is a large protein (750kDa) and consequently may have a slow rate of transfer from the maternal circulation into the amniotic fluid. Alternatively, the EECF and amnion may act as a barrier to the transfer of proteins from the maternal circulation into the amniotic fluid in the first trimester of pregnancy (Gulbis et al 1992, Iles et al 1994).

4.4.2 PAPP-A LEVELS IN DOWN'S SYNDROME PREGNANCIES.

Maternal serum PAPP-A levels are significantly reduced in the first trimester of pregnancies where the fetus has Down's syndrome. A summary of the studies on first trimester maternal serum PAPP-A levels in Down's syndrome pregnancies is given in Table 4.4.1. However, in the second trimester of Down's syndrome pregnancies, maternal serum PAPP-A levels are not significantly different from normal (Table 4.4.2). Berry et al (1997) reported that the median level of PAPP-A in maternal serum from Down's syndrome pregnancies in the first trimester was 0.50 MOM. However, the magnitude of the reduction in median PAPP-A levels decreased with advancing gestation until normal levels were reached in the second trimester (0.94 MOM). As PAPP-A levels are lower at earlier gestations, the overall median MOM reported in individual studies will be affected by the distribution of samples according to gestation.

In the present study, PAPP-A was measured in maternal serum from 58 Down's syndrome pregnancies at 15 to 20 weeks gestation. The overall median level of PAPP-A in maternal serum from the Down's syndrome pregnancies was 0.92 MOM, consistent with previous studies (Table 4.4.2). PAPP-A levels were also measured in amniotic fluid from 32 of these Down's syndrome pregnancies at 15 to 21 weeks

Table 4.4.1 Summary of 13 published studies on maternal serum PAPP-A levels in Down's syndrome pregnancies in the first trimester.

Reference	No. of cases	Gestation	PAPP-A median MOM
Wald et al (1992b)	19	9-12	0.23
Muller et al (1993b)	17	9-14	0.42
Bersinger et al (1994)	29	10-13	0.59
Brambati et al (1994a)	29[a]	6-11	0.31
Brizot et al (1994)	45	10-13	0.50
Macintosh et al (1994)	23[b]	8-14	0.39
Spencer et al (1994)	21[c]	7-14	0.62
Casals et al (1996)	19	10-13	0.42
Krantz et al (1996)	22	10-13	0.41
Qin et al (1996)	39	5-12	0.87
Wald et al (1996b)	77	8-14	0.43
Zimmermann et al (1996)	4	10-13	0.51
Berry et al (1997)	52	7-14	0.50
Total	396		0.50*

* weighted geometric mean.

[a] includes cases previously reported by Brambati et al (1993).

[b] includes cases previously reported by Hurley et al (1993).

[c] includes cases previously reported by Aitken et al (1994).

Table 4.4.2 Summary of five published studies on maternal serum PAPP-A levels in Down's syndrome pregnancies in the second trimester.

Reference	No. of cases	Gestation	PAPP-A median MOM
Cuckle et al (1992)	18	15-20	0.87
Wald & Voller (1992)	16	15-20	1.02
Knight et al (1993)	30	15-19	1.01
Aitken et al (1994)	48	15-20	1.00
Berry et al (1997)	47	15-21	0.94
Total	159		0.97*

* weighted geometric mean.

gestation. Amniotic fluid PAPP-A levels in the Down's syndrome pregnancies were not significantly different from normal (median MOM=0.95). There are no published studies on amniotic fluid PAPP-A levels in Down's syndrome pregnancies.

In this study, the majority of Down's syndrome and control pregnancies from which fetal tissues were collected were terminated during the second trimester. Placental levels of PAPP-A in the Down's syndrome pregnancies (0.96 MOM) were not significantly different than in the controls (0.86 MOM). Despite the similarity between the overall median MOM levels of PAPP-A in placental tissue, maternal serum and amniotic fluid from the Down's syndrome pregnancies, the correlation between PAPP-A levels in the different fetal-maternal compartments was not statistically significant. The results of the present study suggest that in the second trimester of Down's syndrome pregnancies the mechanisms involved in the synthesis, secretion and transport of PAPP-A are not disturbed.

The question still remains as to the cause or causes of reduced maternal serum PAPP-A levels in the first trimester and their apparent normalisation in the second trimester of Down's syndrome pregnancies. Brizot et al (1996a) investigated placental expression of PAPP-A, placental PAPP-A protein levels and maternal serum levels of PAPP-A in 8 pregnancies affected by Down's syndrome and in 15 normal control pregnancies at 12 to 15 weeks gestation. Maternal serum levels of PAPP-A were significantly lower in the Down's syndrome pregnancies than in the controls. Although the levels of placental PAPP-A mRNA and placental PAPP-A protein were also lower in the Down's syndrome pregnancies than in the controls, the reduction was not statistically significant. However, this may be due to the small number of cases and controls studied. Brizot et al (1996a) proposed that the reduction in maternal serum levels of PAPP-A in the first trimester of Down's syndrome pregnancies was not due to a reduction in PAPP-A synthesis by the placenta, but was caused by post-translational

alterations such as impaired placental transport of PAPP-A or changes affecting alternative sources of PAPP-A. However, as the magnitude of the reduction in maternal serum PAPP-A levels in Down's syndrome pregnancies decreases with advancing gestation (Berry et al 1997), a less marked difference in placental PAPP-A levels might be expected at 12 to 15 weeks gestation than at an earlier stage in the first trimester.

4.4.3 PAPP-A LEVELS IN TRISOMY 13 AND TRISOMY 18 PREGNANCIES.

PAPP-A levels in placental tissue, maternal serum and amniotic fluid from the Trisomy 13 and Trisomy 18 pregnancies included in this study are presented in Table 4.4.3. Very low levels of PAPP-A were detected in second trimester maternal serum from two cases of Trisomy 18 and in amniotic fluid from one Trisomy 18 case. PAPP-A levels were also substantially reduced in placental tissue from the Trisomy 13 and Trisomy 18 pregnancies. These results suggest that in the second trimester of Trisomy 13 and Trisomy 18 pregnancies, circulating PAPP-A levels are lower than normal due to reduced placental synthesis of PAPP-A. Very low levels of PAPP-A in Trisomy 18 pregnancies may be a response to imminent abortion as there is a high rate of fetal loss in Trisomy 18 pregnancies (Connor and Ferguson-Smith 1991).

Previous studies have shown that PAPP-A levels in maternal serum from Trisomy 18 pregnancies in the first trimester are lower than normal (Brambati et al 1993, Spencer et al 1994, Brizot et al 1996a, Zimmermann et al 1996). Hurley et al (1993) reported that one Trisomy 13 pregnancy had a maternal serum PAPP-A level of 0.41 MOM in the first trimester.

Brizot et al (1996a) measured placental levels of PAPP-A mRNA, placental PAPP-A protein and maternal serum levels of PAPP-A in seven Trisomy 18 pregnancies at 12

Table 4.4.3 PAPP-A levels (MOM) in placental tissue, maternal serum and amniotic fluid from Trisomy 13 and Trisomy 18 pregnancies.

Case No.	PAPP-A MOM (gestation)		
	Placental	Maternal serum	Amniotic fluid
T13/1	0.39 (23)	-	-
T18/1	0.39 (23)	-	-
T18/2	0.39 (22)	-	-
T18/3	0.45 (21)	0.06 (16)	-
T18/4	-	0.07 (15)	0.52 (17)

to 15 weeks gestation. Maternal serum PAPP-A levels in the Trisomy 18 pregnancies were lower than normal, but there was no statistically significant difference in placental levels of PAPP-A mRNA or PAPP-A protein. The authors propose that abnormal serum PAPP-A levels may be caused by post-translational alterations.

4.5 PREGNANCY SPECIFIC β1 GLYCOPROTEIN.

4.5.3 SP1 LEVELS IN NORMAL PREGNANCIES.

SP1 was measured in placental tissue from 52 control pregnancies at 12 to 20 weeks gestation. SP1 could not be detected in a small number of the placental homogenates. Placental levels of SP1 decreased with advancing gestation. These results are contrary to those of Grudzinskas et al (1980) who found an apparent increase in the concentration of SP-1 in placental tissue with advancing gestation. However, this was based on the analysis of SP1 concentration in homogenates of placental tissue from only five pregnancies terminated in the first trimester and five term deliveries.

In maternal serum from 114 control pregnancies at 15 to 20 weeks gestation, median SP1 levels were found to increase with advancing gestation. This is consistent with previous studies (Wald et al 1989, Graham et al 1992, Sorensen et al 1995, Qin et al 1997). Unlike the other placental products (intact hCG, FβhCG and PAPP-A), the concentration profile of SP1 in the maternal circulation does not appear to reflect the change in placental SP1 levels with gestation. This may be due to the possible differences in the mechanisms which regulate the synthesis and secretion of placental proteins. As discussed previously, it has been proposed that the production rate of placental proteins is related to the mass of the trophoblast, the blood flow into the intervillous space and the concentration of the placental product in the intervillous

space (Gordon and Chard 1979, Chard 1986, Chard 1993). Sorensen et al (1995) demonstrated that the rates of change in the levels of hCG, PAPP-A, SP1 and hPL in maternal serum were different, suggesting that the synthesis of these proteins may be regulated by independent mechanisms. The rates of increase of SP-1 and hPL in the maternal circulation were similar to the rate of growth of the placenta, confirming the results of Braunstein et al (1980) who found that the concentrations of SP1 and hPL in maternal serum were correlated with the estimated growth of the trophoblast. Thus, increasing concentrations of SP1 in the maternal circulation with advancing gestation may closely reflect the increase in trophoblastic mass. However, the decrease with advancing gestation in the placental concentration of SP1 in normal pregnancies is difficult to reconcile with this theory.

In the present study, immunohistochemical analysis demonstrated that SP1 was localised to the syncytiotrophoblast of the placenta. This is in agreement with previous studies which suggest that SP1 is a product of the syncytiotrophoblast (Lin and Halbert 1976, Gosseye and Fox 1984). However, it is possible that there may be other sources of SP1 in the maternal circulation. Some studies have shown that SP1 is present at extremely low concentrations in tissue extracts and serum from non-pregnant healthy adults, while other studies have not been able to confirm this (Sorensen 1984), and the evidence for a maternal source of SP1 during pregnancy is weak. Ho et al (1982) demonstrated the presence of SP1 in the trophoblast cells and amniotic epithelium of the amniochorionic membrane in early and late pregnancy. In a more recent study, Plouzek et al (1993) found that the amniotic and chorionic membranes expressed low levels of SP1 and that there was tissue-specific expression of different SP1 subgroups by the amnion, chorion and placental trophoblast.

The concentration of SP1 in the amniotic fluid at 15 to 20 weeks gestation is approximately 2% of maternal serum levels at the same gestation and a sensitive

immunofluorometric assay was required for amniotic fluid SP1 determination (Qin et al 1997). If the amnion and chorion were major sites of SP1 synthesis, a higher concentration of SP1 in the amniotic fluid might be expected. Amniotic fluid SP1 levels increased with advancing gestation, similar to the concentration profile of SP1 in the maternal circulation. These results are in agreement with previous studies (Grudzinskas et al 1978, Kelly et al 1994). The concentration of SP1 in the fetal circulation is approximately 0.1% of that in the maternal circulation (Grudzinskas et al 1978). Thus the pattern of SP1 concentrations in the different fetal-maternal compartments appears to be similar to that of other placental products (e.g. intact hCG, PAPP-A), suggesting that SP1 is secreted from the placenta into the maternal circulation and is then transported from the maternal decidua across the fetal membranes into the amniotic fluid.

4.5.2 SP1 LEVELS IN DOWN'S SYNDROME PREGNANCIES.

Published studies on SP1 levels in maternal serum from Down's syndrome pregnancies in the first and second trimesters are presented in Table 4.5.1 and Table 4.5.2 respectively. As with PAPP-A, the change in maternal serum SP1 levels in Down's syndrome pregnancies varies with gestation (Qin et al 1997). In the second trimester, maternal serum SP1 levels are elevated in association with fetal Down's syndrome, although reports vary with regard to the magnitude of variation. Qin et al (1997) investigated maternal serum SP1 levels in both the first and second trimesters of Down's syndrome pregnancies. Maternal serum SP1 levels were significantly reduced to 0.29 MOM at 5-9 weeks gestation. At 10-12 weeks gestation maternal serum SP1 levels were not significantly different from normal (0.89 MOM), while at 14-20 weeks maternal serum SP1 levels in the Down's syndrome pregnancies were significantly elevated (1.28 MOM). Thus, in Down's syndrome pregnancies there is a

Table 4.5.1 Summary of six published studies on maternal serum SP1 levels in Down's syndrome pregnancies in the first trimester.

Reference	No. of cases	Gestation	SP1 median MOM
Brock et al (1990)	21	7-14	0.79
Macintosh et al (1993)	14	6-12	0.40
Aitken et al (1994)	14	7-14	0.73
Bersinger et al (1994)	20	10-11	0.95
	9	12-13	0.68
Brizot et al (1995a)	45[a]	10-13	0.96
Qin et al (1997)	25	5-9	0.27
	14	10-12	0.89

[a] includes cases previously published by Bersinger et al (1994).

Table 4.5.2 Summary of eight published studies on maternal serum SP1 levels in Down's syndrome pregnancies in the second trimester.

Reference	No. of cases	Gestation	SP1 median MOM
Bartels & Lindemann (1988)	24	16-19	2.10
Wald et al (1989)	77	13-27	1.20
Knight et al (1989)	24	NS	1.53
Petrocik et al (1990)	46	15-20	1.98[a]
Bartels et al (1990)	43	14-24	1.54
Graham et al (1992)	48	15-20	1.17
Bartels et al (1994)	50	14-21	1.28
Qin et al (1997)	117	14-20	1.28
Total	429		1.41*

* weighted geometric mean.

[a] estimated from publication.

trend of increasing maternal serum SP1 MOM levels with advancing gestation, similar to that described for PAPP-A (Berry et al 1997). In the present study, SP1 levels were found to be significantly elevated to 1.33 MOM in maternal serum from Down's syndrome pregnancies at 15 to 20 weeks gestation.

In placental tissue from the series of Down's syndrome pregnancies in this study, the majority of which were terminated during the second trimester, SP1 levels were significantly higher (1.77 MOM) than in placental tissue from the control pregnancies (0.91 MOM). Examination of placental SP1 MOM levels in relation to gestation (Figure 3.1.12) revealed a trend of increasing placental SP1 MOM levels with advancing gestation in the Down's syndrome pregnancies. This may correspond to the trend of increasing maternal serum SP1 MOM levels with advancing gestation in Down's syndrome pregnancies (Qin et al 1997). However, there are insufficient data on placental SP1 levels at the early and late gestations to investigate this further.

SP1 levels were also measured in amniotic fluid from 32 Down's syndrome and 132 control pregnancies at 15 to 21 weeks gestation. Amniotic fluid SP1 levels were slightly lower in the Down's syndrome pregnancies than in the normal pregnancies, however the difference was not statistically significant. These results are consistent those of Kelly et al (1994) who reported that the overall MOM level of SP1 (0.83 MOM) in amniotic fluid from 46 Down's syndrome pregnancies at 12 to 20 weeks gestation was not significantly different from the controls (n = 106). However, Kelly et al (1994) noted that at earlier gestations (less than 16 weeks) amniotic fluid SP1 levels in the Down's syndrome pregnancies were significantly lower (0.51 MOM) than in the controls, the effect being less marked if all cases of less than 19 weeks gestation were included. Thus, in amniotic fluid from Down's syndrome pregnancies, SP1 may follow a pattern of increasing MOM levels with advancing gestation similar to that seen in maternal serum (Aitken et al 1994, Qin et al 1997). No such association was found in

the present study, although there was a significant correlation between the concentration of SP1 in maternal serum and corresponding amniotic fluid samples. Results from earlier studies also suggest that amniotic fluid SP1 levels in trisomic pregnancies are normal or slightly reduced (Heikinheimo et al 1984, Wurz et al 1981). Bartels and Lindemann (1988) reported that amniotic fluid SP1 levels were elevated in Down's syndrome pregnancies at 16 and 17 weeks gestation, but were not significantly different from normal at 18 and 19 weeks gestation. However, these results were obtained from the analysis of SP1 in amniotic fluid from 44 Down's syndrome pregnancies and only 39 control pregnancies. The small number of controls in this study (Bartels and Lindemann 1988) may account for the different results.

The elevation in placental levels of SP1 in Down's syndrome pregnancies suggests that there is increased synthesis of SP1 by Down's syndrome placentae in the second trimester. This increased synthesis is not a direct result of increased gene dosage as SP1 is encoded by a family of genes on chromosome 19 (Streydio et al 1990). It is possible that increased placental synthesis of SP1 is the underlying cause of increased maternal serum SP1 levels in the second trimester of Down's syndrome pregnancies. However, there was no correlation between placental and maternal serum levels of SP1 in the Down's syndrome pregnancies. This is in marked contrast to the other placental markers (intact hCG, FβhCG, PAPP-A, placental ALP) which all show some degree of association between placental and maternal serum levels. This may suggest that the placenta is not the principle source of SP1 in the maternal circulation and that other sources may contribute to the increase in maternal serum levels in Down's syndrome pregnancies in the second trimester. As noted above, the amnion and chorion are possible sites of SP1 synthesis. However, if synthesis of SP1 by the amnion and chorion was increased in Down's syndrome pregnancies, amniotic fluid SP1 levels would most likely be elevated as opposed to the normal or slightly reduced levels found in the present study and in the study by Kelly et al (1994).

In contrast to other placental markers, the change in maternal serum SP1 concentrations in Down's syndrome pregnancies is not mirrored in amniotic fluid. However, this is similar to inhibin-A, the concentration of which is increased in maternal serum but reduced in amniotic fluid from Down's syndrome pregnancies (Aitken et al 1996b, Wallace et al 1997). Thus, it is possible that the source of SP1 and inhibin-A in amniotic fluid is different from the source of these markers in the maternal circulation (Wallace et al 1997). Furthermore, in Down's syndrome pregnancies there may be tissue-specific changes in the expression of these proteins. Other possible explanations for the difference in maternal serum and amniotic fluid SP1 concentrations in Down's syndrome pregnancies include increased degradation or clearance of SP1 from the amniotic fluid or defective transport of SP1 from the maternal circulation into the amniotic fluid. However, the latter suggestion is unlikely because of the correlation between maternal serum and amniotic fluid SP1 levels in Down's syndrome pregnancies.

Chard (1991) proposed that the changes in marker levels in maternal serum from Down's syndrome pregnancies were the result of a generalised decrease in products of fetal origin and a generalised increase in products of placental origin. However, the demonstration of reduced PAPP-A and SP1 levels in the first trimester of Down's syndrome pregnancies and normal PAPP-A levels in the second trimester contradicts this theory. The reasons for the reduced maternal serum SP1 levels in the first trimester of Down's syndrome pregnancies remain obscure.

4.5.3 SP1 LEVELS IN TRISOMY 13 AND TRISOMY 18 PREGNANCIES.

SP1 levels in placental tissue, maternal serum and amniotic fluid from the Trisomy 13 and Trisomy 18 pregnancies included in this study are presented in Table 4.5.3. Placental levels of SP1 are elevated in all of the cases, while in maternal serum from

Table 4.5.3 SP1 levels (MOM) in placental tissue, maternal serum and amniotic fluid from Trisomy 13 and Trisomy 18 pregnancies.

Case No.	SP1 MOM (gestation)		
	Placental	Maternal serum	Amniotic fluid
T13/1	7.44 (23)	-	-
T18/1	1.41 (23)	-	-
T18/2	1.30 (22)	-	-
T18/3	1.85 (21)	0.90 (16)	-
T18/4	-	0.81 (15)	0.80 (17)

two Trisomy 18 pregnancies and in amniotic fluid from one Trisomy 18 pregnancy SP1 levels do not appear to be substantially different from normal.

Bartels and Lindemann (1988) reported that three out of four cases of Trisomy 18 had maternal serum SP1 levels above the control median. However, the results of subsequent studies on small numbers of affected cases indicate that maternal serum SP1 levels are normal in both the first and second trimesters of Trisomy 18 pregnancies (Bartels et al 1990, Graham et al 1992, Macintosh et al 1993). Graham et al (1992) reported that maternal serum levels of SP1 were not significantly different from normal in four Trisomy 13 pregnancies and Bartels et al (1990) found SP1 levels of 1.07 MOM and 0.36 MOM in maternal serum from two Trisomy 13 pregnancies.

4.6 ALKALINE PHOSPHATASE.

4.6.1 ALP LEVELS IN NORMAL PREGNANCY.

Total and placental ALP activity was measured in homogenates of placental tissue from 52 control pregnancies at 12 to 20 weeks gestation. Total ALP activity may include contributions from the bone, liver, kidney, intestinal and placental isoenzymes. However, there was no difference between total ALP and placental ALP activities in the placental samples indicating that only the placental isoenzyme is expressed in placental tissue. Immunohistochemical analysis using an antibody specific to the placental ALP isoenzyme demonstrated that ALP was localized to the syncytiotrophoblast of the placenta. Placental ALP is synthesised in the syncytiotrophoblast from around 7 weeks of gestation (Okamoto et al 1990).

The alkaline phosphatase isoenzymes are microvillar enzymes which are bound to the microvillar membrane of cells in a variety of tissues (liver, intestine, kidney, placenta) by means of a phosphatidylinositol-glycan anchor (PI-G). During post-translational processing of the ALP protein, PI-G is attached to the peptide with the concomitant cleavage of a COOH-terminal signal peptide (Low et al 1988, Amthauer et al 1992, DeBroe and Moss 1992). In the placenta, ALP is attached to the external surface of the apical border of the syncytiotrophoblast which is in contact with the maternal blood. Placental ALP probably enters the maternal circulation by dissociation from the trophoblast microvillar membrane.

In the present study, total and placental ALP activities in maternal serum from control pregnancies were found to increase with advancing gestation from 15 to 20 weeks. The contribution of the placental isoenzyme to total ALP activity increased with gestation from 1.8% at 15 weeks to 4.5% at 20 weeks. These results are consistent with previous studies (Brock and Barron 1988, Ind et al 1994a, Aitken et al 1996a).

In amniotic fluid, total ALP activity was found to decrease with gestation from 15 to 21 weeks. Total ALP activity in amniotic fluid is extremely low until 13 weeks gestation, at which stage ALP activity increases to a peak at around 18 weeks gestation, followed by a decline to a plateau with a further increase after 28 weeks gestation (Seelen 1978, Mulivor et al 1979, Muller et al 1988, Campbell et al 1992b). In contrast, levels of the placental isoenzyme in amniotic fluid from the series of control pregnancies in this study were found to increase with advancing gestation from 15 to 20 weeks gestation, consistent with the results of Mulivor et al (1979). Ind et al (1993, 1994b) reported that amniotic fluid levels of placental ALP increased from 12 weeks to a peak at around 18 weeks, followed by a decline to 21 weeks. In the present study, it was found that the contribution of the placental isoenzyme to total amniotic fluid ALP activity increased

from 4.5% at 15 weeks gestation to 5.8% at 20 weeks gestation. The rate of increase of placental ALP activity in the amniotic fluid is less than in the maternal circulation, with amniotic fluid placental ALP levels being two thirds of maternal serum levels at 15 weeks but less than one third of maternal serum levels at 20 weeks.

In amniotic fluid in the first half of pregnancy, total ALP is a combination of the placental isoenzyme (4%), the tissue non-specific bone/liver/ kidney isoenzyme (15%) and the intestinal isoenzyme (81%) (Mulivor et al 1979). The initial increase in total ALP activity in amniotic fluid at around 13 weeks gestation appears to be due to the presence of the intestinal isoenzyme, presumably of fetal intestinal origin following the disappearance of the anal membrane (Mulivor et al 1979, Jalanko et al 1985, Potier et al 1985, Muller et al 1988). The proportion of intestinal ALP activity decreases rapidly after 22 weeks gestation coinciding with the time at which innervation of the anal sphincter occurs (Mulivor et al 1979). The subsequent increase in total ALP activity is probably due to an increase in the levels of fetal-derived tissue non-specific ALP and placental ALP (Mulivor et al 1979). The increase in amniotic fluid placental ALP levels with gestation is probably a reflection of the increased contribution of the placental isoenzyme from the maternal circulation. However, the relative concentration of placental ALP in the amniotic fluid compared to the maternal circulation at 15 weeks gestation is greater than for other trophoblast derived proteins. For example, amniotic fluid intact hCG levels are 1/10 of maternal serum levels and amniotic fluid PAPP-A and SP1 levels are 1/50 of maternal serum levels at 15 to 20 weeks gestation. Placental ALP levels in the amniotic fluid are two thirds of maternal serum levels at 15 weeks of gestation decreasing to less than one third of maternal serum levels at 20 weeks gestation. This suggests that in early pregnancy placental ALP may enter the amniotic fluid by an alternative route or that there may be an another source of placental ALP in the amniotic fluid. Like certain other placental proteins (e.g. hCG

subunits, SP1, inhibin-A), there is evidence to suggest that placental ALP is expressed in the amniotic and chorionic membranes (Plouzek et al 1993, Wallace et al 1996a).

4.6.2 ALP LEVELS IN DOWN'S SYNDROME PREGNANCIES.

Brock et al (1990) investigated placental ALP activity in maternal serum from 21 Down's syndrome pregnancies in the first trimester using a specific immunoassay. Placental ALP levels in the Down's syndrome pregnancies (0.93 MOM) were not significantly different from normal. However, all but two of the Down's syndrome samples were obtained at 7 to 12 weeks gestation, a stage at which maternal serum placental ALP levels are very low (Okamoto et al 1990). Ind et al (1994a) also investigated maternal serum levels of placental ALP in 33 cases of Down's syndrome and 300 controls at 14 to 21 weeks gestation using an immunoradiometric assay. They found a small but significant increase (1.2 MOM) in placental ALP levels in the Down's syndrome pregnancies, an increase which was more marked at later gestations. Aitken et al (1996a) measured both heat stable and immunoreactive ALP in maternal serum from 37 Down's syndrome pregnancies at 15 to 20 weeks gestation. There was no significant difference in the levels of total or placental ALP in the Down's syndrome pregnancies compared to normal. The overall median level of total ALP activity in the Down's syndrome pregnancies was 0.93 MOM. The median placental ALP activity was found to be 1.09 MOM by the heat inactivation assay and 0.96 MOM by immunoassay.

In the present study, total and placental ALP activity was measured in second trimester maternal serum from 54 Down's syndrome pregnancies using a fluorometric end point assay similar to that used by Aitken et al (1996a). There was a small but significant reduction in total ALP activity to 0.83 MOM in the Down's syndrome pregnancies. However, placental ALP activity (1.04 MOM) was not significantly

different from normal. These results are consistent with those of Aitken et al (1996a) in that they show that maternal serum placental ALP levels are unchanged in the second trimester of Down's syndrome pregnancies. There was no evidence to support a trend of increasing maternal serum placental ALP levels in Down's syndrome pregnancies in either the present study or in the study by Aitken et al (1996a). Six of the Down's syndrome cases investigated in this study were also included in the study by Aitken et al (1996a).

It is possible that the difference in the maternal serum levels of placental ALP reported for Down's syndrome pregnancies may arise from the use of different types of assays i.e. specific immunoassays compared to biochemical assays measuring enzyme activity. However, Mulivor et al (1985) measured ALP isoenzyme activities in maternal serum and amniotic fluid samples using a heat denaturation-chemical inhibition method and an immunoprecipitation assay that uses monoclonal antibodies specific for the different ALP isoenzymes. They found a close correlation between the values obtained by both methods. Aitken et al (1996a) also demonstrated a high correlation between the levels of placental ALP in maternal serum estimated using the heat-inactivation fluorometric end-point assay with those obtained using the specific immunoassay. This confirms that the ALP activity measured following heat inactivation is the placental isoenzyme.

In the present study, placental ALP activity was measured in homogenates of placental tissue from 51 Down's syndrome pregnancies. Placental tissue levels of placental ALP in the Down's syndrome pregnancies were not significantly different from the controls. The median levels of placental ALP in placental tissue (0.91 MOM) and maternal serum (1.04 MOM) from the Down's syndrome pregnancies were similar, although the correlation was not significant. Ind et al (1994a) proposed increased synthesis by the placenta or increased dissociation from the trophoblast as possible

mechanisms for the increased maternal serum placental ALP activity in Down's syndrome pregnancies noted in their study. However, the results of the present study together with those of Aitken et al (1996a) suggest that placental synthesis and transport of placental ALP into the maternal circulation is unchanged in Down's syndrome pregnancies.

The results of the present study confirm previous reports of substantially reduced total ALP activity in amniotic fluid from Down's syndrome pregnancies (Jalanko et al 1983a, Morin et al 1987). The median total ALP activity in amniotic fluid from 33 Down's syndrome pregnancies was 0.39 MOM. Amniotic fluid placental ALP activity was also significantly reduced in the Down's syndrome pregnancies compared to normal, although the reduction (0.75 MOM) was less than for total ALP activity. This is consistent with the results of Ind et al (1993, 1994b). In a larger series of amniotic fluid samples from 63 Down's syndrome pregnancies and 756 normal pregnancies, including cases from a previous study (Ind et al 1993), Ind et al (1994b) found that amniotic fluid placental ALP levels were significantly reduced to 0.72 MOM in Down's syndrome pregnancies.

The available evidence suggests that in Down's syndrome pregnancies placental ALP activity is reduced in amniotic fluid but is normal, or only marginally elevated in the maternal circulation. In this way, placental ALP is similar to SP1 and inhibin-A as amniotic fluid levels do not reflect maternal serum concentration changes. The reduced amniotic fluid levels in Down's syndrome pregnancies may be due to defective transport of placental ALP from the maternal circulation, across the fetal membranes, into the amniotic fluid (Ind et al 1994a/b) but there is no evidence in the present study to support a similar abnormality between the placenta and maternal serum. There was a small degree of correlation between the levels of placental ALP in placental tissue and in amniotic fluid from the Down's syndrome pregnancies.

However, placental synthesis of placental ALP appears to be normal, while amniotic fluid placental ALP levels are reduced. This may be further evidence for the existence of an alternative source of amniotic fluid placental ALP activity (e.g. amnion or chorion) and as proposed for SP1 and inhibin-A, there may be tissue-specific differences in the expression of placental ALP in Down's syndrome pregnancies.

The magnitude of the reduction of placental ALP activity in the amniotic fluid is not sufficient to be the cause of the overall reduction in total ALP activity, particularly since the placental isoenzyme accounts for about 4% of total ALP activity in the amniotic fluid. Mulivor et al (1979) reported that at 14 to 22 weeks gestation the major component (81%) of total ALP activity in the amniotic fluid is the intestinal isoenzyme. Thus the reduction in total ALP activity in the amniotic fluid in Down's syndrome pregnancies is likely to be due to a reduction in the fetal intestinal isoenzyme component. Morin et al (1987) found that 7 out of 21 amniotic fluid samples from Down's syndrome pregnancies had levels of L-homoarginine-resistant ALP activity (placental/ intestinal) below the normal range. In these cases the fetuses had intestinal obstructions due to compacted meconium. Brock et al (1984) reported that amniotic fluid intestinal ALP activity (determined from the ratio of phenylalanine-resistant to homoarginine-resistant ALP activity) was reduced in only 11% of Down's syndrome cases, compared to reduced GGT and APM activities in ~50% of Down's syndrome cases. If intestinal obstruction was the principle cause of reduced amniotic fluid microvillar enzyme activity in Down's syndrome pregnancies, the intestinal isoenzyme of ALP would be expected to be reduced in the cases with low GGT and APM levels. Giddy et al (1989) reported that, despite a reduction in total ALP activity in amniotic fluid from Down's syndrome pregnancies, there was no significant difference in the levels of phenylalanine-resistant and homoarginine-resistant ALP activity in Down's syndrome pregnancies compared to control pregnancies. Measurement of individual

isoenzyme components of amniotic fluid ALP activity using isoenzyme-specific antibodies would give a clearer picture of the change in ALP isoenzyme activities in Down's syndrome pregnancies.

4.6.3 ALP LEVELS IN TRISOMY 13 AND TRISOMY 18 PREGNANCIES.

ALP activities in placental tissue, maternal serum and amniotic fluid from the Trisomy 13 and Trisomy 18 pregnancies included in this study are presented in Table 4.6.1. There is no clear trend in placental ALP activity in placental tissue. In maternal serum from two Trisomy 18 pregnancies, the activity of placental ALP was low in relation to total ALP activity. This is comparable with the results of Aitken et al (1996a) who found that total ALP activity and placental ALP activity in maternal serum from 28 Trisomy 18 pregnancies was 0.90 MOM ad 0.79 MOM respectively.

In the present study, the activity of placental ALP in amniotic fluid from one Trisomy 18 pregnancy was low compared to total ALP activity which appeared to be unchanged. Previous studies involving larger numbers of cases have shown that amniotic fluid ALP activity (total) is reduced in association with Trisomy 18 (Jalanko et al 1983a, Morin et al 1987). Ind et al (1994b) found that there was no difference in placental ALP activity in amniotic fluid from Trisomy 18 pregnancies (n=15, median MOM=1.09) compared to normal. Brock et al (1984) reported that in 21% of Trisomy 18 cases studied the ratio of phenylalanine-resistant ALP to homoarginine-resistant ALP was outside normal limits. Ind et al (1994b) found that in Trisomy 13 pregnancies (n=8), amniotic fluid placental ALP activity was reduced to 0.75 MOM.

Table 4.6.1 Total ALP (TALP) and placental ALP (PALP) levels (MOM) in placental tissue, maternal serum and amniotic fluid from Trisomy 13 and Trisomy 18 pregnancies.

Case No.	ALP MOM (gestation)				
	Placenta	Maternal serum		Amniotic fluid	
	PALP	TALP	PALP	TALP	PALP
T13/1	1.43 (23)	-	-	-	-
T18/1	0.70 (23)	-	-	-	-
T18/2	1.07 (22)	-	-	-	-
T18/3	2.11 (21)	1.05 (16)	0.64 (16)	-	-
T18/4	-	0.74 (15)	0.34 (15)	1.09 (17)	0.52 (17)

4.7 GAMMA GLUTAMYL TRANSFERASE.

4.7.1 GGT LEVELS IN NORMAL PREGNANCIES.

GGT is an intrinsic membrane protein which is anchored to the microvillar membrane by a single hydrophobic sequence (~20 amino acids) located on the N-terminus of the larger subunit. The hydrophobic segment acts as both a signal sequence and a hydrophobic anchor sequence, therefore it is not cleaved and the N-terminus remains on the cytosolic side of the membrane. The smaller hydrophilic subunit, on which the enzymatically active site is located, extends from the external lumenal surface of the membrane (Semenza 1986). GGT is initially synthesised as a single precursor with approximately 2% of the activity of the mature dimeric form of GGT (Semenza 1986).

In human tissues GGT activity is present at high levels in the kidney, liver and intestine and at lower levels in the placenta (Rosalki 1975, Nemesansky and Lott 1985). In the liver, GGT is present in the canaliculi of the parenchyma and at greater concentrations in the lumenal border of the cells lining the fine biliary ductules (Rosalki 1975). In the present study, GGT activity was measured in homogenates of fetal liver from control fetuses at 12 to 22 weeks gestation. The specific activity of GGT in fetal liver was found to decrease with advancing gestation.

GGT activity can be detected along the length of the intestine but specific activity is highest in the jejunum, followed by the ileum and then the duodenum (Rosalki 1975, Semenza 1986). Jalanko et al (1983a) reported that meconium and bile from second trimester fetuses contained very high levels of GGT activity. In the present study GGT activity was measured in fetal ileum from control fetuses at 12 to 22 weeks gestation. The amount of meconium contained in the sections of ileum varied considerably,

therefore the contents of the tissue section were gently squeezed out prior to processing of the tissues. In contrast to the fetal liver, GGT activity in the fetal intestine was found to increase with advancing gestation.

GGT activity was also measured in homogenates of placental tissue from 52 control pregnancies at 12 to 20 weeks gestation. Placental GGT activity was approximately 2% of that in the fetal liver and activity was found to decrease slightly with advancing gestation. Jalanko et al (1983b) also found low levels of GGT activity in placental tissue from second trimester fetuses.

In amniotic fluid from unaffected control pregnancies, GGT activity was found to decrease from 15 to 21 weeks gestation. Previous studies have show that amniotic fluid GGT activity increases in early pregnancy to a peak at around 15 weeks gestation, followed by a progressive decrease in activity to term (Jalanko et al 1983b, Brock et al 1984, Moniz et al 1984, Muller et al 1988, Campbell et al 1992b).

The activity of GGT in amniotic fluid in the second trimester is approximately 10 to 100 times greater than in the fetal circulation and maternal circulation (Jalanko et al 1983b, Moniz et al 1984). Furthermore, the activity of GGT in the fetal urine is extremely low (Moniz et al 1984, Muller et al 1988) indicating that the maternal circulation and fetal urine are not major sources of amniotic fluid GGT activity in the second trimester. Moniz et al (1984) demonstrated that the major GGT isoenzyme in amniotic fluid had the same electrophoretic mobility as the GGT isoenzyme in the fetal serum. The authors proposed that GGT activity in fetal serum and amniotic fluid was of placental origin, possibly originating from the cellular debris of placental and amnion tissue. However, Jalanko et al (1983b) found that the specific activity of GGT in the placental was much less than in the amniotic fluid and in other fetal tissues.

The presence of high GGT activity in meconium and bile from second trimester fetuses suggests that these are likely sources of GGT activity in the amniotic fluid (Jalanko et al 1983b, Muller et al 1988). Furthermore, Jalanko et al (1983b) demonstrated that the electrophoretic mobilities of GGT in meconium, bile and amniotic fluid were similar. Jalanko et al (1985) and Potier et al (1986) reported that a high proportion of microvillar enzyme activity in the amniotic fluid was bound to fragments of microvillar membrane. Comparison of the patterns of enzyme activity and morphology of the microvillar fragments from amniotic fluid, meconium and homogenates of fetal tissues revealed that most of the microvillar fragments originated from the fetal gastrointestinal tract.

4.7.2 GGT ACTIVITY IN DOWN'S SYNDROME PREGNANCIES.

The association between low amniotic fluid GGT activity and fetal chromosome abnormalities was first reported by Jalanko and Aula (1982). They found that GGT activity in amniotic fluid from Down's syndrome pregnancies was reduced on average to 53% of normal levels, with 84% of affected cases having GGT levels below the control median. Subsequent studies have confirmed these findings (Brock et al 1984, Baumah et al 1984, Morin et al 1987, Macek et al 1987, Jones and Evans 1988, Giddy et al 1989, Zeitune et al 1989, Szabo et al 1990). The results of the present study are consistent with previous studies, with GGT levels reduced to 0.48 MOM in amniotic fluid from 33 Down's syndrome pregnancies. Ninety-one percent of affected cases had amniotic fluid GGT levels below the control median.

The reasons behind the reduction in amniotic fluid GGT activity in Down's syndrome pregnancies are unknown. Morin et al (1987) found deficient microvillar enzyme activities in amniotic fluid from 7 out of 21 Down's syndrome pregnancies. The cases

with abnormal microvillar enzyme activity all had intestinal obstructions and it is feasible that intestinal abnormalities may prevent the passage of microvillar enzymes into the amniotic fluid. However, Brock et al (1984) found that amniotic fluid intestinal ALP levels were reduced in only 11% of Down's syndrome cases compared to reduced GGT and APM activities in approximately 50% of cases. Intestinal ALP activity would be expected to be reduced in those cases with reduced GGT and APM activity if intestinal obstruction was the principle cause of reduced amniotic fluid microvillar enzyme activity in Down's syndrome pregnancies. In contrast, Muller et al (1988) reported that in later pregnancy (> 27 weeks gestation) duodenal atresia was associated with very high levels of GGT activity, possibility caused by fetal regurgitation of bile. In a previous study, Muller at al (1986) reported that 51 out of 54 cases of Down's syndrome had GGT levels below the median, however, in two cases amniotic fluid GGT activity was greater than the control median. The authors proposed that the in these two cases duodenal atresia was present.

In the present study, post mortem reports were available for 25 of the Down's syndrome cases from which amniotic fluid samples were collected. Only three of the cases with reduced amniotic fluid GGT activity were found to have intestinal abnormalities (Meckels diverticulum).

In an attempt to identify the origin of reduced GGT activity in amniotic fluid in association with fetal Down's syndrome, GGT activity was measured in homogenates of fetal liver, fetal ileum and placental tissue from Down's syndrome pregnancies. GGT activity was significantly higher in fetal liver (2.01 MOM) and placental tissue (1.29 MOM) from Down's syndrome pregnancies compared to normal. However, GGT activity in fetal ileum from Down's syndrome pregnancies was significantly lower than in controls. Thus, GGT activity is reduced to a similar extent in fetal ileum and amniotic

fluid from Down's syndrome pregnancies. This is consistent with the results of Gracy (1992) who found that there was a significant reduction in GGT activity in both the microvillar and supernatant fractions of amniotic fluid from 12 Down's syndrome pregnancies. Amniotic fluid GGT activity is reduced in Down's syndrome pregnancies despite the increase in activity in the fetal liver and placenta. These results suggest that the fetal intestine is a major source of GGT activity in the amniotic fluid and that in Down's syndrome pregnancies GGT activity from this source is reduced.

The mechanisms behind the reduction in fetal intestinal and amniotic fluid GGT activity in Down's syndrome pregnancies are not known. One possible explanation is that in Down's syndrome pregnancies there is reduced synthesis of GGT by the fetal intestine, however this is difficult to reconcile with the increased levels of GGT activity observed in the fetal liver and placenta. It is thought that GGT is derived from the expression of a single gene with different isoenzymes arising from tissue-specific differences in post-translational modifications of the GGT peptide (Nemensky and Lott 1986). However, Courtay et al (1994) reported that four GGT genes are expressed in humans in a tissue-specific manner. Tissue specific differences in GGT activity in Down's syndrome pregnancies may be a result of alterations in the tissue-specific expression of GGT activity or defects in post-transcriptional processing of the enzyme or in membrane anchorage or release. The presence of an extra copy of chromosome 21 would not have a direct effect on the expression of GGT as the genes encoding GGT are located on chromosome 22 (Bulle et al 1987). Such changes may be the indirect result of an increase in expression of chromosome 21 genes. The glycosylation of GGT is not altered in trisomic pregnancies. Jalanko et al (1983b) found that the electrophoretic mobility of GGT in second trimester amniotic fluid from trisomic pregnancies was similar to that from normal pregnancies.

Unlike the other markers, GGT is not pregnancy-specific and there are no published studies on maternal serum levels of GGT in Down's syndrome pregnancies. However, there are distinct fetal and adult forms of the enzyme which are distinguishable by their ability to bind to Concanavalin A (Kottegen et al 1976). Rosalki (1975) reported that GGT levels in the maternal circulation decrease with advancing gestation, while other studies have reported that there is no significant change in maternal serum GGT levels during pregnancy (Lum and Gambino 1972, Jalanko et al 1983b, Moniz et al 1984). This would suggest that despite the considerable concentration gradient which exists between the amniotic fluid and maternal circulation, GGT does not appear to cross the fetal membranes into the maternal circulation. This may be due to the fact that much of the GGT activity in the amniotic fluid is bound to microvillar fragments. If the maternal circulation does contain any fetal GGT, it is probably present in only small amounts and is most likely to be of placental origin. Identification of a fetal GGT component in maternal serum from Down's syndrome and unaffected pregnancies may provide further information about the placental synthesis of GGT and the transport of GGT from the placenta to the maternal circulation

4.7.3 GGT IN TRISOMY 13 AND TRISOMY 18 PREGNANCIES.

Jalanko and Aula (1982) reported that amniotic fluid GGT levels were below the 25th centile in seven out of nine pregnancies with a Trisomy 18 fetus. The median value of GGT in the affected pregnancies was one third of that observed in the controls. Low GGT activity was also found in amniotic fluid from one Trisomy 13 pregnancy. Subsequent studies have confirmed that GGT activity is reduced in amniotic fluid from Trisomy 18 and Trisomy 13 pregnancies (Morin et al 1987, Macek et al 1987, Jones and Evans 1988, Zeitune et al 1989). In the present study, amniotic fluid from one

Trisomy 18 pregnancy had a GGT activity of 0.11 MOM, consistent with previous studies.

GGT activity in fetal liver, fetal ileum, placental tissue and amniotic fluid from the Trisomy 13 and Trisomy 18 pregnancies included in this study are presented in Table 4.7.1. The pattern of variation of GGT activity in fetal tissues from the Trisomy 13 and Trisomy 18 pregnancies is similar to that in Down's syndrome pregnancies. GGT levels are elevated in placental tissue and fetal liver from all cases, except one Trisomy 18 pregnancy which has a hepatic GGT level of 1.06 MOM. GGT activity was low in fetal ileum from all cases. Therefore, the mechanisms which give rise to abnormal GGT activity may be similar in Down's syndrome, Trisomy 13 and Trisomy 18 pregnancies.

4.8 PLACENTAL PATHOLOGY.

Post-mortem examination of the placenta was carried out for 31 of the Down's syndrome cases and one of the Trisomy 18 cases included in this study. General pathological findings included fibrinoid degeneration, placental calcification, villous oedema and placental hemorrhage. These are common pathological changes which do not appear to be of particular clinical importance (Fox 1986). However, it has been proposed that villous oedema results in the restriction of maternal blood flow (Fox 1986).

In Down's syndrome pregnancies, there does not appear to be any difference in placental weight compared to normal at equivalent gestations (Shepard et al 1989). However, in Trisomy 18 pregnancies placental weight is generally lower than in normal pregnancies (Shepard et al 1989).

Table 4.7.1 GGT levels (MOM) in fetal liver, fetal ileum, placental tissue and amniotic fluid from Trisomy 13 and Trisomy 18 pregnancies.

Case No.	GGT MOM (gestation)			
	Placenta	Fetal liver	Fetal ileum	Amniotic fluid
T13/1	2.68 (23)	7.58 (23)	0.35 (23)	-
T18/1	1.20 (23)	6.39 (23)	0.30 (23)	-
T18/2	4.18 (22)	3.97 (22)	0.81 (22)	-
T18/3	2.75 (21)	3.14 (21)	0.52 (21)	-
T18/4	-	1.06 (19)	0.33 (19)	0.11 (17)

Honore et al (1976) investigated the association between placental morphology and fetal trisomy in a study of 66 trisomic placentae and 172 controls at 4-22 weeks gestation. On gross examination, two-thirds of the trisomic placentae were found to have cystic villi. On microscopical examination, the trisomic placentae were found to exhibit villous hypovascularity, persistence and irregular distribution of immature stromal cells and failure of development of Hofbaur cells (macrophages). Furthermore, the trisomic placentae often contained cytotrophoblast cells in the villous core, large numbers of syncytial sprouts and showed evidence of reduced trophoblastic growth activity and a lack of villous structural integrity. There did not appear to be an association between placental morphology and specific types of trisomy.

Oberweis et al (1983) reported that in 60% of placentae examined at 28-40 weeks gestation, there was marked retardation of villous maturation with persistence of embryonic forms of villi. Irregular and immature villous maturation, under vascularisation and increased vascular resistance of third trimester trisomic placentae was also reported by Rochelson et al (1990).

Morphological changes of the placenta associated with abnormal maturation of placental villi, trophoblast differentiation and fetal villous and utero-placental blood flow would be expected to result in the impaired synthetic, secretory and transport capacity of the placenta. Thus, placental pathology may play a key role in the production of placental markers, notably intact hCG and FβhCG which is in turn reflected in the concentrations of these markers in Down's syndrome pregnancies.

Impaired maturation of placental villi appears to the characteristic of the Down's syndrome placenta. Villous maturation involves a progressive change from large villi with centrally placed fetal capillaries to smaller villi with fetal capillaries located in close

contact with the syncytiotrophoblast (Fox 1986). The process of trophoblast differentiation is an important part of villous maturation. Initially the cytotrophoblast cells form a continuous layer beneath the syncytiotrophoblast but as gestation advances the cytotrophoblast cells become less prominent. Within the syncytiotrophoblast there are regions which become specially adapted for specific functions (Chard 1986, Fox 1986). There are thin areas of syncytiotrohoblast which are in direct contact with the fetal capillaries and are the principle site of feto-maternal transfer (vasculosyncytial membranes). Thicker areas of syncytiotrophoblast are specialised for protein synthesis. In Down's syndrome placentae there is persistence of immature forms of villi with increased numbers of cytotrophoblast cells. In addition, abnormal villous maturation may prevent the formation of vasculosyncytial membranes. Thus the Down's syndrome placenta may have an increased capacity for protein synthesis and a decreased capacity for feto-maternal transfer.

4.9 SUMMARY AND CONCLUSIONS.

Investigation of endogenous levels of feto-placental markers in tissues involved in synthesis and/ or transport and in corresponding maternal serum and amniotic fluid from chromosomally abnormal pregnancies makes it possible to identify the stage at which the normal process of protein synthesis, secretion and transport is disrupted.

In this study it was shown that the concentration of AFP in fetal liver from Down's syndrome pregnancies was not significantly different from that in unaffected pregnancies. Thus reduced synthesis of AFP by the fetal liver does not appear to be the cause of low maternal serum and amniotic fluid AFP levels during the first half of Down's syndrome pregnancies. Elevated placental levels of AFP in Down's syndrome pregnancies indicate that placental transport of AFP from the fetal circulation to the maternal circulation may be disturbed in Down's syndrome pregnancies resulting in the

accumulation of AFP in the syncytiotrophoblast and reduced maternal serum AFP levels. Abnormal placental morphology in trisomic pregnancies may cause a reduction in the capacity of the placental for feto-maternal transfer. Alternatively, in Down's syndrome pregnancies there may be defects associated with the specific mechanisms of AFP transport across the placenta. Reduced amniotic fluid levels of AFP in Down's syndrome pregnancies may be caused by abnormal fetal kidney function resulting in reduced transfer of AFP from the fetal circulation into the amniotic fluid.

Intact hCG and FβhCG concentrations were significantly elevated in placental tissue from Down's syndrome pregnancies. A significant elevation was also observed for SP1, although the magnitude of elevation was much less than for intact hCG and FβhCG. Thus, there may be increased synthesis of hCG subunits and SP1 by the placenta in Down's syndrome pregnancies. A gene dosage effect can be ruled out as a possible cause of increased hCG, FβhCG and SP1 levels in Down's syndrome pregnancies as the genes encoding these proteins are not located on chromosome 21. PAPP-A and placental ALP levels in placental tissue from Down's syndrome pregnancies were not significantly different from normal suggesting that placental synthesis of these proteins is not altered. There is evidence to suggest that there is abnormal maturation of placental villi in trisomic placentae. Morphological changes to the placenta may play a role in the altered synthesis of certain placental markers in Down's syndrome pregnancies.

Similar trends for each of the markers (intact hCG, FβhCG, PAPP-A, SP1 and placental ALP) were observed in maternal serum from the same series of Down's syndrome pregnancies. This relationship between placental and maternal serum marker levels suggests that transport of these markers from their site of synthesis in the placenta to the maternal circulation in Down's syndrome pregnancies is not

disturbed. Unlike the other placental markers which all show some degree of association between placental and maternal serum levels, there was no correlation between placental and maternal serum SP1 levels in Down's syndrome pregnancies. This may be an indication of an alternative source of SP1 which contributes to SP1 levels in maternal serum.

The change in concentrations of AFP, intact hCG and FβhCG in amniotic fluid in Down's syndrome pregnancies corresponds to the changes observed for each of these markers in maternal serum in the second trimester. AFP is reduced to a similar extent in maternal serum and amniotic fluid from Down's syndrome pregnancies. Intact hCG and FβhCG levels are increased in amniotic fluid from Down's syndrome pregnancies, although the magnitude of elevation is less than in maternal serum. This may reflect differences in the rates of clearance of hCG and free hCG subunits from the two different compartments. In contrast, the change the concentrations of SP1 and placental ALP in amniotic fluid from Down's syndrome pregnancies varies from that observed in maternal serum. SP1 levels are elevated in maternal serum but unchanged or slightly reduced in amniotic fluid, while placental ALP activity is unchanged in maternal serum and low in amniotic fluid from Down's syndrome pregnancies. This suggests that the sources of amniotic fluid SP1 and placental ALP and maternal serum SP1 and placental ALP may be different, and that in Down's syndrome pregnancies there may be tissue specific differences in the expression of these proteins.

GGT activity was found to be reduced to a similar extent in fetal ileum and in corresponding amniotic fluid from Down's syndrome pregnancies, suggesting that amniotic fluid GGT activity may originate from the fetal intestine. GGT activity was elevated in fetal liver and placental tissue from Down's syndrome pregnancies. Tissue

specific changes in GGT activity in Down's syndrome pregnancies maybe the result of tissue specific alterations in GGT expression or defects in post-transcriptional processing of the enzyme, or in membrane anchorage or release.

The pattern of marker changes in fetal tissues, maternal serum and amniotic fluid from Trisomy 13 and Trisomy 18 pregnancies varied from the changes seen in Down's syndrome pregnancies. Therefore, the mechanisms giving rise to marker variations in Down's syndrome, Trisomy 13 and Trisomy 18 pregnancies are not the same.

4.10 FUTURE WORK.

The present study has revealed significant differences in fetal tissue and placental marker levels between Down's syndrome and unaffected pregnancies, but it is not clear how the presence of an extra copy of chromosome 21 leads to the observed changes. As none of the markers has a structural gene on chromosome 21, a direct gene dosage effect can be excluded. However, it is possible that the observed changes in marker concentrations are the result of altered cellular metabolism due to increased dosage of chromosome 21 genes.

A fuller picture of marker variation in Down's syndrome pregnancies could be obtained by the investigation of other markers in fetal tissues and in corresponding maternal serum and amniotic fluid. UE_3 levels are known to be reduced in first and second trimester maternal serum and in amniotic fluid from Down's syndrome pregnancies. Estriol is produced by the syncytiotrophoblast from the fetal precursor 16α-hydroxy-dehyroepiandrosterone-sulphate and is metabolised via conjugated forms by the maternal liver. Reduced synthesis due to a diminished supply of fetal precursor, rather than altered placental secretion, has been proposed as the underlying cause of low

UE_3 in Down's syndrome pregnancies (Cuckle et al 1991). Inhibins and activins are involved in the regulation of hCG synthesis. Maternal serum inhibin, and more specifically inhibin-A levels are elevated in Down's syndrome pregnancies. With the development of assays specific for inhibin-A, inhibin-B, activin-A, activin-B and α-subunit precursors (Wallace and Healy 1996), it will be possible to investigate the physiology of inhibins and activins in Down's syndrome and unaffected pregnancies. Further information may also be obtained from the study of other components of cell regulation and differentiation e.g. epidermal growth factor, fibroblast growth factor and transforming growth factors-α and -β. These growth factors may play a role in the regulation of trophoblast differentiation and hCG secretion. Certain markers have been identified which have a chromosome 21 gene location e.g. heat shock protein 70, superoxide dismutase. It would be of particular interest to examine the levels of these markers in fetal tissues, maternal serum and amniotic fluid from Down's syndrome pregnancies.

Perhaps a clear comparison of the rate of production of placental markers could be obtained by measurement of mRNA levels in fetal tissues. However, this may prove to be unsatisfactory in post-abortion material where the lability of mRNA may lead to difficulties in interpretation. Such investigations would be better carried out using trophoblast cultures from Down's syndrome and unaffected pregnancies, matched for gestational age and stage of culture (Eldar-Geva et al 1995). Systems for the isolation and culture of placental trophoblast have been developed (Ringler and Strauss 1990, Bloxam et al 1997a/b), and such an approach could also be used to further investigate the process of differentiation of cytotrophoblast into syncytiotrophoblast and the relative effects of exogenous materials (e.g. growth factors, cyclic AMP, inhibin, activin, IFNα) on the production of placental proteins, in particular hCG.

Feto-maternal transfer of substances across the placenta could also be studied using trophoblast culture systems. Sophisticated two-sided syncytiotrophoblast cultures may provide the basis of an investigation into the mechanisms of placental transfer/ release of AFP, hCG and other markers in Down's syndrome and unaffected pregnancies (Bloxam et al 1997a/b). Characterisation of cell surface receptors for AFP may also provide further insight into possible receptor-mediated transport mechanisms for AFP.

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