AN EVALUATION OF FETAL GROWTH IN HUMAN IMMUNODEFICIENCY VIRUS INFECTED WOMEN AT KHAYELITSHA AND GUGULETHU MIDWIFERY OBSTETRIC UNITS IN THE WESTERN CAPE

by

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DECLARATION:

I, Ferial Isaacs, hereby declare that the content of this thesis represent my own work, and that the thesis has not previously been submitted for academic examination towards any qualification.

Signed: Isaacs

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ABBREVIATIONS

AC	Abdominal Circumference
AFI	Amniotic Fluid Index
AIDS	Acquired Immunodeficiency Syndrome
ANOVA	Analysis of Variance
AO	Acoustic Output
APH	Antepartum Haemorrhage
ARVT	Antiretroviral Therapy
BMI	Body Mass Index
BPD	Biparietal Diameter
CD4	CD4 T-lymphocyte count
CI	Confidence Interval
CPD	Cephalo-Pelvic Disproportion
CS	Caesarian Section
DOB	Date of Birth
EDD-LMP	Estimated Due Date according to Last Menstrual Period
EDD-US	Estimated Due Date according to Ultrasound
EFW	Estimated Fetal Weight
ELISA	Enzyme-linked immunosorbant assay
FL	Femur Length
GMOU	Gugulethu Midwifery Obstetric Unit
GA-US	Gestational Age by Ultrasound
GPH	Gestation Proteinuria Hypertension
GSH	Groote Schuur Hospital

HAART	Highly Active Antiretroviral Therapy
HC	Head Circumference
HELLP	Haemolysis, Elevated Liver Enzymes, Low Platelet
HIV	Human Immunodeficiency Virus
IOL	Induction of Labour
IUD	Intrauterine Death
IUGR	Intrauterine Growth Restriction
KMOU	Khayelitsha Midwifery Obstetric Unit
LBW	Low Birth Weight (<2500 grams)
LNMP	Last Normal Menstrual Period
MMH	Mowbray Maternity Hospital
NVD	Normal Vertex Delivery
NVP	Nevirapine
PCR	Polymerase chain reaction – testing for presence of HIV in the body / viral load test
PMTCT	Prevention of Mother-to-Child Transmission
PPH	Postpartum Haemorrhage
PROM	Premature Rupture of Membranes
PTD	Preterm Delivery
PTL	Preterm Labour
RPOC	Retained Products of Conception
RVD	Retroviral Disease
SB	Stillbirth
SFH	Symphysis Fundal Height

TGC	Time Gain Compensation
TOP	Termination of Pregnancy
VDRL	Venereal Diseases Reference Laboratories
VTC	Voluntary Testing and Counselling
ZDV	Zidovudine

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1. SUMMARY

A prospective cohort study was done on Human Immunodeficiency Virus (HIV) infected and uninfected women attending Khayelitsha Midwifery Obstetric Unit (MOU) and Gugulethu MOU from June 2003 to December 2004, primarily to establish whether there is an association between HIV infection and Intra-uterine growth restriction (IUGR). B-Mode real time ultrasound imaging was used to monitor fetal growth from ±22 weeks to 36 weeks gestational age. Birth weight, gestational age at delivery, gender, placental weight, and maternal complications were also included. Maternal factors considered included age, weight, parity, singleton versus multiple pregnancy, previous IUGR or preterm delivery, previous fetal abnormality, social habits viz. cigarette smoking, alcohol and drug use, and vascular disease viz. Diabetes, hypertension, renal disease, cardiac disease and collagen disease. A secondary objective was to establish whether the CD4 T-lymphocyte count possibly modulated the presence of IUGR. All HIV infected women were given antiretroviral therapy according to the standard Protocol of the Provincial Government of Western Cape (2002).

The research questions were:

- Does maternal HIV infection increase the risk of intrauterine growth restriction and associated preterm delivery?
- Does the immune status of (CD4 T-lymphocyte count) of HIV infected pregnant women modulate fetal growth?

The primary objective of this study was to establish whether there is an association between HIV infection and IUGR, and hence that HIV infection leads to an adverse perinatal outcome.

Ultrasound was used as a diagnostic tool to establish normal or abnormal fetal growth patterns. Anecdotal reports from health workers in the obstetric field suggested that IUGR and preterm delivery may be associated with low birth weight infants in HIV infected pregnant women. However, preterm delivery is associated with various other factors including low socio-economic status (poor nutrition), cigarette smoking, drug and alcohol abuse, previous history of preterm delivery, over distention of the uterus (hydramnios, multiple gestation), premature rupture of membranes, cervical incompetence, vaginal infections (bacterial vaginosis) and maternal disease e.g. hypertension, heart disease (Lizzi, 1993; Symmonds, 1992; Odendaal et al, 2002). HIV is now thought to be an added factor. After doing a systematic review and meta-analysis of 31 studies, Brocklehurst and French (1998) reported that there is an association (although not strong) between HIV infection and adverse perinatal outcome in developed countries; but in developing countries, there is an increased risk of infant death. By excluding or controlling for confounding variables that could affect fetal growth, this study aimed to determine whether there is a significant association between HIV and fetal growth by comparing fetal growth in HIV infected and uninfected women from mid-second trimester to the time of delivery.

A secondary objective was to establish whether there is an association between the immune status (CD4 T-lymphocyte count) of the mother and IUGR. The immune status of the mother is probably one of the most important factors affecting the fetus and perinatal outcome. As the mother's viral load increases, her immune system is increasingly compromised, resulting in the occurrence of HIV-related diseases, and a concurrent increase in fetal complications. In this study a CD4 T-lymphocyte count was used to assess the level of immunodeficiency of all

the HIV infected participants. Ideally the test should have been done each time the participant was scanned so that the CD4 T-lymphocyte count could be monitored simultaneously with the fetal growth parameters, however due to financial constraints and ethical considerations, one test was done on each HIV infected women.

This study was based at two MOU's where different antiretroviral therapy (ARVT) regimens were used. The one MOU offered Zidovudine (ZDV) to mothers from 34 weeks gestation to the onset of labour, and the other MOU offered Nevirapine (NVP) as a single dose to the mother at the onset of labour and to the neonate within 72 hours of birth (Provincial Government Western Cape, 2002). This presented an opportunity to compare two groups of HIV infected women on different regimes. The intention was to establish whether ZDV had an adverse effect on fetal growth and resulted in low birth weight. However, 6 months after the study started a revised Prevention of Mother to Child Transmission (PMTCT) Protocol was implemented where women at both MOU's received the same ARVT i.e. ZDV and NVP. This objective was therefore abandoned due to a change in the PMTCT Protocol in the Western Cape.

The study was based at two Midwife Obstetric Units (MOU) in the Western Cape where the prevalence of HIV in pregnant women is relatively high i.e. 20 – 24 % (Mother-to-child-transmission Monitoring Team, 2001), viz. Gugulethu MOU and Khayelitsha MOU.

A prospective cohort study was done with the intention of recruiting a sample of 400 pregnant women, 200 HIV infected and 200 uninfected. The actual sample size was 415. The study

group was 194 HIV infected women and the control group was 221 uninfected women. Confounding variables such as cigarette smoking, alcohol and drug abuse, multiple gestation, grand multipara pregnancy, history of IUGR or preterm delivery, fetal abnormality detected at the time of the first scan in the current pregnancy, and maternal vascular disease – were excluded. Confounding variables such as maternal age, maternal weight and gestational age were controlled.

Ultrasound imaging was used as a diagnostic tool to establish normal and abnormal fetal growth patterns. A B-mode real time ultrasound unit was used to confirm the gestation age and rule out any obvious fetal abnormalities at 20-24 weeks gestation. Fetal growth scans were done at 28 weeks, 32 weeks and 36 weeks gestation to compare fetal growth patterns in the study and control groups. Fetal biometry used to monitor fetal growth included biparietal diameter (BPD), head circumference (HC), femur length (FL), abdominal circumference (AC) and estimated fetal weight (EFW). Amniotic fluid index (AFI), placental thickness & placental grading were also included.

The following variables were analyzed post delivery:

- Gestation age at delivery: Normal term delivery is considered to be at 37 – 42 weeks and premature delivery is considered to be less than 37 weeks gestation. The HIV infected and uninfected groups were compared to assess if there was a significant difference in the number of preterm deliveries.
- Birth weight: The HIV infected and uninfected groups were compared to assess if there was a significant difference in the number of infants with low birth weight.

- Perinatal complications: The HIV infected and uninfected groups were compared to assess if there was a significant difference in the number of perinatal complications and to assess if there was an association between the immune status (CD4 T-lymphocyte count) of HIV infected women and perinatal complications.

Appropriate ethical principles in medical research were applied. The participant's autonomy, rights and best interests were always considered a priority. Informed consent was obtained from all the participants. Strict confidentiality was adhered to regarding any data collected throughout the study. The Research Ethics Committees at Cape Peninsula University of Technology and University of Cape Town granted ethics approval for the study.

Statistical analysis was performed using the statistical package SPSS 12.0.

2. LITERATURE REVIEW

2.1 HUMAN IMMUNODEFICIENCY VIRUS (HIV)

2.1.1 HIV Statistics in the Western Cape

HIV prevalence in South African women attending antenatal clinics in 2002 was 26.5% (CI: 25.5-27.6). HIV prevalence in the Western Cape among women attending antenatal clinics in 2002 was 12.4% (CI: 8.8-15.9) (Department of Health, South Africa, 2002). HIV and AIDS are more commonly found in low socio-economic and poverty-stricken communities. Khayelitsha and Gugulethu are 2 such peri-urban areas located 20-30 km outside of Cape Town. According to statistics from a Prevention of Mother-To-Child-Transmission (PMTCT) Monitoring Team, 24.3% of pregnant women presenting at Khayelitsha MOU, and 20.8% presenting at Gugulethu MOU, were HIV infected (Provincial Government Western Cape, 2002). This study took place at Gugulethu MOU and Khayelitsha MOU in the Western Cape. The PMTCT Programme Protocol in the Western Cape (Provincial Government Western Cape, 2002) includes voluntary testing and counselling (VTC) and treatment. At the start of the study the PMTCT Programme was running at both MOU's. However it seemed to be better established at the Khayelitsha MOU because it had been implemented a lot earlier at this centre than at the other MOU.

There are many factors that could adversely affect fetal wellbeing and perinatal outcome in HIV infected women. These may include the maternal immune status in HIV infected women, mother-to-child-transmission and antiretroviral therapy (ARVT).

2.1.2 Maternal Immune Status in HIV Infected Women

When pregnant women present with major symptoms of HIV-related disease (i.e. when CD4 T-lymphocyte count drops to 200 – 350 cells / mm^3) or with clinical signs of AIDS (i.e. when CD4 T-lymphocyte count < 200 cells / mm^3), there may be an increase in fetal complications including IUGR (Evian, 2000). Dreyfuss et al (2001) found that a low CD8 count was associated with low birth weight. It is generally accepted that symptomatic HIV infected pregnant women present with increased fetal complications and adverse neonatal outcome, but there is little evidence to suggest that asymptomatic HIV infected women are also at risk of fetal complications. In this study, both symptomatic and asymptomatic women were monitored. A CD4 T-lymphocyte count was done for all HIV infected participants in this study.

2.1.3 Mother-to-Child Transmission (MTCT)

MTCT of the virus may occur at various stages of pregnancy i.e. intrauterine (trans-placental), intrapartum (during labour and delivery) or postpartum (breast-feeding). There is little scientific evidence of exactly when or how transmission of the virus from the mother to the fetus or child occurs but according to Cronje (2003) 50% or more HIV infections occur during labour or delivery.

Transmission of the virus from mother to fetus or child depends on the HIV status of the mother and the availability of ARVT. A low CD4 T-lymphocyte count or high viral load will increase the risk of transmission. Interventions like caesarean section delivery, vaginal lavage and formula feeding will reduce the risk of MTCT (Evian, 2000). The incidence of MTCT is 20 – 40% with no antenatal, intrapartum or postpartum intervention, reducing to less

than 10% with ARVT (monotherapy) and to less than 2% with ARVT and caesarean section (Cronje, 2003). Elective caesarean section is not considered a good option for developing countries like South Africa because of financial constraints and the risk of post-operative complications in HIV infected women who have a higher mortality rate (Coovadia & Coutsoudis, 2000). In the developed world (USA & Europe) the transmission rate is reported to be less than 5% since the implementation of the AIDS Clinical Trials Group 076 (ACTG 076) regimen (Guay et al, 1999) that focuses on ARVT as the most effective option (Coovadia & Coutsoudis, 2000).

2.1.4 Antiretroviral Therapy (ARVT)

A randomized controlled study in Thailand (Shaffer et al, 1999) and the HIVNET 012 randomized trial in Uganda (Guay et al, 1999) have demonstrated that short course ARVT reduced the risk of MTCT by 50% to 30% in developing countries. In developed countries (USA and France) long course ZDV administered to pregnant women from as early as 14 weeks gestation, during labour and to the infant for 6 weeks, has been shown to reduce the risk for transmission by two thirds (ACTG 076) (Guay et al, 1999). This method of ARVT was considered too costly and complex to administer in developing countries. The randomized controlled trial in Thailand demonstrated that short course ZDV administered from 36 weeks gestation and during labour reduces the risk of transmission by 50% (Shaffer et al, 1999). A study in West Africa has suggested that ZDV is a safe and beneficial intervention for reducing the risk of MTCT (Dabis et al, 2001). A study in Uganda (United States of America: National Institute of Allergy & Infectious Diseases, 2000) has also shown that NVP is more effective than ZDV. NVP is also more cost effective and being simpler to administer (single dose

compared to 4 weeks for ZDV), was the preferred choice in developing countries. Single dose NVP however has since been found to be unsuitable because of the increased risk of resistance when used as a single dose. Single dose NVP was initially used at one of the MOU's but the regimen was later changed to a combination of NVP and ZDV.

A Cochrane review of four trials (Brocklehurst & Volmink, 2005) has shown evidence that ZDV reduces the risk of MTCT. It was also shown that a "short-short" course of ZDV (from 35 weeks gestation for the mother and 72 hours after birth for the infant) is associated with a higher risk of transmission than a "long-long" course (from 28 weeks gestation for the mother and 6 weeks after birth for the infant). With reference to NVP, this review has shown that when given to mothers as a single dose at the onset of labour and as a single dose to the infant within 72 hours of birth, it is more effective than ZDV used intrapartum or postpartum. The authors concluded that there was sufficient evidence to suggest that short course ZDV and a single dose NVP are effective for reducing MTCT. This regimen of ZDV and NVP is currently used in the Western Cape.

Short course ARVT used at the two MOU's (Provincial Government Western Cape, 2002):

- Short course ZDV is administered from 34 weeks gestation through delivery. However due to the high number of preterm deliveries resulting in a shorter period for ZDV to be effective, women at risk for preterm deliveries are given ZDV earlier than 34 weeks. Women should receive at least 2 - 3 weeks of ZDV before delivery.
- Women receive a single dose of NVP when in labour.
- The neonate receives a single dose of NVP after delivery and a 7-day course of ZDV.

2.2 INTRAUTERINE GROWTH RESTRICTION (IUGR)

It is important to distinguish between small-for-gestation fetus and IUGR. Both can be defined as a fetus with a weight less than 10% for gestational age. Small-for-gestation can however refer to a small but healthy fetus whereas IUGR is a pathological condition that implies abnormal fetal growth (Bamberg & Kalache, 2004).

IUGR is closely associated with low birth weight and preterm delivery.

2.2.1 Low Birth Weight

Low birth weight is commonly defined as an infant born with weight less than 2500g (Sanders, 1998) in developed countries. However Steyn (2003) suggests that in developing countries the average birth weight is lower and therefore low birth weight can be defined as less than 2000g or even less than 1500g. Low birth weight according to the protocol at the 2 MOU's is defined as less than 2500g (Perinatal Education Programme: Maternal Care, 1998).

2.2.2 Preterm Delivery

Preterm delivery (PTD) is defined as an infant born before 37 completed weeks (Lizzi, 1993; Steyn, 2003). Accurate gestational ageing as early as possible is very important for estimating the time of delivery, and hence effectively diagnosing preterm labour and the management thereof.

In developed countries there does not seem to be an association between HIV infection and IUGR, preterm delivery, low birth weight and stillbirth (Brockelhurst & French, 1998). However, studies in developing countries have shown an association between HIV infection and IUGR, preterm delivery and low birth weight with a much higher rate of perinatal

mortality in women with advanced HIV infection (Brockelhurst & French, 1998). A study in Tanzania (Coley et al, 2001) has shown that there is a significant increase in the risk of low birth weight and preterm delivery for symptomatic HIV infected women. A study in Rwanda (Weng et al, 1998) showed a significant difference in fetal growth, birth weight and gestation age at birth when comparing HIV infected to uninfected mothers.

Birth weight and term / preterm delivery were compared between the HIV infected and uninfected women in this study.

2.2.3 Intrauterine Growth Restriction Classification

Reece et al. (1994) categorizes IUGR into two clinical types determined mainly by the cause, time of onset and duration of the restricted growth:

Type I: Symmetric or Early Onset IUGR

Type I IUGR refers to intrinsic fetal problems that are usually caused by genetic disease, chromosomal anomaly or early intrauterine infection. The potential of fetal growth is hampered from early on in the pregnancy (Jaffe & Abramowicz, 1997). All fetal measurements are usually smaller for gestation.

Type II: Asymmetric or Late Onset IUGR

Type II IUGR may be caused by extrinsic factors like maternal disorders (e.g. hypertension, renal/ cardiac disease), alcohol, drugs, cigarettes, multiple gestation, and placental abnormalities. Most of the complications relate to placental insufficiency (lack of oxygen and

nutrients to the fetus). It is referred to as late onset IUGR because it usually begins after 24 weeks gestation. The parameter most likely to be affected is the abdominal circumference with a normal biparietal diameter and head circumference. This is probably due to the "brain sparing effect" where the fetus directs blood flow to the vital organs, especially the brain, with a decreased blood flow to the liver [decreased abdominal circumference] (Jaffe & Abramowicz, 1997).

Some suggest that differentiating symmetric and asymmetric IUGR have limited value because there is quite a lot of overlap between the two types (Gardosi, 2005). Taking this study as an example, HIV infection can be associated with symmetric IUGR where a very low CD4 T-lymphocyte count can cause early onset IUGR, or it can be associated with asymmetric IUGR caused by placental insufficiency.

2.2.4 Placental Insufficiency

The placenta can be seen as the source of nutrition, respiration, hormonal synthesis and immunological protection for the fetus. The main functions of the placenta are: (Symmonds, 1992; Sadler, 1995; Wallach, 1993)

- To supply nutrition and remove waste products from the fetus; it plays an important role in metabolism and the removal of metabolic waste.
- Exchange of gases (oxygen, carbon dioxide and carbon monoxide) by diffusion.
- Hormone production. Human Chorionic Somatomammotropin (protein hormone) is a "growth hormone" significant in the 2nd and 3rd trimester and can be used to test placental function; Estriol (steroid hormone) is an oestrogen produced in large quantities necessary

for a complex process of metabolism involving the mother, placenta and fetus. A good utero-placental blood flow is necessary for the optimal functioning of the placenta. If the mother's condition is compromised by HIV or AIDS the fetus may not be provided with the nourishment necessary for normal growth.

2.2.5 Clinical Diagnosis of Intrauterine Growth Restriction

Fetal growth is usually monitored clinically by serial symphysis fundal height (SFH) measurements. Clinical criteria for suspecting IUGR according to Steyn (2003) include:

- 2 serial SFH measurements below the 10^{th} percentile
- Any 3 SFH measurements below the 10^{th} percentile
- 3 SFH measurements demonstrating a plateau i.e. no fetal growth, not necessarily below the 10^{th} percentile
- SFH measurement that is below that of 2 previous visits, not necessarily below the 10^{th} percentile

However the accuracy of this clinical diagnosis can be hampered by factors like unknown or uncertain last normal menstrual period, difficult palpation due to obesity or uterine anomalies. Ultrasound is a more precise and objective method of diagnosing IUGR (Manning, 1996). A patient presenting with a significant discrepancy between symphysis fundal height and gestational age is the most common clinical scenario suggesting IUGR and is then referred for an ultrasound examination to confirm IUGR. An accurate diagnosis of IUGR is dependent on the accurate estimation of gestational age as early as possible in the pregnancy.

2.3 ULTRASOUND AND THE MANAGEMENT OF INTRAUTERINE GROWTH RESTRICTION

2.3.1 Estimating Gestational Age

It is essential that accurate gestational age is determined as early as possible for IUGR or preterm delivery to be diagnosed. It is generally accepted that estimating gestational age by ultrasound is more accurate than by last normal menstrual period (LNMP). Inaccuracies derived from LNMP include a wide variation in menstrual cycle, inaccurate dates and vaginal bleeding in early pregnancy (Benson & Doubilet, 2005). However, it is important to note that the accuracy of gestational age estimation by ultrasound is inversely related to gestational age (Manning, 1996). Benson & Doubilet (2005) recommend ultrasound to be used for gestational ageing up to 24 weeks thereafter fetal biometry should only be used for assessing fetal growth. A single parameter, crown rump length is the most accurate method of estimating gestational age by ultrasound between 5 and 12 weeks with an estimate error of ± 3 days (Manning, 1996). After 12 weeks estimating gestational age by ultrasound should be based on more than one parameter. Biparietal diameter can be used from 12 to 20 weeks with an estimated error of < 7 days, and femur length and humeral length can be measured accurately from 14 to 16 weeks gestation (Manning, 1996). According to Jaffe and Abramowicz (1997) biparietal diameter has a good correlation with gestational age between 11 and 26 weeks with an estimate error of 10 to 14 days. The same authors also suggest that combining several measurements (biparietal diameter, head circumference and femur length) can decrease the estimate error to ± 5 days.

The initial scan in this study was done at 20-24 weeks to confirm gestational age using the average of 4 parameters: biparietal diameter (BPD), Head Circumference (HC), Femur Length

(FL) and Abdominal Circumference (AC). Chitty et al. Fetal Charts were used (Chitty et al., 1994 (a), (b), (c)).

2.3.2 Monitoring Fetal Growth

Abdominal circumference is an important parameter used to assess fetal growth and predict birth weight (Reece et al, 1994). The fetal liver is the region most affected by a decrease in nutrition hence a decrease in the abdominal circumference is often the first sign of decrease in fetal growth. A decrease in femur length may occur in severe cases of IUGR (Jaffe & Abramowicz, 1997). However single parameters should not be used for assessing fetal growth, as it is associated with high measurement error when estimating fetal weight. Serial measurements of the AC, FL, BPD and HC are commonly used to estimate fetal weight antenatally. Growth parameters should not be measured less than two weeks apart to allow for measurement error (Manning, 1996).

2.3.3 Estimated Fetal Weight

There are various formulae used to calculate estimated fetal weight (EFW) using ultrasound. More commonly used are Shepard, Campbell and Hadlock's formulae (Benson & Doubilet, 2005). Chien et al (2000) concluded in a study assessing the validity of different methods of estimating fetal weight that Shepard, Campbell and Hadlock's formulae had a high level of validity. Shepard et al's formula (Shepard et al, 1987) for estimating the fetal weight includes two parameters (AC and BPD) with an accuracy of within 10-15% of the actual weight. However the error could be increased if the fetus is very small (Jaffe & Abramowicz, 1997; Reece et al, 1994). In the third trimester the BPD can have an estimate error of $\pm$ 3.5 weeks,

making it an unreliable parameter. If however, a normal cephalic index can be confirmed, the BPD can be used to estimate fetal weight. A normal cephalic index (biparietal diameter / occipito-frontal diameter) ranges from 0.74 to 0.83, and will exclude brachycephaly (cephalic index ≥ 0.83) or dolichocephaly [cephalic index ≤ 0.74] (Jaffe & Abramowicz, 1997). Hadlock et al (1984) suggest firstly that HC should be used rather than BPD because the former is less shape dependent and measures the head area which is a more accurate measurement of head size; and secondly that femur length should be added to the equation when calculating estimated fetal weight because it has a linear relationship to crown-heel length. The authors conclude that four measurements (AC, BPD, HC and FL) produced the best results however the outcome of three measurements of the fetal head, abdomen and femur was not significantly different to four measurements. Hadlock et al's formula was used for the purposes of the study.

2.3.4 Amniotic Fluid Index

From the second trimester the amniotic fluid is mainly produced by the fetal kidneys and to a lesser degree by the fetal lungs. By about 20 weeks gestation the fetal kidneys are the main source of amniotic fluid (Manning, 1996). Reduced amniotic fluid, in the absence of fetal genito-urinary abnormalities or premature rupture of membranes (PROM), is often associated with a decrease in fetal growth. A consequence of placental insufficiency is the decrease of blood flow to the fetal abdominal organs including the kidneys and hence the decrease in the production of urine resulting in oligohydramnios (Jaffe & Abramowicz, 1997). Amniotic fluid index (AFI) is the sum of the measurements of the largest vertical cord-free pocket of amniotic fluid in each of the four quadrants of the uterine cavity. The normal AFI at term is 12.9 ± 4.6

cm. (Reece et al, 1994). Oligohydramnios is diagnosed with an AFI < 5 and polyhydramnios with an AFI >24 (Jaffe & Abramowicz, 1997). Many studies have reported a high positive predictive accuracy (79 – 100%) of association between oligohydramnios and IUGR (Manning, 1996).

2.3.5 Placental Size and Maturity

Placental size can be an indication of an abnormal placenta. A thick placenta can be associated with fetal abnormalities, maternal Diabetes, fetal hydrops, maternal and fetal anaemia, multiple gestation and chronic infection. A small placenta can be associated with IUGR, pre-eclampsia, chromosomal abnormalities, severe maternal vascular disease and Diabetes (Jaffe & Abramowicz, 1997).

Placental maturity can be graded on ultrasound according to its homogeneous or non-homogeneous echopattern, which is determined by the degree of calcification of the placenta and the ultrasound appearances of the basal and chorionic plates. It ranges from Grade 0 (homogeneous placenta without calcifications and a smooth hyperechoic chorionic plate) to Grade 3 (non-homogeneous placenta which has many calcifications and chorionic plate indentations) (Grannum, et al, 1979). A Grade 3 placenta can normally be seen from 36 weeks. Early placental maturity can be associated with IUGR and pre-eclampsia (Jaffe & Abramowicz, 1997).

2.3.6 Doppler Ultrasound

Doppler ultrasound is a useful tool in the management of high-risk pregnancies. It is used as an additional test in diagnosing IUGR. Pulsed-wave Doppler ultrasound can be used to assess

placental insufficiency and IUGR. Assessment of the blood flow velocity of the umbilical artery is commonly used to establish whether there is placental blood flow resistance. A marked increase in systolic to diastolic ratio for gestational age can signify a resistance to placental blood flow. Absent or reversed end diastolic flows are strongly associated with IUGR. According to Manning (1996) the incidence of IUGR with absent end diastolic flow is 94% and with reversed end diastolic flow is 100%.

Doppler ultrasound was not used in this study. However, Doppler ultrasound of the umbilical artery was used in the management of patients referred to secondary (Mowbray Maternity Hospital) or tertiary care (Groote Schuur Hospital) with diagnosed IUGR.

3. RESEARCH DESIGN & METHODOLOGY

A prospective cohort study was done on HIV infected and uninfected women attending Khayelitsha MOU and Gugulethu MOU from June 2003 to December 2004. This was primarily to establish whether there is an association between HIV and IUGR. B-Mode real time ultrasound imaging was used to monitor fetal growth from ±22 weeks to 36 weeks gestation. Birth weight, gestational age at delivery, gender, placental weight and perinatal complications were also recorded. A secondary objective was to establish whether there was an association between the CD4 T-lymphocyte count and IUGR in the HIV infected group.

3.1 NULL HYPOTHESES

3.1.1 There is no significant difference in the fetal growth in HIV infected pregnant women compared to uninfected pregnant women at Khayelitsha MOU and Gugulethu MOU.

3.1.2 There is no significant correlation between the immune status (CD4 T-lymphocyte count) of HIV infected pregnant women and IUGR.

3.1.3 There is a significant difference in fetal growth in HIV infected pregnant women using ZDV from 34 weeks gestation compared to those who do not.*

(Level of significance for rejecting the null hypothesis: $p = 0.05$)

* *This hypothesis had to be excluded due to the changes in the PMTCT Protocol in the Western Cape regarding ARVT.*

3.2 SAMPLE SELECTION

Women who tested positive for HIV were recruited for the study groups and women who tested negative for HIV were recruited for the control groups. The same selection criteria were applied to both groups from each MOU. The nursing staff at the antenatal clinics was responsible for the recruitment and obtaining informed consent (Appendix I: English Consent, Appendix II: Xhosa Consent) of the participants.

3.2.1 Criteria to Control Confounding Variables Affecting Fetal Growth

- Maternal age: Women between the age 18 and 32 years were included. Teenage pregnancies as well as pregnancies in women over 32 years of age were not included as they are associated with an increased risk for perinatal mortality and morbidity (Fawcus, 2003; Pattison, 1998).
- Gestational Age: Women who attended the MOU before an estimated 20 weeks gestation by LNMP and / or symphysis fundal height measurement were booked for the first ultrasound examination at 20-24 weeks gestation.
- Weight: Women with weight ≤ 95 kg at the first scan were included. Women with weight more than 100 kg are considered obese (Theron, 2003). Obesity in pregnant women is associated with a high risk for obstetric complications like hypertension, pre-eclampsia and Diabetes mellitus. These complications could affect fetal growth.

3.2.2 Exclusion Criteria

Patients with the following clinical history were excluded from the study:

- Cigarette smoking, alcohol and drug users: These substances are associated with a high risk for placental insufficiency. The placenta is thus unable to full-fill its important functions of providing oxygen and nutrition to the fetus, resulting in IUGR.
- An obstetric history of IUGR and preterm delivery: These problems can recur in a current pregnancy. A history of one preterm delivery increases the risk for preterm delivery four fold and a history of two preterm deliveries increases the risk six fold (Lizzi, 1993).
- Fetal abnormality in the current pregnancy such as chromosomal and other structural abnormalities could have a direct or indirect effect on fetal growth. All fetal abnormalities detected on the first scan were excluded.
- Grand multipara women: Women with a parity of 5 or more were excluded. High parity is associated with an increased risk for maternal complications e.g. antepartum haemorrhaging. These complications could have an adverse effect on fetal growth (Fawcus, 2003; Beck, 1993).
- Multiple pregnancy: Multiple pregnancy is associated with low birth weight. Preterm delivery is frequent in multiple pregnancy and is associated with increased perinatal mortality and morbidity (Howarth, 2003).
- Other maternal vascular diseases (severe Diabetes, chronic hypertension, renal disease, cardiac disease, collagen disease e.g. systemic lupus erythematosus). These complications may be associated with placental insufficiency i.e. fetal growth is at risk because of a problem with maternal utero-placental supply of oxygen and nutrients to the fetus (Steyn, 2003).

3.2.3 Study and Control Groups

- Two study groups were selected from women who were tested HIV positive at the MOU (Abbott Determine Rapid Test / GAIFAR Instant Screen / ELISA test):

  Group K1: Khayelitsha MOU

  Group G1: Gugulethu MOU

- Two control groups were selected from women who were tested HIV negative (HIV Rapid Screening Test):

  Group K2: Khayelitsha MOU

  Group G2: Gugulethu MOU

3.2.4 Sample Size

A proposed sample size was calculated using EPI Info 2000 as follows:

"Sample size calculated for unmatched cohort cross-sectional studies (exposed and unexposed)"

Assumption: The prevalence of IUGR in uninfected women is 10%, and 25% in HIV infected women. There is a 15% difference between the 2 groups.

Confidence interval: 95%

Power: 80%

Sample size: 151 (uninfected) + 151 (HIV infected) = 302

Although the sample size calculated is 302, the target sample size for this study was 400 to allow for an expected ± 25% drop out rate.

3.2.5 Protocol: Criteria for Recruiting Participants

See Appendix III: Protocol for HIV-US Study

3.3 ULTRASOUND EQUIPMENT

GE LogiqBook digital ultrasound system, a B-Mode real time scanner was used with the following features (LogiqBook Advanced Reference Manual, GE Medical):

- 2-D black and white imaging.
- A lightweight laptop making it easily transportable from venue to venue.
- Raw data processing, patient database and image archiving, which facilitated data management for research purposes (CPU Pentium III, 256MB with USB ports for a CD-writer and printer).
- Software that enabled optimum obstetric ultrasound imaging, including annotation, calculation and report packages. Chitty et al's Fetal Charts (1994 a, b, c) had to be added as these are the standard fetal biometry charts used in the Western Cape.
- Multi-frequency (2-5 MHz) transducer allowing optimum visualization of superficial and deeper structures. The choice of lower frequency was particularly useful for scanning obese patients for optimum depth visualization. Convex probe with an aperture of 58mm and field of view of 55 degrees allowing optimum visualization of bigger areas that is especially useful for obstetric ultrasound imaging.
- Time Gain Compensation (TGC) amplifies returning echoes to compensate for attenuation caused when the sound beam travels through tissues, i.e. it compensates for loss of intensity of the beam at increasing depth. TGC is best set while scanning the patient's

liver longitudinally. Adjustments can be made until the liver has an even mid-grey echogenicity from its superior to inferior border.

- Gain controls the amount of echo information. Adjusting the overall Gain allows a general increase or decrease in echogenicity of the image.
- Dynamic Range determines the way returning echoes are converted to shades of grey. Image contrast can thus be adjusted.
- Focal Zones: There is an option of selecting 1 – 4 Focal Zones. Selecting a specific Focal Zone is useful when improved image resolution is needed for a specific area of interest. Lateral resolution is improved at the specific depth.
- Acoustic Output (AO) should be set as low as possible without hampering image quality, to avoid adverse bio-effects to human tissue. All other options should be set appropriately before increasing the AO to improve image quality, e.g. a transducer frequency that provides optimum penetration and focal depth; set Gain and TGC.

3.4 ULTRASOUND EXAMINATIONS

3.4.1 Scanning took place 2 days per week (1 day at each antenatal clinic) from June 2003 to December 2004. 15 - 20 women were scanned at each clinic each week. Each woman had to have 4 scans: 20-24 weeks, 28 weeks, 32 weeks and 36 weeks gestation. Two experienced sonographers scanned the women. The researcher scanned all women from June 2003 to February 2004. A second sonographer assisted at Gugulethu MOU from March to December 2004.

3.4.2 On the day of the first ultrasound examination (20-24 weeks gestation) the following were done:

- Confirmation of Informed Consent by the sonographer.
- Confirmation that the selection criteria were adhered to.
- Weight and height documented.
- Blood test to assess CD4 T-lymphocyte count was initially part of the study protocol. Six months into the study the Western Cape's PMTCT Protocol was revised to include blood tests for CD4 T-lymphocyte count. These results were made available for the purposes of the study and the protocol for the study was thus revised to stop blood tests taken for CD4 T-lymphocyte count.

3.4.3 Scanning Protocol

3.4.3.1 Scan 1 at 20-24 weeks Gestation: Detailed Fetal Anomaly Scan

- Intrauterine gestation was confirmed by identifying the internal cervical os and visualizing the uterine wall surrounding the gestation sac.
- A singleton pregnancy was confirmed by visualizing one fetus in-utero.
- Fetal presentation (cephalic, breech or transverse) was identified. Identifying a breech presentation becomes more critical in late third trimester as the woman may need to deliver by caesarean section.
- Identifying fetal lie assists the scanner to identify the fetal right and left sides. Abnormalities like dextrocardia (the heart located on the right side of the thorax) and situs inversus (transposition of abdominal organs) can be excluded.
- Positive fetal heartbeat was identified.

- **Placenta**
    - Location of the placenta was noted – anterior, posterior or fundal. A low placenta (inferior tip of the placenta measuring < 4 cm from the internal cervical os) was reviewed at 32 weeks gestation and if still low, was referred to secondary level care. Placenta praevia (placenta covering the internal cervical os) was referred to secondary level care.
    - Size of the placenta was measured at the center of the placenta, the distance from the chorionic plate to the basal plate, perpendicular to the chorionic plate (Figure 3.1). A placenta > 4cm before 24 weeks can be associated with increased perinatal risk (Alkazaleh, et al, 2005). A small placenta has been associated with IUGR (Jaffe & Abramowicz, 1997; Dähnert, 1993).

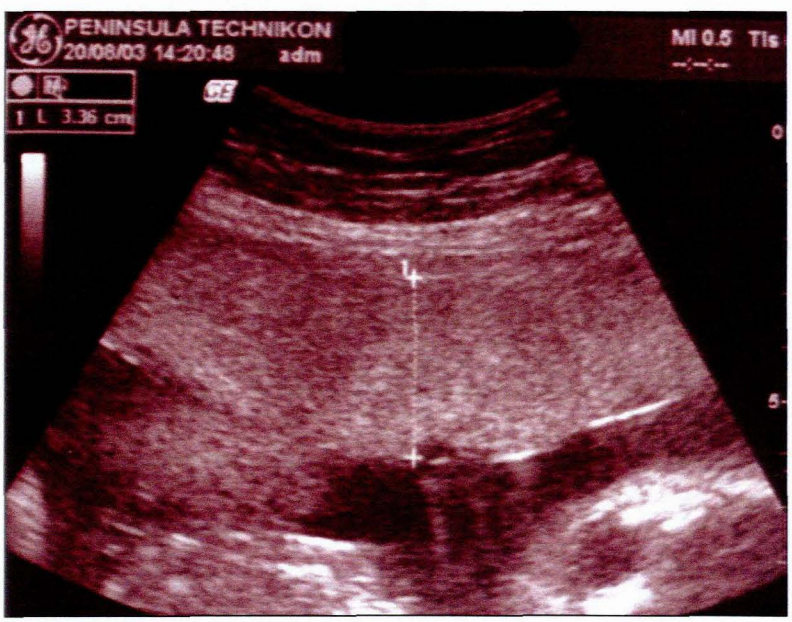

Figure 3.1: Placental thickness measured in mid-second trimester.

- Placental maturity was assessed using Grannum's Classification (Grannum et al, 1979), which is based on the degree of calcification in the placenta (Figure 3.2). This can be seen on ultrasound as follows:
  - Grade 0: In early pregnancy the placenta appears completely homogeneous (no calcifications) with a smooth chorionic plate (first and second trimester) (Grannum et al, 1979) (Figure 3.2A & 3.3).
  - Grade 1: A few echogenic densities (calcifications) are seen with subtle indentations in the chorionic plate (early third trimester) (Figure 3.2B) (Grannum et al, 1979).
  - Grade II: There is an increased number of echogenic densities with more marked indentations (without reaching the basal plate) in the chorionic plate. Some echogenic areas are seen along the basal plate (Figure 3.2C) (Grannum et al, 1979). These appearances are usually seen from the second trimester until 34 to 36 weeks gestation (Jaffe & Abramowicz, 1997).
  - Grade III: The echogenic areas become denser and can cast acoustic shadows. The indentations in the chorionic plate appear to extend to the basal layer (Figure 3.2D & 3.4) (Grannum et al, 1979). These appearances are usually seen at 36 weeks gestation (Jaffe & Abramowicz, 1997).

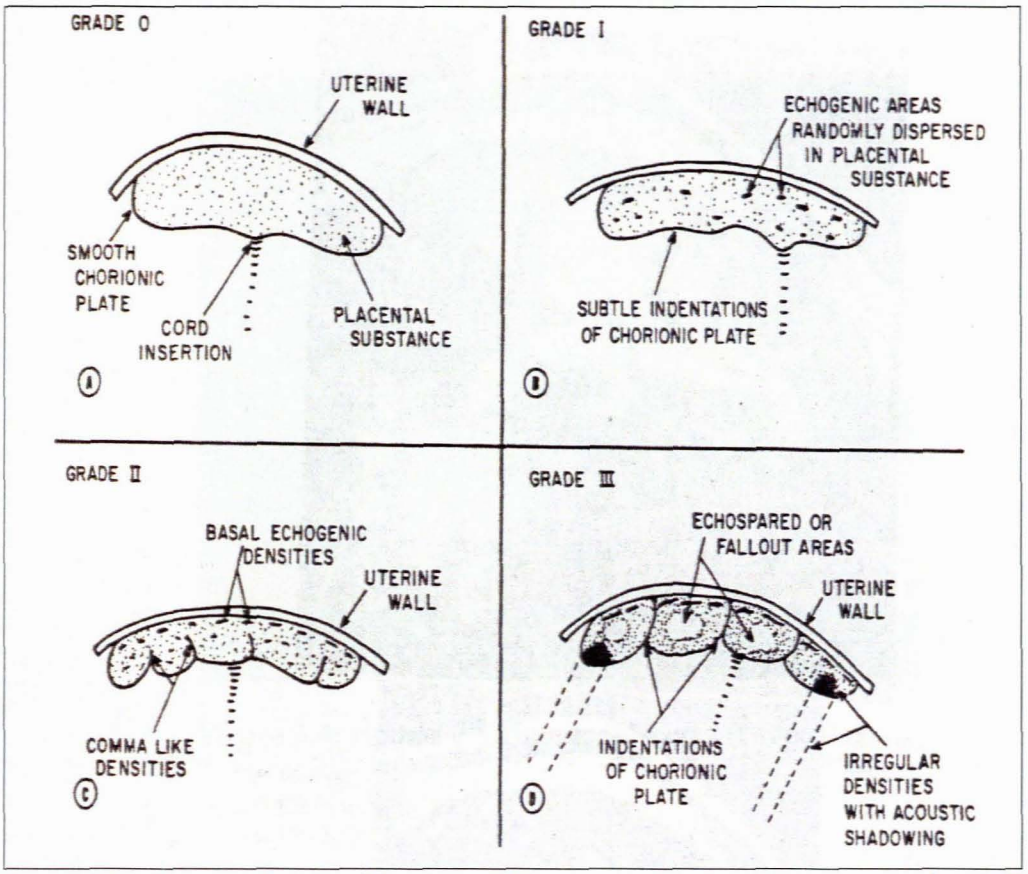

Figure 3.2: Grannum's Classification of Placental Maturity (Grannum et al., 1979)

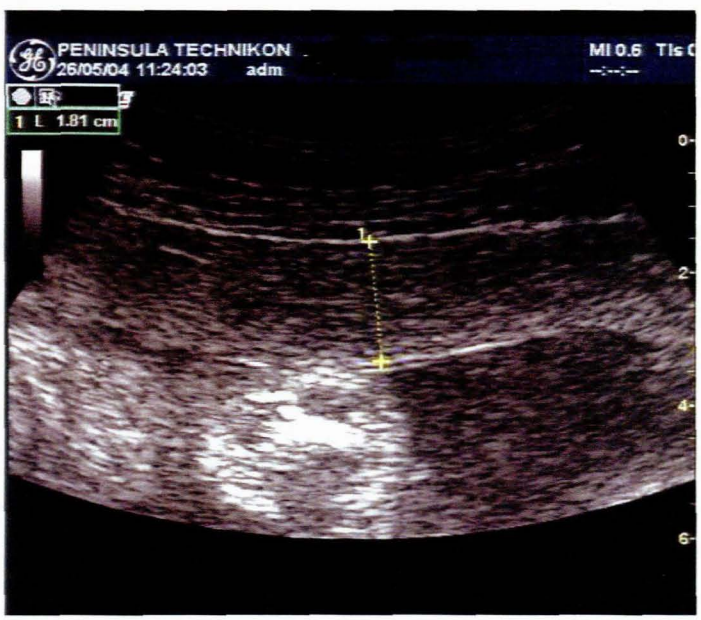

Figure 3.3: Normal 2nd trimester placenta (Grade 0)

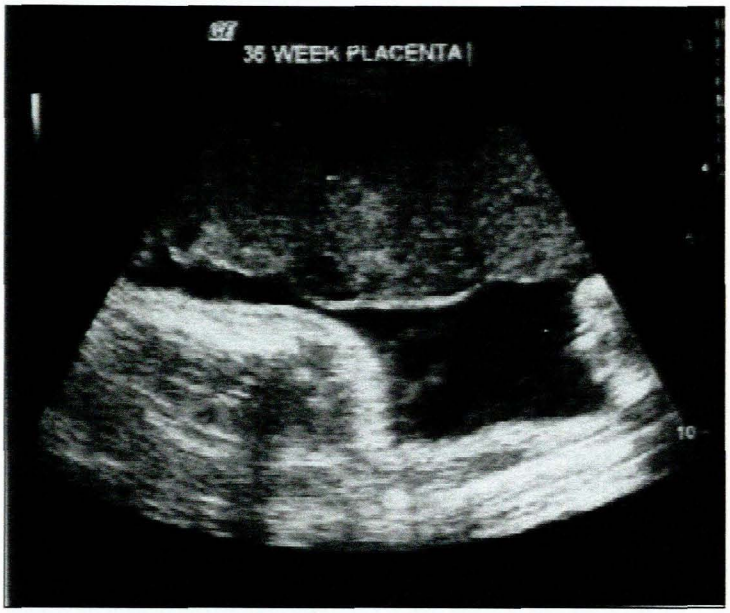

Figure 3.4: Normal 3rd trimester placenta (Grade III)

- When IUGR was suspected especially in third trimester, placental grading (maturity) according to Grannum's Classification assisted in the confirmation of IUGR.

- **Amniotic fluid volume** was measured using the Amniotic Fluid Index (AFI). A normal AFI is 5 - 20cm. Oligohydramnios (AFI<5) is associated with IUGR. The uterus is divided into 4 quadrants. The vertical depth of the largest cord-free pocket of amniotic fluid in each quadrant was measured and added together.

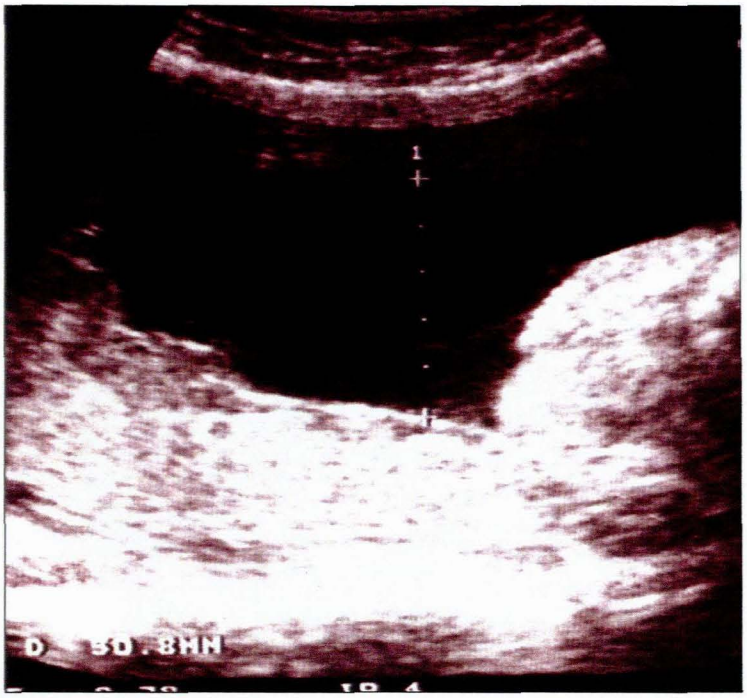

Figure 3.5: Amniotic Fluid Index: Measurement of one pocket of amniotic fluid.

- Fetal biometry for estimating gestation age: Chitty et al Fetal Biometry Charts (50th percentile) were used for the purposes of clinical management of the participants (Chitty et al, 1994).
    - **Biparietal Diameter**
        - The BPD measurement is taken in the axial plane of the fetal head at the level of the thalami and cavum septum pellucidi. The cranium must be oval-shaped and the measurement is taken from the outer border of the cranium closest to the transducer to the inner border of the cranium distal to the transducer (Figure 3.6). The BPD is thought to be the most

accurate measurement for estimating gestation age up to 22 weeks (CI: 1 – 1.5 weeks) (Jaffe & Abramowicz, 1997).

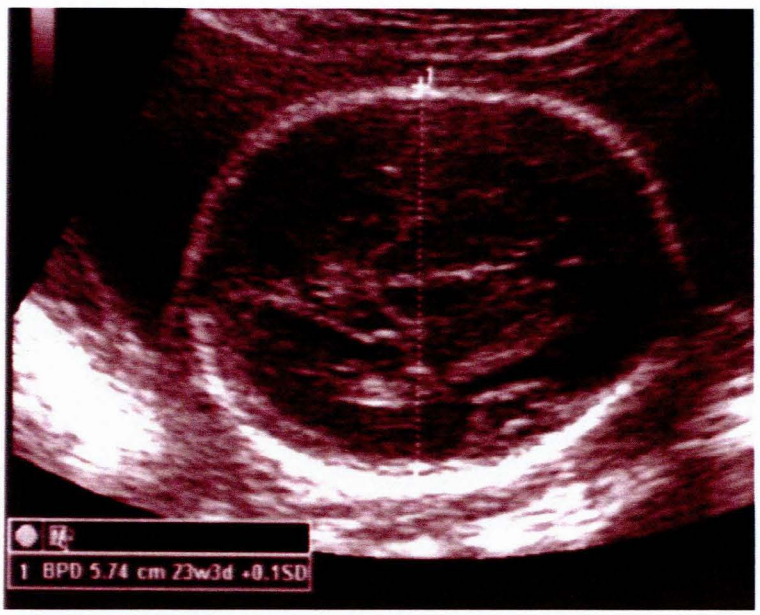

Figure 3.6: Biparietal Diameter measurement

- The BPD however does not take into account the shape of the head. Measuring the head circumference is therefore also done.

- **Head Circumference (HC)**
  - The HC measurement is made on the same image as that of the BPD. Calipers are placed on the outer borders of the cranium and the electronic ellipse is used to take the measurement (Figure 3.7). The HC is considered to be more accurate because it is not shape dependent (Jaffe & Abramowicz, 1997; Benson &

Doubilet, 1998). HC measures area and is therefore a more accurate method of head measurement (Hadlock et al, 1984).

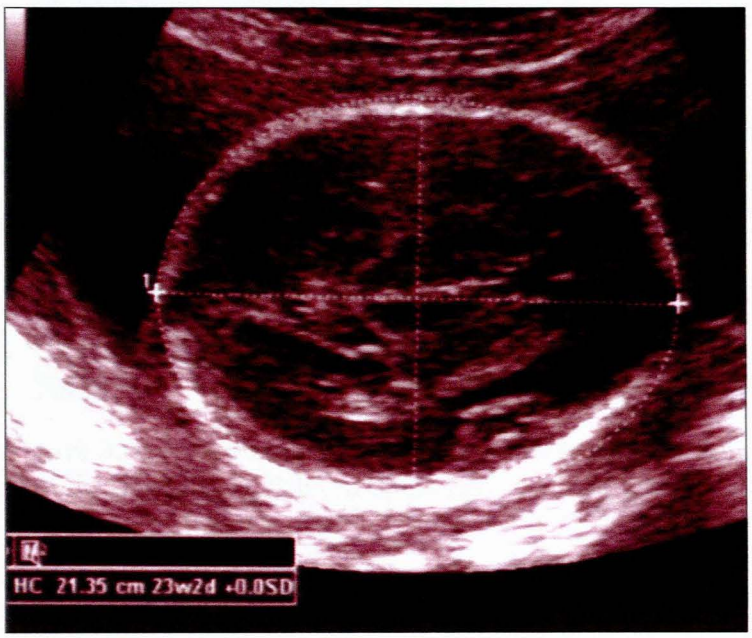

Figure 3.7: Head Circumference measurement

- **Abdominal Circumference (AC)**
  - The abdominal circumference measurement is made in the axial plane of the fetal abdomen at the level of the stomach and the intrahepatic part of the umbilical vein. Electronic ellipse is used for measuring the perimeter of the fetal abdomen (Figure 3.8)

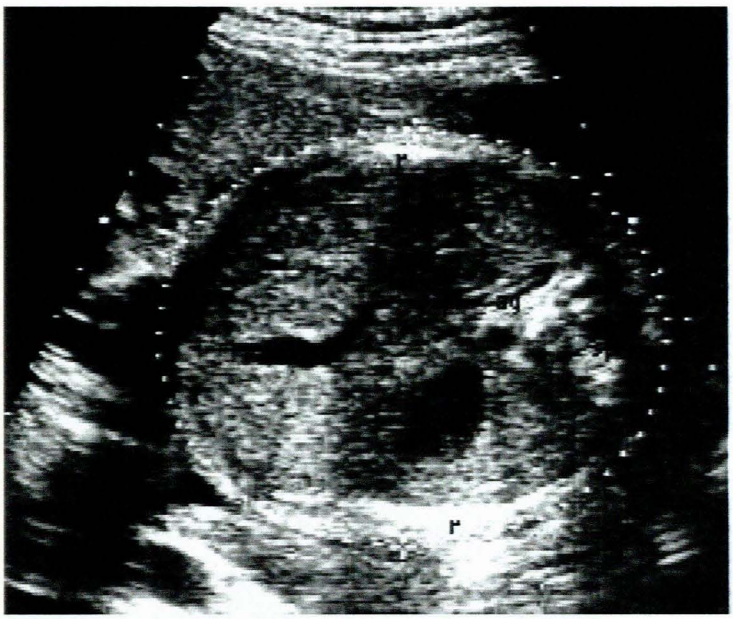

Figure 3.8: Abdominal Circumference Measurement: Stomach (st); Umbilical vein (uv); Rib (r); Spine (sp); Adrenal gland (ag)

- The abdominal circumference is a good indicator of the amount of subcutaneous fat and fetal nutritional changes and hence a good indicator of fetal growth (Jaffe & Abramowicz, 1997).

- **Femur Length (FL)**
  - The length of the ossified diaphysis of the fetal femur is commonly used for estimating gestation age (Figure 3.9) (Benson & Doubilet, 1998).

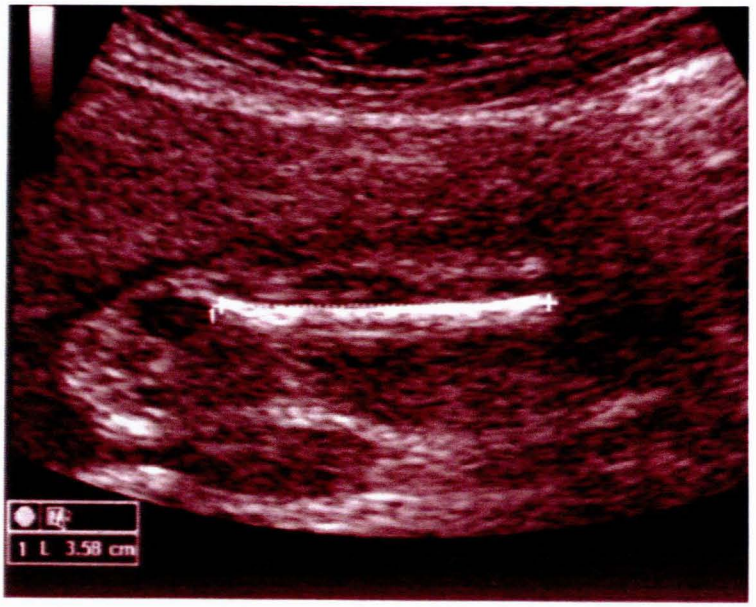

Figure 3.9: Femur Length measurement

- **Fetal anatomy** was assessed to exclude obvious abnormalities:
    - Fetal intracranial structures assessed included:
    - Atrial width to exclude ventriculomegaly
    - Posterior fossa for the cerebellum and cisterna magna to exclude abnormalities like Dandy-Walker Syndrome and Arnold Chiarri malformation (Jaffe & Abramowicz,1997; Toi & Sauerbrei, 1998)
    - Fetal facial structures in the sagittal plane (profile view of the forehead, nose, lips and chin) and/or coronal plane (cleft lip and palate)
    - Fetal thorax including the diaphragm, lungs and cardiac structures
    - Fetal spine in at least two scanning planes (sagittal and axial / coronal and axial) to exclude neural tube defects e.g. spina bifida
    - Fetal stomach bubble

- Fetal bladder and both kidneys
- Fetal upper and lower extremities identifying the humeri, radii and ulna, femora, tibia and fibula. Due to time constraints the digits of hands and feet were only assessed if easily visualized
- Umbilical cord insertion into the anterior abdominal wall of the fetus to exclude herniation complications like omphalocele or gastroschisis. Umbilical blood vessels (two umbilical arteries and one umbilical vein) were also identified

3.4.3.2 Fetal Growth Scans at 28 weeks, 32 weeks and 36 weeks gestation

- Fetal biometry:
  - Biparietal diameter, Head Circumference, Femur Length and Abdominal Circumference measurements were taken
  - Estimated Fetal Weight (EFW) was calculated using Hadlock et al's chart (Hadlock et al, 1984)
  - Amniotic fluid volume was measured using Amniotic Fluid Index (normal AFI: 5 - 20cm)
- Placenta
  - Placental size was measured (Figure 3.1)
  - Placental grading was noted (according to Grannum's classification (Grannum et al, 1979) (Figure 3.2)

Women presenting with the following complications were referred to Secondary (Mowbray Maternity Hospital) or Tertiary (Groote Schuur Hospital) Health Care for further management:

- HIV infected women who had a CD4 T-lymphocyte count of < 200 cells/mm^3
- Suspected fetal abnormalities or other complications
- Suspected IUGR at the 28-, 32- or 36-week scan. Ultrasound markers for IUGR included:
    - Estimated Fetal Weight below the 10th percentile for gestational age
    - Abdominal Circumference below the 10th percentile for gestational age
    - Femur Length below the 10th percentile for gestational age
    - Oligohydramnios (AFI < 5 cm)
    - Grade III placenta (Grannum's Classification) at 28-week or 32-week scan

3.5 POST DELIVERY FOLLOW UP

Data recorded from the participants' hospital folders post delivery included:

- Newborn's birth weight: < 2500 grams was considered low birth weight.
- Gestational age (weeks) at delivery i.e. normal term delivery (37- 40 weeks) or preterm delivery (<37 weeks)
- Type of delivery (normal vertex delivery, caesarean section or other)
- Gender of newborn: male or female
- Placental weight (grams)
- Perinatal complications

3.6 DATA COLLECTION & STATISTICAL ANALYSIS

3.6.1 Data Collection

A data form was designed for data collection (see Appendix IV: Data Form).

3.6.2 Statistical Analysis

Statistical analysis was performed using the statistical package SPSS 12.0.

3.6.2.1 Attrition Rate

Analysis of the attrition rate was performed to evaluate whether there was a trend in the drop out rate:

- The t-test for unequal variance was used to assess whether there was a trend in the attrition rate of a particular variable viz. age, mass and height ($p = 0.05$).
- The Chi-squared (χ^2) test was used to assess whether there was a trend in the attrition rate of a particular sample (group) viz. HIV infected and uninfected groups and Gugulethu and Khayelitsha MOU's ($p = 0.05$).

3.6.2.2 Descriptive Analysis of the Sample

Descriptive statistics viz. mean and standard deviation were used for analyzing the variables age, mass and height.

3.6.2.3 Comparative Analysis

The t-test was used to compare variables (age, mass and height) of the HIV infected and uninfected groups ($p = 0.05$).

3.6.2.4 Analysis of Variance (ANOVA)

- ANOVA (t-test / χ^2 test) was used to compare the multiple measurements (scans 1 – 4) of biparietal diameter, head circumference, femur length, abdominal circumference, estimated fetal weight, placental size and amniotic fluid index of the HIV infected and uninfected groups ($p = 0.05$).
- ANOVA (t-test / χ^2 test) was also used to compare birth weight, preterm delivery, type of delivery and maternal complications of the HIV-infected and the uninfected groups ($p = 0.05$).

3.6.2.5 The Chi-squared (χ^2) test was used to assess whether there was an association between a low CD4 T-lymphocyte count < 200 cells/mm^3 and maternal complications and preterm delivery.

(Underhill & Bradford, 1996; Campbell & Machin, 1999)

3.7 ETHICS

3.7.1 Ethics in Medical Research

The purpose of medical research is to improve health care to the public. Research must have diagnostic and therapeutic value, in other words there must be benefits to the management of health care to patients. Because research (especially biomedical research) often involves hazards to humans, the well-being and rights of any participant must be a priority over the benefit to science or society. Critical consideration of the risks and benefits to humans (environment and animals where applicable) must be taken into account before embarking on any medical research (Helsinki Declaration, 2000).

In 1964 the World Medical Association published the "Declaration of Helsinki" which was aimed at providing principles for good ethical practice in medical research. These principles have been reviewed and updated many times. The update of the Declaration of Helsinki (2000) included the following basic principles that were considered in this study:

3.7.1.1 ***"Clinical research should be conducted only by scientifically qualified persons and under the supervision of a qualified medical person"***(Helsinki Declaration, 2000)

This research was conducted by a postgraduate ultrasound student with 5 years clinical experience. The supervisors were appointed on the basis of their knowledge and experience in Obstetric Ultrasound as well as medical research. The researcher also worked closely with sonographers and doctors at the two secondary and tertiary referral centres.

3.7.1.2 ***"Every clinical research project should be preceded by careful assessment of inherent risks in comparison to foreseeable benefits to the subject or to others"***(Helsinki Declaration, 2000)

Ultrasound can have biological effects (thermal and mechanical effects) on human tissue if very high acoustic intensities are used for a long period of time. However diagnostic ultrasound applied in obstetrics usually uses low level acoustic intensities and studies have shown that there are no adverse biological effects to human tissue when using the required frequencies for diagnostic ultrasound imaging in obstetrics. All participants were scanned using a B-mode real time ultrasound unit. Based on scientific evidence, B-mode real time ultrasound uses a low enough acoustic output to render it a safe diagnostic tool that can be

used in the management of pregnant women (European Committee for Medical Ultrasound Safety, 1998; International Society of Ultrasound in Obstetrics and Gynaecology, 2003). Doppler ultrasound uses a higher level energy with associated higher temperatures and should therefore only be used when clinically necessary and scanning time should be kept to a minimum (International Society of Ultrasound in Obstetrics and Gynaecology, 2003). Doppler ultrasound was not used in this study.

Diagnostic ultrasound, especially Doppler ultrasound should always be done by experienced scanners with a good operational knowledge of the ultrasound unit keeping the scanning time and acoustic output to the lowest possible level.

The benefits of the research, viz. to improve the management of the HIV infected pregnant woman therefore outweighs the minimal risk of B-mode real time ultrasound imaging.

3.7.1.3 "*If at all possible, consistent with patient psychology, the doctor* (researcher) *should obtain the patient's freely given consent after the patient has been given a full explanation. ..... Consent should, as a rule, be obtained in writing. However, the responsibility for clinical research always remains with the research worker; it never falls on the subject even after consent is obtained"*

(Helsinki Declaration, 2000)

The nursing staff recruiting the participants obtained informed consent. The objectives and procedure of the study was explained in their language of choice. The majority of the participants as well as the nursing staff at the MOU's were Xhosa-speaking. All women who agreed to participate in the study had to sign the consent form in one of the following languages: English or Xhosa (Appendices I & II respectively). The majority of participants

had Xhosa as their first language and hence preferred the Xhosa consent form. Informed consent was confirmed by the researcher on the day of the first scan.

An interpreter was used where the researcher was unable to communicate in Xhosa for confirmation of consent.

3.7.1.4 "*The doctor* (researcher) *can combine clinical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that clinical research is justified by its therapeutic value for the patient*"(Helsinki Declaration, 2000)

This study was integrated as far as possible with the normal antenatal care the participant would receive. Visits for the study were booked on the same day of the routine antenatal visits. Unfortunately this did not always happen due to suboptimal working conditions at the one MOU. All data collected by the researcher (Ultrasound Report and Immunology Report) were included in the participant's hospital folder so that the information could assist with the management of the pregnancy. Immunology reports (CD4 T-lymphocyte count) were of particular significance to clinical management of HIV infected participants. Participants with a CD4 T-lymphocyte count < 200 cells / mm^3 were referred to secondary level care. Participants with suspected fetal anomalies or other complications were referred to secondary or tertiary level care.

3.7.1.5 "*The investigator must respect the right of each individual to safeguard his/her personal integrity, especially if the subject is in a dependent relationship to the investigator*" (Helsinki Declaration, 2000)

The participant's *"personal integrity"* is closely linked with her autonomy and rights as a

patient.

The First World has seen a turn-around in the relationship between the patient and the health worker in the last 30 years. There has been a major shift in attitudes from "medical paternalism" to "patient-centred" healthcare (Hope et al, 2003). In the past it was common practice for health workers to make decisions based on what they thought was in the patient's best interest. Medical information was often considered "too complicated" for the lay person to understand. This was the justification for the non-transparent approach to patients' medical information. Patients were not fully informed and were therefore not able to decide what their 'best interests' were. Today "The Patient's Rights Charter" (Department of Health, South Africa, 2000) protects patients' rights and autonomy.

Medical research is about accessing and using patients' health records. Inherent in this is the risk of violating a patient's privacy. When a patient agrees, by informed consent, to participate in a study, it is assumed that the researcher will keep all information strictly confidential. Researchers need to have a comprehensive plan of how the confidentiality of patients' records will be dealt with to ensure the strictest confidentiality and anonymity. In this study another issue had to be considered i.e. the HIV status of the women which will be discussed later.

Criteria for ensuring Confidentiality within the Study:

- In order to easily identify participant's hospital folders a sticker labelled "Peninsula Technikon – Ultrasound Study" was placed on the cover of each participant's folder. There was no mention of the HIV aspect of the study.
- Participants' names had to be included on the Ultrasound and Immunology reports because these were included in the hospital folders and contributed towards clinical management.

For research purposes the participants' names were deleted and hospital numbers were used for identification. The hospital policy regarding the use of hospital numbers as identification of participants in research has subsequently changed. Hospital numbers are now considered confidential and cannot be used for research purposes.

- Access to the research data was limited to the researcher, research assistant, supervisors and statistician.
- The participant was ensured of complete confidentiality at all times (see Appendix I: English Consent).

3.7.1.6 *"At any time during the course of clinical research the subject or the subject's guardian should be free to withdraw permission for research to be continued. The investigator or the investigation team should discontinue the research if in their judgment, it may, if continued be harmful to the individual"* (Helsinki Declaration, 2000)

Participants had an option of withdrawing from the study at any time (Appendix I: English Consent). This study was not seen to be harmful to the participants at any stage and therefore there was no need for the researcher or medical team to discontinue the study.

3.7.2 Ethics HIV/AIDS

3.7.2.1 Ethics Affecting Participants in HIV Research

HIV/AIDS is an epidemic in South Africa and the leading cause of adult deaths. In the absence of a vaccine, the only way to control this epidemic is the prevention of spread of the virus (Medical Research Council, 2003). Many preventive strategies have been put into place for both the public and the health worker. This ranges from public education programmes on

HIV/AIDS, Voluntary Testing and Counseling (VTC) programmes and the PMTCT programme (Provincial Government Western Cape, 2002) that provides guidelines for reducing the spread of the virus from the pregnant mother to child. The biggest stumbling block in reducing HIV infection rates is the stigmatization of this disease. South Africans affected by HIV/AIDS are often at the receiving end of stigmatization, discrimination and violation of other basic human rights. This could be because HIV is associated with sex, homosexuality, commercial sex and drug abuse (Medical Research Council, 2003). Marginalized communities from low socio-economic and poverty-stricken areas are the most affected by HIV. Researchers embarking on studies in these communities must take heed of the vulnerability of people living with HIV/AIDS especially women, a marginalized group in our society. Researchers must ensure that the benefits to participants outweigh the risks. Strict confidentiality and authentic informed consent are needed to protect the participants. In this study pregnant women were recruited from antenatal clinics. Informed consent was taken by the nursing staff and confirmed by the researcher. The researcher found that on many occasions the participants had given their consent but weeks later on the first day of meeting with the researcher, seemed uninformed or partially informed of the study. This could have been due to two main factors:

- The participant was not given a proper explanation about the objectives of the study. The consent could therefore not be considered 'informed'.
- The participant in many cases had received the news of her positive HIV status quite recently and may have been too distressed to fully comprehend information given to her about the study.

It is therefore recommended that researchers recruiting vulnerable participants for studies in HIV should give the prospective recruits all the relevant information, allow them to take it home, discuss it with their partner or family, and on return, give their consent.

3.7.2.2 Ethics Affecting the Researcher in HIV Research

Any researcher involved in HIV studies must have good background knowledge of not only the medical issues around HIV but also the psychological, social, and cultural issues relevant to the specific community being researched (Medical Research Council, 2003). A holistic approach to dealing with HIV infected participants is essential. In this study the researcher was often faced with questions about HIV but unrelated to the study. At Khayelitsha MOU an established HIV clinic with a good support group was in place and participants could easily be referred. However at Gugulethu MOU the HIV clinic was not well-established and there were no support groups at the clinic. In the latter case the researcher had to try to counsel the participants where necessary and refer them to support groups in their community.

The dilemma that the health care worker faces is the conflict between the rights of the HIV infected person to privacy, confidentiality and a choice of disclosure of HIV status on the one hand, and the battle of a society to overcome the epidemic. Health care workers can run the risk of being authoritarian for the 'good of society' when caring for HIV infected patients (Cogan et al, 2003). HIV/AIDS is not a notifiable disease and the health worker therefore has no statutory right to disclose the HIV status of a patient. Policies and guidelines have been put in place by the Department of Health in a document, "Ethics in Health Research: Principles, Structures and Processes" to protect both the public and the health professional involved in research (Act No. 61 of 2003, Department of Health, South Africa).

3.7.2.3 The Right of the HIV Infected Person to Health Care

South Africa is facing serious ethical issues regarding HIV infected people's accessibility to adequate health care. The Department of Health in the Western Cape has implemented the Prevention of Mother-to-child Transmission (PMTCT) Programme to address the accessibility of optimum health care to pregnant women and their babies. According to a report by the Department of Health in March, 2002 (Provincial Government Western Cape, 2002) 60% of pregnant women attending state hospitals or clinics have access to the PMTCT Programme, and by 2003, 90% should have access. At the start of the study the PMTCT Programme was running at both MOU's. However it seemed to be better established at the Khayelitsha MOU because it had been implemented a lot earlier than at the other MOU.

The PMTCT Programme Protocol (Provincial Government Western Cape, 2002) includes Voluntary Testing and Counselling (VTC) and treatment:

(i) Voluntary Testing and Counselling

Only health care professionals and lay counsellors who are trained to do the testing and counselling may do so.

- HIV Pre-test Counselling: The first step is usually the sharing of information with a group regarding HIV transmission and its prevention, PMTCT, treatment, feeding options, HIV testing, positive and negative results and confidentiality. At the two MOU's in this study this information was usually given to a large group of women sitting in the waiting room at the beginning of a clinic. VTC was offered to all women. Women who opted for HIV testing were then offered individual pre-test counselling.
- HIV Testing: Written informed consent is necessary for HIV testing. The Abbott Determine Rapid Test is done which detects antibodies to HIV-1 and HIV-2 from

serum, plasma or whole blood. If this test is negative the women is considered negative. If the test is positive a second rapid test (GAIFAR Instant Screen) is done. If both tests are positive the woman is considered HIV-infected. If the first test is positive and the second test is negative, a third ELISA test must be done. All results are recorded in a MOU register which is kept strictly confidential.

- Post-test Counselling: All women who have been tested, regardless of the outcome, must receive post-test counselling.

  Counselling for women who test negative includes:

  - Maintaining a HIV negative status
  - Safe sex
  - Risks of becoming HIV infected during the pregnancy

  Counselling for women who test positive includes:

  - Immediate and continuous support
  - Participation in the PMTCT programme
  - Referrals to support groups
  - Education about the HIV disease and healthy living
  - ARVT and its side effects
  - Feeding options (formula feeding / breast feeding)
  - Disclosure, stigma and discrimination
  - Safe sex
  - Partner testing

(ii) Treatment

When the study commenced in June 2003 the PMTCT Protocol regarding ARVT was different at the two MOU's: Zidovudine (ZDV) was administered to women at Khayelitsha MOU from 34 weeks gestation until delivery. Nevirapine (NVP) was administered to women at the onset of labour Gugulethu MOU.

In October 2003 PMTCT Protocol was revised. Changes affecting testing and treatment were implemented by December 2003:

- Testing: All women testing HIV positive had a CD4 T-lymphocyte count. Women with CD4 < 200 cells / mm^3 were referred to secondary health care and received Co-trimoxazole (prophylaxis). All mothers were also offered to have their babies tested (PCR) for HIV status at 14 weeks.
- Treatment: There was evidence of a high risk of toxicity associated with NVP administered as monotherapy. The ARVT regimen was thus changed at all MOU's to include both ZDV and NVP. Mothers received ZDV from 34 weeks gestation until delivery (ZDV was started earlier where preterm delivery was suspected) plus a single dose of NVP in labour. Infants received one dose of NVP 48-72 hours after delivery and a 7-day course of ZDV.

These changes to the PMTCT Protocol resulted in the following changes in the study protocol:

- Blood tests for CD4 T-lymphocyte count were discontinued in the study because the test was offered to all HIV infected pregnant women. The researcher was allowed access to the results of the blood tests done at the MOU.

- One of the objectives of the study had to be abandoned viz. the comparison of the two different ARVT regimens offered at the two MOU's. One regimen was later used for all MOU's.

3.7.3 Ethics Approval

The research proposal including the protocol was submitted to the Ethics Committees at Cape Peninsula University of Technology (formerly Peninsula Technikon) and University of Cape Town. Ethics approval was granted before the fieldwork began.

4. RESEARCH RESULTS

Statistical analysis was performed using the SPSS software package, the significance level accepted as $p = 0.05$.

4.1 SAMPLE SIZE

4.1.1 Total Sample Recruited

A total of 415 patients were recruited from the two MOU's: 173 patients from Khayelitsha MOU and 242 patients from Gugulethu MOU. 194 HIV infected women were included in the study group and 221 uninfected women were included in the control group as seen in Table 4.1.

Table 4.1: Sample Size

Midwifery Obstetric Unit	No. of patients HIV+	No. of patients HIV-	Total no. of patients
Khayelitsha	77	96	173
Gugulethu	117	125	242
Total	194	221	415

4.1.2 Exclusions from the Sample

It was not always possible to find all the data of each patient. Where gaps (e.g. maternal age, mass, CD4 T-lymphocyte count, etc.) existed in data for specific patients, the statistical

package SPSS 12.0 used "case-wise" deletion of the specific patient. This resulted in a variable sample size for different variables.

4.2 ATTRITION RATE

There appeared to be an attrition rate that progressively increased along the course of the study with the highest drop out rate at the 36 week scan. A sample $n = 415$ entered the study at 20 – 24 weeks gestation. The attrition rate at 28 weeks gestation was 12% ($n = 59$), at 32 weeks gestation it was 23% ($n = 95$), at 36 weeks gestation it was 39% ($n = 162$) and at follow-up post-delivery it was 30% ($n = 124$) as seen in Table 4.2.

Table 4.2: Attrition Rate at Scans 1-4 and Post-delivery

Scans/ Follow Up	**Scan 1 20-24 weeks** *n = 415*	**Scan 2 28 weeks** *n = 415*	**Scan 3 32 weeks** *n = 415*	**Scan 4 36 weeks** *n = 415*	**Follow-up Post-delivery** *n = 415*
No. Dropout	0	59 *(12%)*	95 *(23%)*	162 *(39%)*	124 *(30%)*
No. Scanned / Followed up	415	356 *(88%)*	320 *(77%)*	253 *(61%)*	291 *(70%)*

The first step was to establish if there was a particular trend in the attrition rate, i.e. was there a particular variable/s that affected the dropout rate? The variables that were considered were the age, mass, body mass index, HIV status, MOU, time of delivery (preterm delivery) and low CD4 T-lymphocyte count.

4.2.1 Attrition by Maternal Age, Mass, Height and Body Mass Index

The t-test for unequal variance (level of significance $p = 0.05$) was used to assess whether there was a trend in the attrition rate of particular variables viz. maternal age, mass and body mass index.

There was no significant difference in the maternal age ($p = 0.26$), maternal mass ($p = 0.79$) and body mass index ($p = 0.91$) of the group that remained in the study and those who were lost to follow-up at the end of the study as seen in Tables 4.3, 4.4 and 4.5.

Table 4.3: Attrition Rate: Maternal Age

Attrition	* $n = 403$	Mean Age (years)	Standard Deviation (years)	p-value (t-test)
Lost	116	23.97	3.68	0.26
In study	287	24.41	3.52	

* "Case-wise" deletion due to missing data of 12 cases: $n = 415 - 12 = 403$

Table 4.4: Attrition Rate: Maternal Mass

Attrition	*$n = 388$	Mean Maternal Mass (kg)	Standard Deviation (kg)	p-value (t-test)
Lost	112	66.95	10.48	0.79
In study	276	67.28	11.18	

* "Case-wise" deletion due to missing data of 27 cases: $n = 415 - 27 = 388$

Table 4.5: Attrition Rate: Maternal Body Mass Index (BMI)

Attrition	*n = 389	Mean BMI (kg)	Standard Deviation (kg)	p-value (t-test)
Lost	105	26.76	4.28	0.91
In study	284	26.82	4.82	

*"Case-wise" deletion due to missing data of 26 cases: $n = 415 - 26 = 389$

Because of the high attrition rate at the 36 week scan (39%) it was important to establish whether there was a particular variable/s associated with this high attrition rate. There was no significant difference in the maternal mass ($p = 0.72$) and body mass index ($p = 0.40$) when comparing the group that remained in the study at the 36 week scan and the group that was lost to follow-up at the end of the study as seen in Tables 4.6 and 4.7.

Table 4.6: Attrition Rate at the 36-week Scan: Maternal Mass

Attrition 36 weeks	*n = 388	Mean Maternal Mass (kg)	Standard Deviation (kg)	Standard Error Mean	p-value (t-test)
Lost	144	66.91	10.13	0.84	0.72
In study	244	67.34	11.45	0.73	

* "Case-wise" deletion due to missing data of 27 cases: $n = 415 - 27 = 388$

Table 4.7: Attrition Rate at the 36-week Scan: Body Mass Index (BMI)

Attrition 36 weeks	***n* = 389**	**Mean BMI**	**Standard Deviation**	**Standard Error Mean**	***p*-value (t-test)**
Lost	147	26.56	3.71	0.30	0.40
In study	242	26.95	5.17	0.33	

* "Case-wise" deletion due to missing data of 26 cases: $n = 415 - 26 = 389$

4.2.2 Attrition by HIV status

The Chi-squared test (χ^2) (level of significance $p = 0.05$) was used to assess whether there was a significant difference in the attrition rate between the study group (HIV infected) and the control group (uninfected). The calculation of $\chi^2 = 1.11$ yielded a $p = 0.29$. There was therefore no significant difference in the attrition rate at the follow-up stage of the study between the study group and the control group as seen in Table 4.8.

Table 4.8: Attrition Rate (at the follow up stage) According to HIV Status

Attrition - Follow-up post-delivery	**HIV + *n* = 194**	**HIV – *n* = 221**	**Total *n* = 415**
Lost	63 (32%)	61 (28%)	124 (30%)
In Study	131 (68%)	160 (72%)	291 (70%)

$\chi^2 = 1.11$ yielded a p-value = 0.29

4.2.3 Attrition by health facility (MOU)

The Chi-squared test (χ^2) (level of significance $p = 0.05$) was also used to assess whether there was a significant difference in the attrition rate between the two groups from the two antenatal clinics. The calculation of $\chi^2 = 0.25$ yielded a p-value = 0.6. There was therefore no significant difference in the attrition rate at the follow-up stage of the study between the groups from the MOU's as seen in Table 4.9.

Table 4.9: Attrition Rate at Khayelitsha and Gugulethu MOU's

Attrition - Follow-up post-delivery	**Khayelitsha MOU** $n = 173$	**Gugulethu MOU** $n = 242$	**Total** $n = 415$
Lost	54 (31%)	70 (29%)	124 (30%)
In Study	119 (69%)	172 (71%)	291 (70%)

$\chi^2 = 0.25$ yielded a p-value = 0.6.

4.2.4 Attrition by CD4 T-lymphocyte count in the HIV Infected Group

The t-test (level of significance $p = 0.05$) was used to assess whether there was a significant difference in the CD4 T-lymphocyte count of the HIV infected group lost in the study compared to the group that remained in the study. The calculation of $t = 1.15$ yielded a p-value = 0.25 as seen in Table 4.10. The CD4 T-lymphocyte count therefore had no significant effect on the attrition rate in the HIV infected group.

Table 4.10: Attrition Rate According to CD4 T-lymphocyte Count in the HIV Infected Group

Attrition **Follow up post delivery**	**CD4 (cells / mm³)** **Mean**	**Standard** **Deviation**	***n = 173**
Lost	448	208	50 (29%)
In Study	408	211	123 (71%)

* "Case-wise" deletion due to missing data (CD4) of 21 cases: $n = 194\text{-}21 = 173$
$t = 1.15$ yielded a p-value = 0.25

4.3 DESCRIPTIVE ANALYSIS OF THE SAMPLE (maternal age, mass and height)

4.3.1 Maternal Age

Maternal age was taken at the first visit. The sample for maternal age was $n = 403$. The minimum and maximum ages were 18 (17.9) years and 32 (31.5) years respectively with a mean of 24.3 and standard deviation (SD) of 3.5 as seen in Table 4.11 and in the histogram in Figure 4.1.

Table 4.11: Age Distribution of the Sample at the First Visit

n	**Minimum** **Age** (years)	**Maximum** **Age** (years)	**Mean** **Age** (years)	**Standard** **Deviation**
403 *	18 (17.9)	32 (31.5)	24.3	3.5

*"Case-wise" deletion due to missing data of 12 cases: $n = 415\text{-}12 = 403$

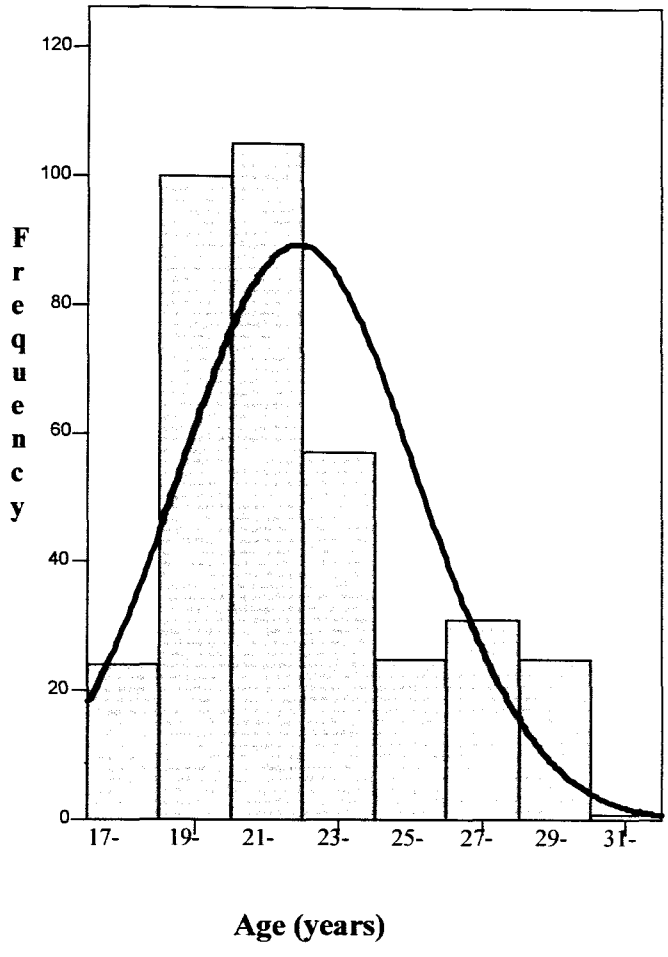

Figure 4.1: Age Distribution of the Sample n = 403 *

4.3.2 Maternal Mass

Maternal mass was taken at the first visit. The sample for maternal mass was n = 388 due to missing data of 27 patients (total n = 415). The mean maternal mass was 67.2 kg with a SD = 3.6. The minimum and maximum mass was 42 kg and 95 kg respectively, shown in Figure 4.2.

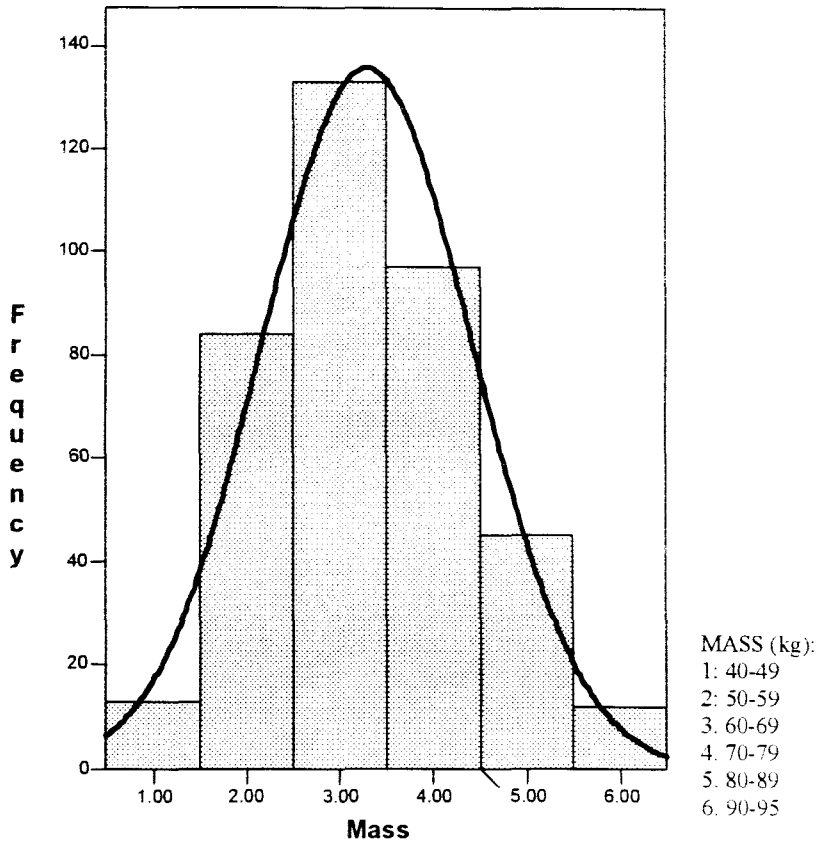

Figure 4.2: A Normal Distribution of the Mass (kg) of the Sample n = 388*
* "Case-wise" deletion due to missing data of 27 cases: n = 415- 27 = 388

4.3.4 Maternal Height

Maternal height was taken at the first visit. The sample for maternal height was n = 367 due to missing data of 48 patients (total n = 415). The mean patient height was 1.58 m with a SD = 0.06. The minimum and maximum height was 1.43 m and 1.78 m respectively as seen in Figure 4.3.

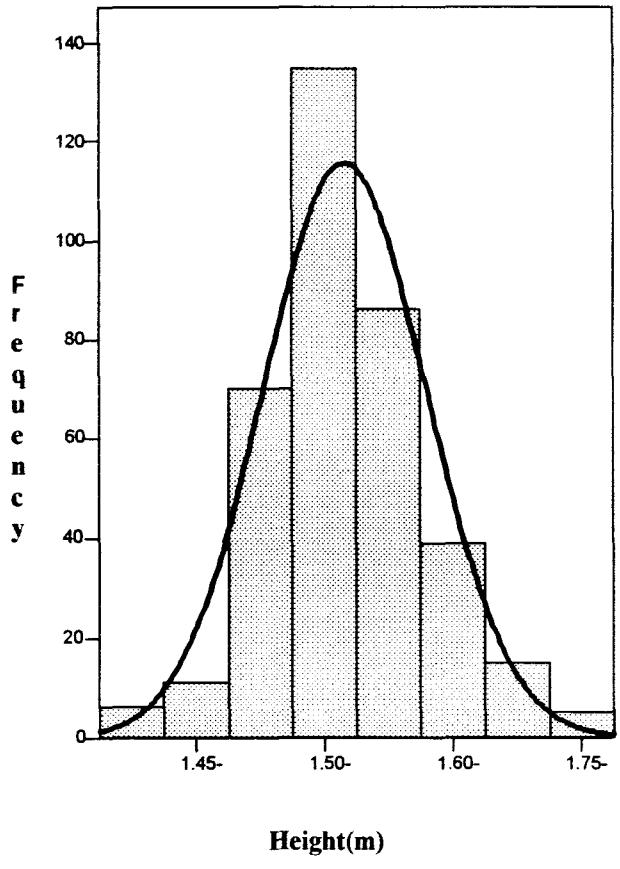

Figure 4.3: A Normal Distribution of the Height of the Sample
* "Case-wise" deletion due to missing data of 48 cases: $n = 415 - 48 = 367$

4.4. COMPARATIVE ANALYSIS of MATERNAL AGE, MASS, HEIGHT and BODY MASS INDEX

The t-test (significance level $p = 0.05$) was used to compare variables age, mass, height and body mass index of the HIV infected group and the uninfected group taken at the first scan (Table 4.12). There was a statistically significant difference in the age ($p = 0.003$) of the two groups. The HIV infected group was ± 1 year older than the uninfected group. This difference in maternal age is not clinically relevant but because it was found to be statistically significant,

it was used as a covariate when comparing repeated measurements (analysis of variance) of the study and control groups.

There was no significant difference in the maternal mass ($p = 0.97$), maternal height ($p = 0.47$) and body mass index ($p = 0.31$) of the two groups.

Table 4.12: Comparison of Maternal Age, Mass, Height and BMI

Taken at 1st Scan	**HIV Status**	***n* = 415**	**Mean**	**Standard Deviation**	***p*-value *t*-test**
Age (years)	HIV +	187	24.87	3.32	**0.003**
	HIV –	216	23.83	3.59	
	*Missing data	12			
Mass (kg)	HIV +	187	67.22	10.46	0.97
	HIV –	201	67.18	11.47	
	*Missing data	27			
Height (m)	HIV +	175	1.58	0.06	0.47
	HIV –	192	1.58	0.06	
	*Missing data	48			
BMI	HIV +	184	27.06	4.91	0.31
	HIV –	204	26.59	4.46	
	*Missing data	27			

* "Case-wise" deletion: cases were excluded where data was missing

4.5 ANALYSIS OF VARIANCE (ANOVA) FOR FETAL BIOMETRY

Tests of Within-Subjects Effects and Within-Subject Contrasts were performed when analyzing the repeat measurements of the BPD, HC, AC and FL because the HIV infected group was found to be statistically significantly older than the uninfected group.

4.5.1 Biparietal Diameter (BPD)

The mean and standard deviation of the BPD at Scans 1 to 4 were compared between the HIV infected (HIV+) and uninfected (HIV-) groups as seen in Table 4.13. Only cases where all 4 x BPD measurements were available were included in the analysis. One hundred and seventy-eight cases were excluded due to missing data. The sample for the comparison of the BPD measurements was $n = 415 - 178 = 237$. There was no significant difference between the HIV infected and uninfected groups in the mean of the BPD at each scan as well as the overall mean (marginal means) ($p = 0.31$).

Table 4.13: Comparison of Means of Biparietal Diameter (BPD)

	HIV Status	Mean (cm)	Standard Deviation	*$n = 237$
BPD1	HIV+	5.39	0.43	106
20- 24 weeks	HIV-	5.46	0.43	131
	Total	5.43	0.43	237
BPD2	HIV+	7.12	0.27	106
28 weeks	HIV-	7.14	0.26	131
	Total	7.13	0.26	237
BPD3	HIV+	7.99	0.33	106
32 weeks	HIV-	8.03	0.28	131
	Total	8.01	0.30	237
BPD4	HIV+	8.62	0.34	106
36 weeks	HIV-	8.73	0.28	131
	Total	8.68	0.31	237

* "Case-wise" deletion: There was missing data in 178 cases. The sample for the comparison of the BPD measurements was $n = 415 - 178 = 237$. $p = 0.31$

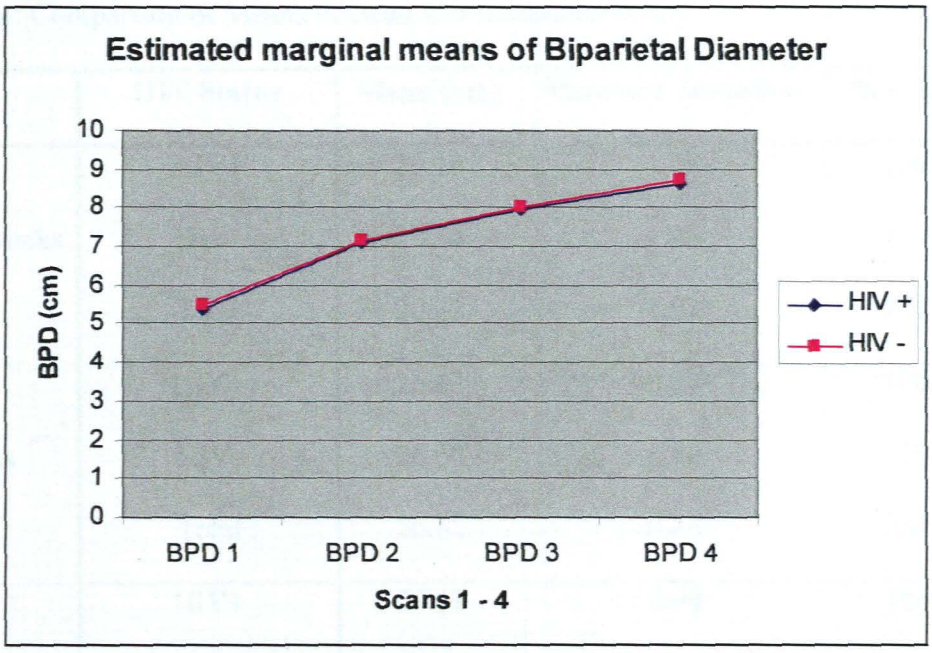

Figure 4.4: The estimated marginal means of the BPD at the four scans. There was no significant difference in the BPD between HIV infected (HIV+) and uninfected (HIV-) overall (marginal means) and at each scan ($p = 0.31$).

4.5.2 Head Circumference (HC)

The mean and standard error of the HC at Scans 1 to 4 were compared between the HIV infected (HIV+) and uninfected (HIV-) groups. Only cases where all 4 x HC measurements were available were included in the analysis. One hundred and eighty-four cases were excluded due to missing data. The sample for the comparison of the HC measurements was $n = 415 - 184 = 231$. There was no significant difference between the HIV infected and uninfected groups in the mean of the HC at each scan as well as the overall mean (marginal means) ($p = 0.64$) (Table 4.14 & Figure 4.5).

Table 4.14: Comparison of Means of Head Circumference (HC)

	HIV Status	Mean (cm)	Standard Deviation	*n = 231
HC1	HIV+	20.10	1.58	106
20- 24 weeks	HIV-	20.54	1.48	129
	Total	20.32	1.53	231
HC2	HIV+	26.63	0.87	106
28 weeks	HIV-	26.60	0.80	129
	Total	26.62	0.83	231
HC3	HIV+	29.68	0.98	106
32 weeks	HIV-	29.83	0.75	129
	Total	29.75	0.86	231
HC4	HIV+	32.03	0.86	106
36 weeks	HIV-	32.33	0.72	129
	Total	32.18	0.80	231

* "Case-wise" deletion: There was missing data in 184 cases. The sample for the comparison of the HC measurements was $n = 415 - 184 = 231$.
$p = 0.64$

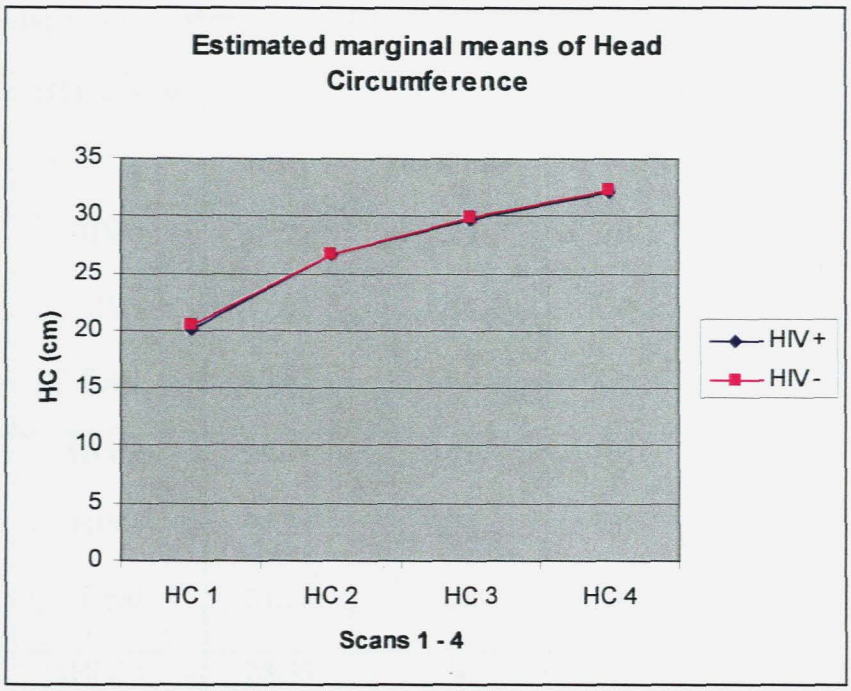

Figure 4.5: The estimated marginal means of the HC at the four scans. There was no significant difference in the HC between HIV infected (HIV+) and uninfected (HIV-) overall (marginal means) and at each scan ($p = 0.64$).

4.5.3 Abdominal Circumference (AC)

The mean and standard deviation of the AC at Scans 1 to 4 were compared between the HIV infected (HIV+) and uninfected (HIV-) groups. Only cases where all 4 x AC measurements were available were included in the analysis. One hundred and eighty cases were excluded due to missing data. The sample for the comparison of the AC measurements was $n = 415 - 180 = 235$.

There was no significant difference ($p = 0.44$) between the HIV infected and uninfected groups in the mean of the AC at each scan as well as the overall mean (marginal means) as seen in Table 4.15 & Figure 4.6.

Table 4.15: Comparison of Means of Abdominal Circumference (AC)

	HIV Status	Mean (cm)	Standard Deviation	*n = 235
AC1	HIV +	17.72	1.29	104
20-24 weeks	HIV -	17.95	1.48	131
	Total	17.85	1.40	235
AC2	HIV +	24.11	0.91	104
28 weeks	HIV -	24.14	0.74	131
	Total	24.13	0.82	235
AC3	HIV +	28.36	1.04	104
32 weeks	HIV -	28.14	1.02	131
	Total	28.24	1.03	235
AC4	HIV +	31.98	1.11	104
36 weeks	HIV -	31.93	1.24	131
	Total	31.95	1.19	235

* "Case-wise" deletion: There were missing data in 180 cases. The sample for the comparison of the AC measurements was $n = 415 - 180 = 235$.
$p = 0.44$

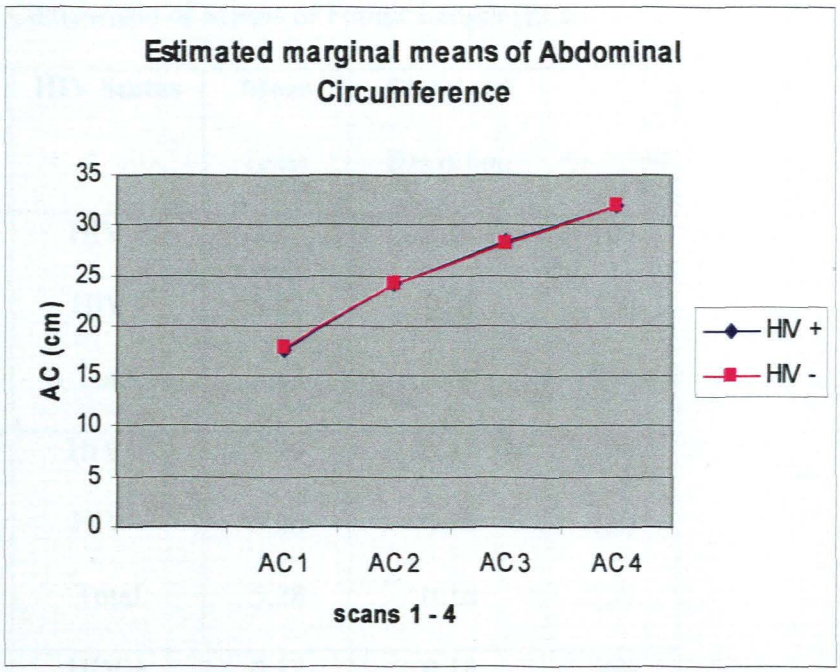

Figure 4.6: The estimated marginal means of the AC at the four scans. There was no significant difference ($p = 0.44$) in the means of the AC between HIV infected (HIV+) and uninfected (HIV-) overall (marginal means) and at each scan.

4.5.4 Femur Length (FL)

The mean and standard deviation of the FL at Scans 1 to 4 were compared between the HIV infected (HIV+) and uninfected (HIV-) groups. Only cases where all 4 x FL measurements were available were included in the analysis. One hundred and seventy-six cases were excluded due to missing data. The sample for the comparison of the FL measurements was $n = 415 - 176 = 239$. There was no significant difference ($p = 0.9$) between the HIV infected and uninfected groups in the mean of the FL at each scan as well as the overall mean (marginal means) as seen in Table 4.16 & Figure 4.7.

Table 4.16: Comparison of Means of Femur Length (FL)

	HIV Status	Mean (cm)	Standard Deviation	*n = 239
FL1	HIV +	3.83	0.38	109
20-24 weeks	HIV -	3.82	0.38	130
	Total	3.83	0.38	239
FL2	HIV +	5.29	0.17	109
28 weeks	HIV -	5.26	0.16	130
	Total	5.28	0.16	239
FL3	HIV +	6.12	0.18	109
32 weeks	HIV -	6.10	0.15	130
	Total	6.11	0.17	239
FL4	HIV +	6.78	0.33	109
36 weeks	HIV -	6.78	0.31	130
	Total	6.78	0.32	239

* "Case-wise" deletion: There were missing data in 176 cases. The sample for the comparison of the FL measurements was $n = 415 - 176 = 239$.
$p = 0.9$

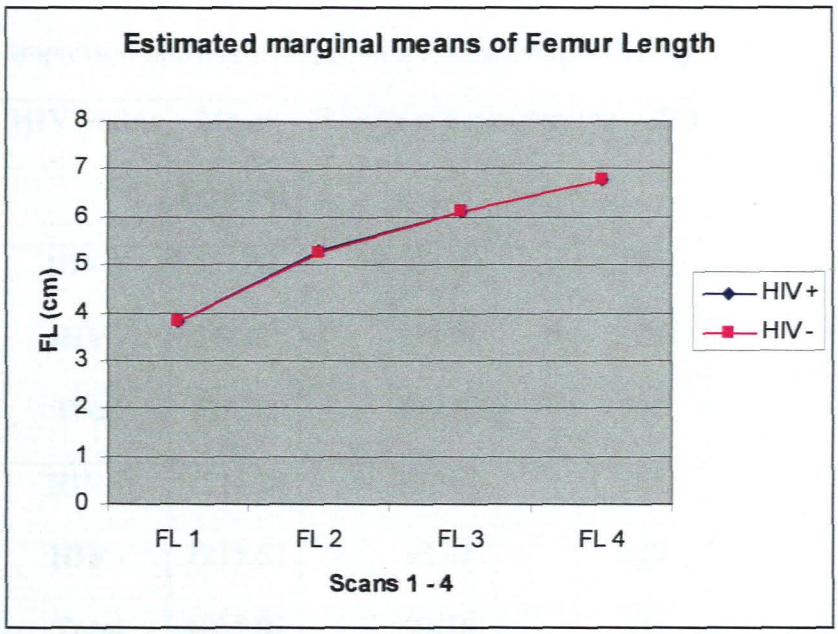

Figure 4.7: The estimated marginal means of the FL at the four scans. There was no significant difference ($p = 0.9$) in the means of the FL between HIV infected (HIV+) and uninfected (HIV-) overall (marginal means) and at each scan.

4.5.5 Estimated Fetal Weight (EFW)

The mean and standard deviation of the EFW at Scans 1 to 4 were compared between the HIV infected (HIV+) and uninfected (HIV-) groups. Only cases where all 4 x EFW measurements were available were included in the analysis. One hundred and eighty-two cases were excluded due to missing data. The sample for the comparison of the EFW measurements was $n = 415 - 182 = 233$.

There was no significant difference ($p = 0.8$) between the HIV infected and uninfected groups in the mean of the EFW at each scan as well as the overall mean (marginal means) as seen in Table 4.17 & Figure 4.8.

Table 4.17: Comparison of Means of Estimated Fetal Weight (EFW)

	HIV Status	Mean (grams)	Standard Deviation	*n = 233
EFW1	**HIV +**	527.50	101.86	104
20-24 weeks	**HIV -**	547.05	111.50	129
	Total	538.32	107.52	233
EFW2	**HIV +**	1214.25	103.94	104
28 weeks	**HIV -**	1215.62	92.31	129
	Total	1215.01	97.46	233
EFW3	**HIV +**	1895.82	245.40	104
32 weeks	**HIV -**	1907.49	176.92	129
	Total	1902.28	209.86	233
EFW4	**HIV +**	2709.65	223.99	104
36 weeks	**HIV -**	2723.55	223.35	129
	Total	2717.35	223.26	233

* "Case-wise" deletion: There were missing data in 182 cases. The sample for the comparison of the EFW measurements was $n = 415 - 182 = 233$.

$p = 0.8$

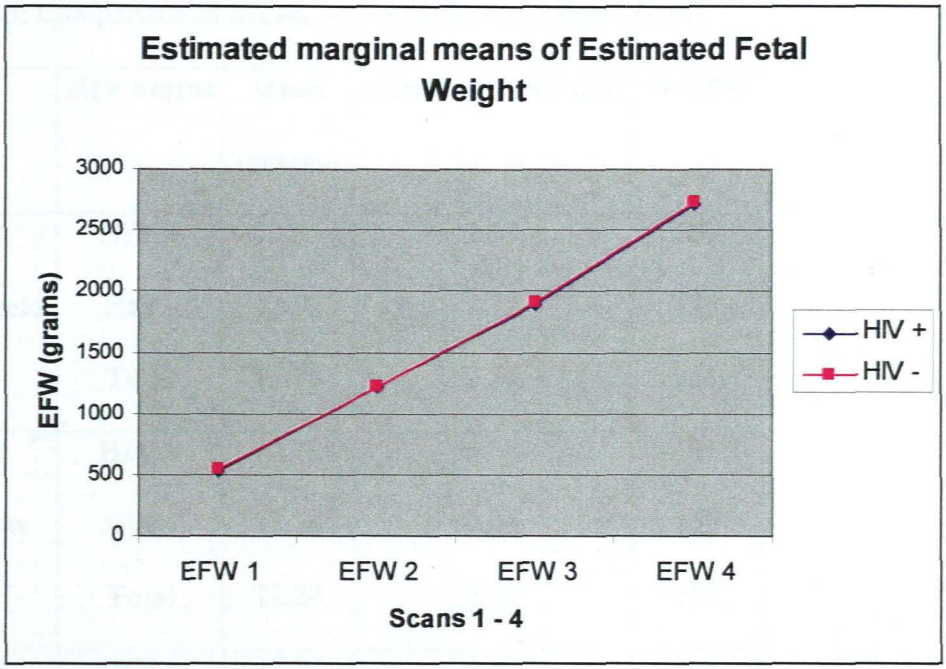

Figure 4.8: The estimated marginal means of the EFW at the four scans. There was no significant difference ($p = 0.8$) in the means of the EFW between HIV infected (HIV+) and the uninfected (HIV-) groups.

4.6 ANALYSIS OF VARIANCE OF THE AMNIOTIC FLUID INDEX (AFI)

The mean and standard deviation of the AFI at Scans 1 to 4 were compared between the HIV infected (HIV+) and uninfected (HIV-) groups. Only cases where all 4 x AFI measurements were available were included in the analysis. One hundred and eighty-four cases were excluded due to missing data. The sample for the comparison of the AFI measurements was $n = 415 - 184 = 231$. There was no significant difference ($p = 0.26$) between the HIV-infected and uninfected groups in the mean of the AFI at each scan, nor for the overall mean (marginal means) as seen in Table 4.18 & Figure 4.9.

Table 4.18: Comparison of Means of Amniotic Fluid Index (AFI)

	HIV Status	Mean (grams)	Standard Deviation	*n = 231
AFI 1	**HIV +**	12.55	2.33	101
20-24 weeks	**HIV -**	13.02	2.72	130
	Total	12.78	2.56	231
AFI 2	**HIV +**	12.12	5.34	101
28 weeks	**HIV -**	12.36	4.96	130
	Total	12.24	5.14	231
AFI 3	**HIV +**	11.32	5.47	101
32 weeks	**HIV -**	11.81	5.64	130
	Total	11.56	5.56	231
AFI 4	**HIV +**	10.63	5.73	101
36 weeks	**HIV -**	10.59	5.56	130
	Total	10.61	5.63	231

* "Case-wise" deletion: There were missing data in 184 cases. The sample for the comparison of the AFI measurements was $n = 415 - 184 = 231$.
$p = 0.26$

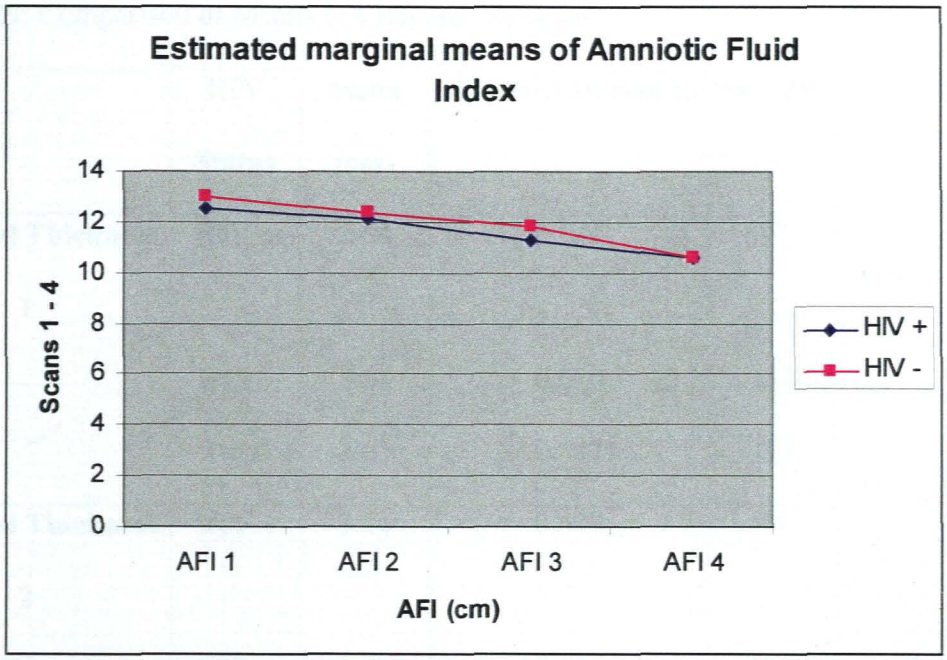

Figure 4.9: The estimated marginal means of the AFI at the four scans. There was no significant difference ($p = 0.26$) in the means of the AFI between HIV infected (HIV+) and uninfected (HIV-) overall (marginal means) and at each scan.

4.7 COMPARISON ANALYSIS OF VARIANCE OF THE PLACENTAL THICKNESS

The mean and standard deviation of the placental thickness at Scans 1 to 4 were compared between the HIV infected (HIV+) and uninfected (HIV-) groups. Only cases where all 4 x placental thickness measurements were available were included in the analysis. Two hundred and eighteen cases were excluded due to missing data. The sample for the comparison of the placental thickness measurements was $n = 415 - 218 = 197$. There was no significant difference ($p = 0.71$) between the HIV infected and uninfected groups in the mean of the placental thickness at each scan nor in the overall mean (marginal means) as seen in Table 4.19 and Figure 4.10.

Table 4.19: Comparison of Means of Placental Thickness

	HIV Status	Mean (cm)	Standard Deviation	*n = 197
Placental Thickness 1	**HIV +**	2.64	0.547	87
	HIV -	2.66	0.620	110
	Total	2.65	0.587	197
Placental Thickness 2	**HIV +**	3.11	0.670	87
	HIV -	3.05	0.711	110
	Total	3.07	0.692	197
Placental Thickness 3	**HIV +**	3.40	0.882	87
	HIV -	3.23	0.746	110
	Total	3.31	0.811	197
Placental Thickness 4	**HIV +**	3.47	0.927	87
	HIV -	3.50	0.780	110
	Total	3.49	0.846	197

* "Case-wise" deletion: There were missing data in 218 cases. The sample for the comparison of the EFW measurements was $n = 415 - 218 = 197$.
$p = 0.71$

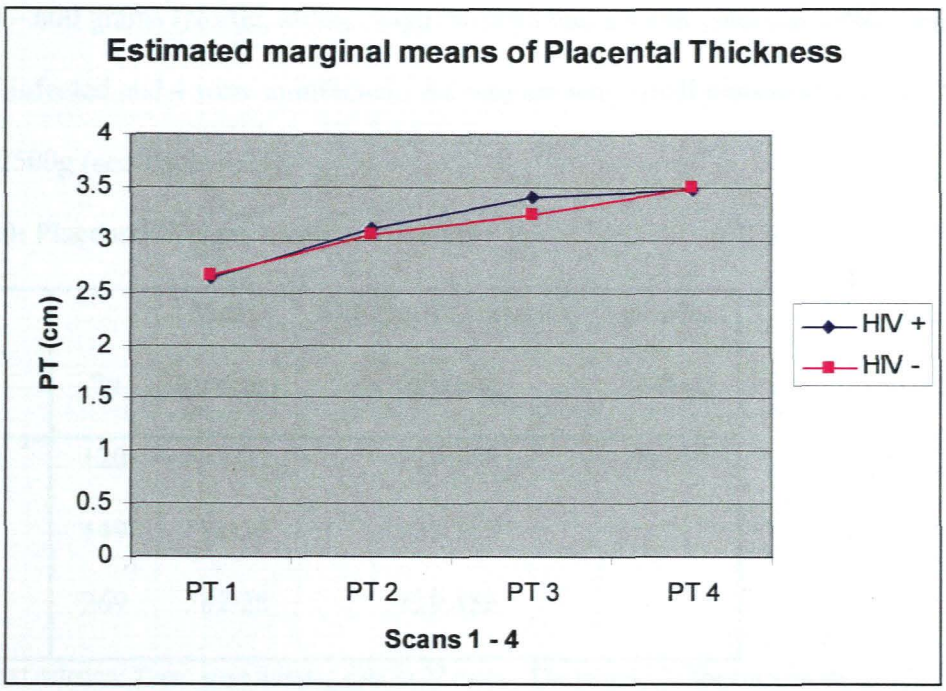

Figure 4.10: The estimated marginal means of the placental thickness at the four scans. There was no significant difference ($p = 0.71$) in the means of the placental thickness between HIV infected (HIV+) and the uninfected (HIV-) groups.

4.8 COMPARATIVE ANALYSIS OF VARIABLES POST DELIVERY

4.8.1 Placental Weight

A t-test was used (level of significance $p = 0.05$) to establish whether there was a significant difference in the placental weight measured at the time of delivery between the HIV infected group and the uninfected group. The number of cases followed up post delivery was $n = 291$. There were missing data in 22 cases. The actual sample for the comparison of the placental weight was $n = 291 - 22 = 269$.

There was no significant difference in the placental weight between the two groups ($p = 0.92$) as seen in Table 4.20. The means of the placental weight of the HIV infected group and uninfected group were 583 grams and 581 grams respectively which was within the normal

range 400 – 600 grams (Nortjé, 2003). Eight women had a small placenta < 400 grams: 4 were HIV infected and 4 were uninfected. All women with small placentas also had low birth weight < 2500g (see Table 4.21).

Table 4.20: Placental Weight Measured at Birth

HIV Status	*n	Mean (grams)	Standard Deviation (grams)	p-value (t-test)
HIV +	120	583.03	127.658	0.92
HIV -	149	581.54	131.255	
Total	269	582.28	129.456	

* "Case-wise" deletion: There were missing data in 22 cases. The sample for the comparison of the placental weight was $n = 291 - 22 = 269$.
$p = 0.92$

Table 4.21: Patients with Small Placentas (< 400 grams)

	Placental weight (grams)	HIV Status	Maternal Complications
1.	320	HIV +	LBW (2280g) at 40 weeks
2.	320	HIV -	APH PTD at 27 weeks LBW (950g)
3.	300	HIV-	IUD at 37 weeks GHD Placenta abruption LBW(2300g)
4.	300	HIV+	PTD at 33 weeks LBW (1400g)
5.	380	HIV+	PTD at 28 weeks LBW (980g)
6.	360	HIV+	CD4 = 166 cells / mm^3 GHD HELLP IUD at 31 weeks LBW (1535g)
7.	240	HIV-	IUD at 25 weeks LBW (600g)
8.	380	HIV-	Delivered at 37 weeks LBW (2340g)

KEY: GHD – Gestational Hypertensive Disease; **LBW** – Low birth weight; **IUD** - Intrauterine Death; **APH** - Antepartum Haemorrhage (unclassified); **HELLP** - Haemolysis, Elevated Liver Enzymes, Low Platelet; **PTD** - Preterm delivery.

4.8.2 Birth Weight

A t-test was used (level of significance $p = 0.05$) to establish whether there was a significant difference in the birth weight of the neonates of the HIV infected group and the uninfected group. There was no significant difference in the birth weight between the two groups ($p = 0.8$) as seen in Table 4.22. There was also no significant difference ($p = 0.9$) in the birth weight of males compared to females as seen in Table 4.23. The mean birth weight of males was 2981 grams (SD: 673) and that of females was 2972 grams (SD: 567).

Table 4.22: Birth Weight

HIV Status	*n	Birth Weight Mean (grams)	Standard Deviation	p-value t-test
HIV +	131	2969.95	583.81	0.8
HIV -	158	2981.84	646.39	
Total	289	2975.89	615.10	

* "Case-wise" deletion: There were missing data in 2 cases. The sample for the comparison of birth weight was $n = 291 - 2 = 289$.

Table 4.23: Gender Specific Birth Weight

Gender	*n	Birth Weight Mean (grams)	Standard Deviation	p – value t-test
Male	136	2981.40	672.96	0.9
Female	153	2972.31	566.50	
Total	289			

* "Case-wise" deletion: There were missing data in 2 cases. The sample for the comparison of gender specific birth weight was $n = 291 - 2 = 289$.

4.8.3 Preterm Delivery

The Chi-squared test (χ^2) (level of significance $p = 0.05$) was used to assess whether there was a significant difference in the time of delivery between the HIV infected and the uninfected groups. The calculation of $\chi^2 = 0.62$ yielded a $p = 0.43$. There was therefore no significant difference in the time of delivery of the two groups as seen in Table 4.24.

Table 4.24: Time of Delivery

	HIV + $n = 131$	**HIV –** $n = 160$	**Total** $n = 291$
Preterm delivery	17*(13%)*	13 *(8%)*	30 *(10%)*
Term delivery	114 *(87%)*	147*(92%)*	261 *(90%)*

$\chi^2 = 0.62$ yielded a $p = 0.43$

4.8.4 Type of Delivery

The Chi-squared test (χ^2) (level of significance $p = 0.05$) was used to assess whether there was a significant difference in the type of delivery between the HIV infected and the uninfected groups. The different types of delivery included normal vertex delivery (NVD), caesarean section (CS) and other. The calculation of $\chi^2 = 4.52$ yielded a $p = 0.1$. There was therefore no significant difference in the type of delivery of the two groups as seen in Table 4.25.

Table 4.25: Type of Delivery

Type of Delivery	**HIV +** $n = 131$	**HIV –** $n = 160$	**Total** $n = 291$
NVD	89 *(68%)*	122 *(76%)*	211 *(73%)*
CS	38 *(29%)*	29 *(18%)*	67 *(23%)*
Other	4 (3%)	9 *(6%)*	13 (4%)

$\chi^2 = 4.52$ yielded a $p = 0.1$

4.8.5 Perinatal Complications

4.8.5.1 Comparative Analysis of Perinatal Complications

The Chi-squared test (χ^2) (level of significance $p = 0.05$) was used to assess whether there was a significant difference in the number of perinatal complications between the HIV infected and the uninfected groups. Perinatal complications considered are included in Table 4.27. There was no significant difference ($p = 0.1$) in the number of perinatal complications of the two groups as seen Table 4.26.

Table 4.26: Perinatal Complications in HIV+ and HIV- Women

		HIV + $n = 125$	HIV – $n = 166$	Total $n = 291$
Perinatal Complications	No	68 *(54 %)*	100 *(60%)*	168 (58%)
	Yes	57 *(46%)*	66 *(40%)*	123*(42%)*

$p = 0.1$

4.8.5.2 Types of Perinatal Complications

The number of women in this study with perinatal complications was $n = 123$. The complications included gestational hypertensive disease (31.7%), fetal distress (29.2%), preterm delivery (24.3%), prolonged labour (17.8%), low birth weight (16.2%), cephalopelvic disproportion (8.9%), intrauterine death (8.1%), infections (7.3%), VDRL+ (7.3%), intrauterine growth restriction (5.6%), premature rupture of membranes (5.6%), breech presentation (4.8%), low APGAR (4.0%), unclassified antepartum haemorrhage (3.2%), placenta abruptio (3.2%), post dates (3.2%), herpes zoster (2.4%), HELLP (2.4%), postpartum haemorrhage (1.6%), retained products of conception (1.6%), TB (1.6%), placenta praevia (0.8%), pulmonary oedema (0.8%) (see Table 4.27).

Table 4.27: Types of Perinatal Complications

	Perinatal Complications	HIV+ $N = 57$	HIV- $n = 66$	Total Perinatal Complications $n = 123$	TOTAL $n = 291$
1	Unclassified antepartum haemorrhage	2	2	4(3.2%)	1.3%
2	Placenta Abruptio	3	1	4 (3.2%)	1.3%
3	Breech	2	4	6 (4.8%)	2.0%
4	Cephalo-pelvic disproportion	5	6	11 (8.9%)	3.7%
6	Fetal distress	20	16	36 (29.2%)	12.3%
7	Prolonged labour	9	13	22 (17.8%)	7.5%
8	Gestational Hypertensive Disorders	17	22	39 (31.7%)	13.4%
10	HELLP	1	2	3 (2.4%)	1.0%
11	Herpes Zoster	3	0	3 (2.4%)	1.0%
12	Intrauterine death	4	6	10 (8.1%)	3.4%
13	Intrauterine growth restriction	4	3	7 (5.6%)	2.4%
14	Low Apgar	1	4	5 (4.0%)	1.7%
16	Placenta Praevia	0	1	1 (0.8%)	0.3%
18	Post dates	0	4	4 (3.2%)	1.3%
19	Postpartum haemorrhage	2	0	2 (1.6%)	0.6%
20	Premature rupture of membranes	5	2	7 (5.6%)	2.4%
21	Preterm delivery	17	13	30 (24.3%)	10.3%

	Perinatal Complications	HIV+ *N* = 57	HIV- *n* = 66	Total Perinatal Complications *n* = 123	TOTAL *n* = 291
22	Infections	6	3	9 (7.3%)	3.0%
23	Pulmonary oedema	0	1	1 (0.8%)	0.3%
24	Retained products of conception	1	1	2 (1.6%)	0.6%
25	TB	2	0	2 (1.6%)	0.6%
27	VDRL+	7	2	9 (7.3%)	3.0%
28	Low birth weight	11	9	20 (16.2%)	6.8%

4.8.5.3 Intrauterine Growth Restriction (IUGR)

All of the 7 women with IUGR had associated complications as seen in Table 4.28.

Table 4.28: IUGR and Associated Complications

Diagnosed with IUGR	HIV Status	Associated complication/s
1.	HIV +	Low CD4 Low Birth Weight
2.	HIV+	Pulmonary Tuberculosis Gestational hypertensive disease Preterm delivery at 33 weeks
3.	HIV+	Antepartum Haemorrage Prolonged labour Low birth weight Preterm delivery at 36 weeks
4.	HIV-	Fetal distress Low birth weight
5.	HIV+	Breech Gestation Hypertensive disease Low birth weight Preterm delivery at 35 weeks
6.	HIV-	Gestational hypertensive disease
7.	HIV-	Low birth weight

4.8.5.4 Preterm Delivery (PTD)

30 women were diagnosed with preterm delivery. Seventeen with PTD were HIV infected and 13 were uninfected. Associated complications are listed in Table 4.29

Table 4.29: Preterm Delivery (< 37 weeks gestation) and Associated Complications

	GA at Birth (weeks)	HIV Status	GHD	LBW	SB/ IUD	APH	IUGR	FD	PROM	PL ABR	HELLP	BR	INF	PL	PTB
1.	33	+	✔				✔								✔
2.	36	+		✔		✔	✔							✔	
3.	34	+		✔				✔	✔						
4.	35	+	✔												
5.	36	+													
6.	36	+													
7.	34	+		✔					✔						
8.	36	+	✔	✔	✔										
9.	31	+	✔							✔					
10.	33	+													
11.	28	+													
12.	27	+											✔		
13.	31	+	✔		✔					✔	✔	✔			
14.	35	+	✔	✔			✔								
15.	36	+													
16.	36	+	✔	✔											
17.	35	+	✔	✔		✔									

	GA at Birth (weeks)	HIV Status	GHD	LBW	SB/ IUD	APH	IUGR	FD	PROM	PL ABR	HELLP	BR	INF	PL	PTB
18.	32	-		✔											
19.	27	-				✔									
20.	36	-		✔					✔			✔			
21.	35	-													
22.	28	-	✔		✔										
23.	36	-				✔		✔							
24.	36	-	✔					✔			✔				
25.	33	-	✔		✔										
26.	35	-	✔	✔											
27.	36	-	✔												
28.	36	-	✔												
29.	25	-			✔										
30.	36	-	✔	✔											

KEY: GA – Gestational Age; **GHD** – Gestational Hypertensive Disease; **LBW** – Low birth weight; **SB** - Stillbirth; **IUD** - Intrauterine Death; **APH** - Antepartum Haemorrhage (unclassified); **IUGR** – Intrauterine Growth Restriction; **FD** – Fetal distress; **PROM** – Premature rupture of membranes; **PL ABR** - Placental abruptio; **HELLP** - Haemolysis, Elevated Liver Enzymes, Low Platelet; **BR** – Breech; **INF** - Infection; **PL** - Prolonged labour; **PTB** - Pulmonary Tuberculosis

4.9 CD4 T-LYMPHOCYTE COUNT IN HIV INFECTED WOMEN AND PERINATAL COMPLICATIONS

4.9.1 CD4 T-lymphocyte count < 200 cells / mm³ and perinatal complications

The Chi-squared test (χ^2) (level of significance $p = 0.05$) was used to assess whether there was a significant difference in the number of perinatal complications in the HIV infected group with a CD4 T-lymphocyte count of more and less than 200 cells / mm³. Eight cases were deleted due to missing data. The sample used was $n = 131 - 8 = 123$. Perinatal complications considered are included in Table 4.27. The calculation of $\chi^2 = 1.53$ yielded a $p = 0.22$. There was therefore no significant difference in the number of perinatal complications in the HIV-infected group with a CD4 T-lymphocyte count < 200 cells / mm³ compared to those with a count > 200 cell / mm³ as seen in Table 4.30.

Table 4.30: Perinatal Complications and CD4 T-lymphocyte Count in HIV+ Women

		CD4 T-lymphocyte count (cells/mm³)		**Total**
		< 200	**> 200**	
		***n* = 21**	***n* = 102**	****n* = 123**
Perinatal Complications	**No**	*8 (39%)*	*54 (53%)*	*62 (50%)*
	Yes	*13 (61%)*	*48 (47%)*	*61 (50%)*

* "Case-wise" deletion: The CD4 T-lymphocyte count was not available in 8 cases. The sample was therefore $n = 131 - 8 = 123$.
$\chi^2 = 1.53$ yielded a $p = 0.22$

4.9.2 CD4 T-lymphocyte Count and Intra-uterine Growth Restriction in HIV Infected Women

One of the 4 HIV infected women with IUGR had a low CD4 T-lymphocyte count < 200 cells / mm^3 (see Table 4.27).

4.9.3 CD4 T-lymphocyte Count and Preterm Delivery in HIV Infected Women

The Chi-squared test (χ^2) (level of significance $p = 0.05$) was used to assess whether there was a significant difference in the number of term / preterm deliveries in the HIV infected group with a CD4 T-lymphocyte count of more than 200 cells / mm^3 compared to a CD4 count < 200 cells / mm^3. There was no significant difference $p = 0.4$ as seen in Table 4.31.

Table 4.31: Preterm Delivery and CD4 T-lymphocyte Count

	Time of Delivery		**Total**
CD4 Cells / mm^3	**Preterm Delivery < 37 weeks** ***n* = 17**	**Term Delivery 37 – 42 weeks** ***n* = 106**	***n* = *123**
< 200	4 (19%)	17 (81%)	21
> 200	13 (13%)	89 (87%)	102

* "Case-wise" deletion: The CD4 T-lymphocyte count was unavailable in 8 cases. The sample was therefore $n = 131 - 8 = 123$.
χ^2 test yielded $p = 0.4$

4.10 MATERNAL DEATHS

There were 2 (1%) maternal deaths in the HIV infected group (n = 194).

4.11 SUMMARY OF RESULTS

A sample n = 415 was recruited. The attrition rate was 12% at the 28 week scan, 23% at the 32 week scan, 39% at the 36 week scan and 30% at follow-up post-delivery. There was a high attrition rate at the 36 week scan and follow up post-delivery.

The age of the participants ranged from 18 – 32 years with a mean = 24.3 and SD = 3.5. The maternal mass taken at the first scan ranged from 42 kg – 95 kg with a mean = 67.2 kg with a SD = 3.6. The maternal height ranged from 1.43 m – 1.78 m with a mean = 1.58 and SD = 0.06.

A t-test (p=0.05) showed no significant difference in the mass, height and body mass index of the HIV infected and uninfected groups. There was however a statistically significant difference (p=0.003) in the age of the 2 groups. Although the difference in age (± 1 year) had no clinical relevance it nevertheless had to be used as a covariate when comparing repeat measurements for analysis of variance of the 2 groups.

Analysis of variance of the repeat measurements (Scan 1 – 4) of the fetal parameters (BPD, HC, AC and FL), estimated fetal weight, amniotic fluid index and placental thickness showed no significant difference between the HIV infected and uninfected groups.

There was no significant difference (p=0.05) in the birth weight, placental weight, type of delivery, time of delivery (term / preterm) and prevalence of perinatal complications of the HIV infected and the uninfected groups.

There was a 42% prevalence of perinatal complications in both groups ($n = 291$) with 46% prevalence in the HIV infected group ($n = 123$) and 40% in the uninfected group ($n = 166$). Perinatal complications included gestational hypertensive disease (31.7%), fetal distress (29.2%), preterm delivery (24.3%), prolonged labour (17.8%), low birth weight (16.2%), intrauterine death (8.1%), cephalopelvic disproportion (8.9%), infections (7.3%), VDRL positive (7.3%), intrauterine growth restriction (5.6%), premature rupture of membranes (5.6%), unclassified antepartum haemorrhage (3.2%), breech presentation (4.8%), low APGAR (4%), placenta abruptio (4%), post dates (3.2%), herpes zoster (2.4%), HELLP (2.4%), postpartum haemorrhage (1.6%), retained products of conception (1.6%), PTB (1.6%), placenta praevia (0.8%) and pulmonary oedema (0.8%).

There was no significant difference ($p = 0.22$) in the number of perinatal complications in the HIV infected group with a CD4 T-lymphocyte count < 200 cells / mm^3 compared to those with a count > 200 cells / mm^3. Perinatal complications occurred in 61% of the low CD4 count group ($n = 21$) (< 200 cells / mm^3) while 48% occurred in the group with CD4 > 200 cells / mm^3 ($n = 102$).

One of the four HIV infected women with IUGR had a low CD4 T-lymphocyte count < 200 cells / mm^3.

The χ^2 test ($p = 0.4$) showed no significant difference in the number of preterm deliveries in the HIV infected group with a CD4 T-lymphocyte count > 200 cells / mm^3 compared to a CD4 count < 200 cells / mm^3. Preterm delivery occurred in 19% of women with CD4 < 200 cells / mm^3 and 13% in women with CD4 > 200 cells / mm^3.

There were 2 (1%) maternal deaths in the HIV infected group.

5. DISCUSSION AND RECOMMENDATIONS

5.1 REVISION OF RESEARCH HYPOTHESES AND METHODOLOGY

Changes in the protocol of the PMTCT Programme (Provincial Government of Western Cape, 2002) were implemented 6 months after the study started. The changes that affected the study included:

- The exclusion of a secondary hypothesis – *"there is a significant difference in fetal growth in HIV infected pregnant women using ZDV from 34 weeks gestation compared to those who do not"*. At the start of the study the two MOU's used different ARVT regimens. Gugulethu MOU used NVP as a single dose to the mother at the onset of labour while Khayelitsha MOU used ZDV from 34 weeks gestation. This difference in ARVT protocol presented two groups of pregnant women: one group using ZDV from 34 weeks and the other not – hence the opportunity to assess whether ZDV had an effect on fetal growth from 34 weeks gestation. Six months into the study the ARVT regimen was revised: both MOU's now use a combination of short course ZDV and a single dose NVP. This improvement in ARVT regimen was supported by a Cochrane review (Brocklehurst & Volmink, 2005) that suggests NVP had a higher risk of resistance when used as monotherapy and that combination therapy - short course ZDV and a single dose NVP are effective for reducing MTCT.
- There was a variation in the time that the blood test was done for the CD4 T-lymphocyte count for HIV infected women. The study protocol included a blood test done at 20-24 weeks gestation (first scan) for CD4 T-lymphocyte count for HIV infected women. The time that the blood test was done was therefore controlled. The revised PMTCT Protocol offered a blood test for CD4 T-lymphocyte count to all pregnant women testing HIV

positive at the MOU's. This blood test was usually done on the day of the first or second visit to the MOU. For the women participating in the study the blood test could have been taken any time before 20-24 weeks gestation. It is uncertain whether the variation in time of the blood test had any effect on the CD4 T-lymphocyte count. This study showed no evidence of an association between CD4 T-lymphocyte count and perinatal complications, IUGR or preterm delivery. These findings will be discussed later.

5.2 SAMPLE INCLUDED PARA 0 (Primagravida)

Women with a history of Para 0 (first birth) were included in this study. According to Fawcus (2003) Para 0 may be associated with many complications e.g. gestational proteinurea hypertension, cephalopelvic disproportion and postpartum haemorrhage. These complications may also have an adverse effect on fetal well-being. However if Para 0 participants were excluded from this study there would have been a problem in getting a large enough sample within the specified time. Sixty-eight percent ($n = 281$) of the women included in the study were Para 0.

5.3 RELIABILITY OF ULTRASOUND MEASUREMENTS WITH MULTIPLE SCANNERS

A second scanner was introduced midway through the study because of the extended time needed to complete the study. Although both sonographers were experienced scanners there may have been a significant variation in measurements taken by the two.

A systematic review of the accuracy of EFW by Dudley (2005) suggests there is too large an intra- and inter-observer variability for EFW. The main problem seemed to be the

variability of the AC. An intra- and inter-observer variability study should have been done to assess the degree of variation in the ultrasound measurements taken by the two scanners which would have resulted in a more reliable outcome of the research results. It is recommended that whenever more than one scanner is used for fetal measurements in ultrasound research, an intra- and inter-observer variability study must be included.

5.4 ATTRITION RATE

There was a high attrition rate in the study. There was no specific variable/s found to have a significant effect on the high attrition rate.

A sample $n = 302$ was statistically determined for the study using EPI Info 2000. To allow for a 25% attrition rate the target sample was increased to 400. The sample recruited was 415, however there was >25 % attrition rate at two stages of the study. Attrition rate at the different scanning stages of the study varied. At the 28-week and 32-week scan the attrition rate was within the acceptable range (12% and 23% respectively). At the 36-week scan and follow-up post-delivery it was 39% and 30% respectively. The 36-week scan had the highest attrition rate. However the preterm delivery cases were included in the analysis. If the preterm delivery cases ($n = 30$) were excluded, the attrition rate for the 36-week scan would be reduced to 32% ($n = 162 - 30 = 132$).

When participants were referred to Secondary (MMH) and Tertiary (GSH) Level Care with suspected complications, they were often lost to follow-up i.e. they did not return for the follow-up scans. All health professionals at the referral centres were not necessarily aware of the research protocol. It is uncertain whether better communication between the researcher and the referral centres could have reduced this drop out rate.

The assumed statistical power for the study was 80%. Due to the higher than expected attrition rate it was reduced to 78%. The high attrition rate at the 36-week scan was unavoidable. The high attrition rate at the follow-up stage of the study was due to difficulties encountered locating hospital folders post-delivery. Due to time constraints and staff shortages in the Medical Records Departments, locating hospital folders became a huge administrative stumbling block. It was suggested by some hospital staff members that HIV infected patients removed their own hospital folders to destroy them. The fear of disclosure of HIV status and breech of confidentiality still plagues our communities living with HIV.
It is recommended that researchers embarking on studies dependent on patients' data in public health institutions in the Western Cape allow for ± 35% attrition rate to avoid an inadequate sample size.

5.5 AGE AS A COVARIATE

Although the age of the women in the study was controlled (18 – 32 years), there was still a statistically significant difference ($p = 0.003$) in age of the 2 groups. The mean age of the HIV infected group was ±25 years and that of the uninfected group was ±24 yrs. The difference in age had no clinical relevance however because it was statistically significant it was used as a covariate when comparing repeat mean measurements of the BPD, HC, AC, FL, EFW and AFI.

5.6 FETAL BIOMETRY

Fetal biometric parameters used to test the main hypothesis "*there is no significant difference in fetal growth in HIV infected and uninfected women*" included BPD, HC, AC and FL. All

four parameters were incorporated in estimating gestational age, which is in keeping with current trends of combining several measurements. Jaffe & Abramowicz (1997) suggest combining the BPD, HC and FL can decrease the estimate error by ± 5 days. Chitty et al Fetal Charts (Chitty et al, 1994 a, b, c) are the reference charts currently used in public hospitals in the Western Cape and were used in this study. These charts were derived from populations in Europe and North America and are used because no charts have been developed for a South African or Western Cape population. Studies have shown that there is variation in fetal growth and birth weight not only in different populations but also within populations with different ethnic groups (Gardosi, 1995; Wilcox et al, 1993; Spencer et al, 1995). Based on a study on a Dutch population with different ethnic groups, Drooger et al (2005) suggest that there are factors like maternal height, weight, parity and fetal gender that may affect variation in fetal growth. Developing a formula for estimating fetal weight for an ethnically diverse population is a challenge. McCowan et al (2004) developed customized charts for Maoris, Tongans and Samoans in the New Zealand population. Birth weight coefficients are used in growth charts so that customized charts can be used for specific groups within a population. The question arises: are the Chitty et al Fetal Charts (1994 a, b, c) used in the Western Cape appropriate for such a diverse ethnic population? It is therefore recommended that the Western Cape develop its own reference charts taking into account its diverse ethnic population and the factors suggested above by Drooger et al (2005).

The use of Chitty et al Fetal Charts (1994 a, b, c) had no effect on the outcome of this study because the main hypothesis was testing the difference between the HIV infected and the uninfected groups – the same charts were used for both groups. It could however have an effect on accurate estimation of gestational age and estimated birth date.

Results showed no significant difference in the mean at each scan as well as the overall mean of all four parameters (BPD, HC, AC, and FL). The overall means of the BPD, HC, AC and FL for the HIV infected and uninfected groups were very similar suggesting HIV infection irrespective of CD4 T-lymphocyte count, has no significant effect on fetal growth. This supports the argument that > 50% of MTCT of HIV occurs during labour and delivery and not antenatally (Silva & Jeanty, 1997; Cronje, 2003).

5.7 ESTIMATED FETAL WEIGHT (EFW) AND BIRTH WEIGHT

It is recommended in literature that the difference between EFW and actual birth weight should not exceed 15% and that the choice of formula for estimating fetal weight should include a minimum of 3 fetal measurements (head, abdomen and femur) (Benson & Doubilet, 1998). Hadlock et al (1984, 1985) charts were used in this study because of its high level of validity (Chien et al, 2000). Hadlock et al's formula has an accuracy of fetal weight prediction of 15% (Hadlock, 1984 & 1985). The formula incorporates 4 fetal measurements (BPD, HC, AC and FL) as opposed to Shephard et al's formula (Shephard et al, 1987) that uses 2 fetal measurements (AC and BPD / AC and FL).

The study showed no significant correlation between EFW and birth weight ($p = 0.579$) (Appendix VIa: Birth Weight and EFW Correlation), which is in contradiction to what most of the literature states. The reason for this could be that the EFW was done at 36 weeks gestation i.e. 2 to 4 weeks before the actual birth date in most cases. EFW is usually done just before birth.

A subjective view of the means of EFW at scans 1-4 compared to an EFW chart proposed by Doubilet et al (1997) is shown in Appendix VIb: Comparison of EFW of Study and Doubilet

et al. The mean values of the EFW in the study seem higher than Doubilet et al's charts (1997). This raises the question again: do we need to develop fetal reference charts for the Western Cape population?

A lack of reproducibility of fetal measurements cannot be ruled out because no inter- and intra-observer study was included in this study. Recent studies have found that there is high rate of errors in the prediction of fetal weight by ultrasound (Dudley, 2005). The main reason given by Dudley (2005) after completing a systematic review was that the inaccuracies in ultrasound estimation of fetal weight were caused by a large inter-and intra-observer variability. Gull et al (2002) found that the high rate of errors were due to the inter-observer variability of the AC. Measurement of the AC in the 3rd trimester can be challenging when fetal breathing can make a difference in the AC. To improve the accuracy of EFW Dudley (2005) proposed the following:

- Multiple fetal measurements (especially AC) must be done and the average must be used
- Ensure optimum image quality
- Uniform calibration of all ultrasound machines that are used in a unit
- Scanners must acknowledge a long learning curve
- There must be a regular audit of the quality of scanning

Some studies have shown that there is a need for gender-specific fetal growth charts (Schild, et al, 2004; Schwärzler et al, 2004). This study showed no significant difference between birth weight of male and female fetuses ($p = 0.9$). However the sample ($n = 289$) was small and a larger study may yield different results.

The average normal birth weight for males is ±3400 grams and for females it is ±3150 grams (Cilliers, 2003). In this study the average birth weight for males was 2981 grams (SD: 673) and for females was 2972 grams (SD: 566). The overall average birth weight (2975 grams) in the study was lower than the normal overall average birth weight (3275 grams) suggested by Cilliers (2003). Cilliers does however state that birth weight can vary widely citing an example where it was found that the average birth weight for black women in Bloemfontein was 2900 grams (2003). The questions arise: is the lower average birth weight found in this study due to socio-economic or genetic factors; and are we using appropriate birth weight reference charts for the Western Cape population? As mentioned earlier, studies have shown that there is variation in fetal growth and birth weight not only in different populations but also within populations with different ethnic groups (Gardosi, 1995; Wilcox et al, 1993; Spencer et al, 1995). It is recommended that birth weight reference charts be developed for the diverse population in Western Cape.

5.8 AMNIOTIC FLUID INDEX

A low AFI < 5 cm is reported as oligohydramnios and is a marker for IUGR (Manning, 1996). It is therefore important to include AFI in the ultrasound assessment for suspected IUGR. In this study the mean AFI measurements taken at each scan (Table 4.18) was within normal limits according to Jaffe & Abramowicz (1997) (Appendix V). There was also no significant difference in the overall mean as well as the mean at scans 1 – 4 of the AFI measurements. Oligohydramnios was not associated with any of the 7 women with IUGR which is contrary to what many studies suggest. Many studies reported a high positive predictive accuracy (79-100%) for an association between oligohydramnios and IUGR (Manning, 1996). The sample

$n = 7$ for IUGR cases in this study was small. A larger study with a bigger sample may yield different results.

5.9 PLACENTA

Ultrasound assessment of the placenta posed some challenges in this study.

There was difficulty in measuring the placental thickness in the 3rd trimester when a larger fetus fills the uterine cavity. The placenta was often squashed and not seen separate from the fetus resulting in sub-optimal visualization of the chorionic and basal plates. Ideally the placental thickness is measured at the centre of the placenta from the chorionic plate to the basal plate, perpendicular to the chorionic plate (Figure 3.1). Difficulties were also encountered when measuring posterior placentas at the 36-week scan. Inaccurate measurements may have affected the reliability of the assessment of the placenta. However in this study where the focus was to compare measurements of two groups, both groups were exposed to the same potential errors in measurement. Placental size has recently been assessed using placental volume as a method of measurement with 3D ultrasound (Wegrzyn, et al, 2005). Placental volume is a more accurate method of measurement and should be used in future studies of placental size.

Images of the placenta in each case were digitally saved so that the researcher could access the information later to assess placental maturity according to Grannum's Classification (Grannum et al, 1979). Unfortunately due to an archival fault of the ultrasound unit this information was not accessible and the evaluation of the placental maturity could not be included in the results of the study.

Placental weight measured at birth is a good indication of a normal or abnormal pregnancy. Normal placental weight measured at birth is 400 – 600 grams (Nortjé, 2003). The placental weight in the study showed no significant difference (p = 0.92) between the HIV infected and uninfected groups (Table 4.20). The means of the placental weight of the HIV infected group and uninfected groups were 583 grams and 581 grams respectively which was within the normal range 400 – 600 grams (Nortjé, 2003). Eight women had a small placenta < 400 grams: 4 were HIV infected and 4 were uninfected. All of the women with small placentas were associated with complications (Table 4.21). Small placentas can be associated with IUGR, PTD and LBW (Nortjé, 2003). In this study all the woman with a small placenta (n = 8) had associated low birth weight (< 2500 g) and 6 women had associated preterm delivery (< 37 weeks); 3 of the preterm deliveries were stillbirths; none with a small placenta were diagnosed with IUGR (Table 4.21).

5.10 PRETERM DELIVERY

The incidence of preterm delivery is thought to be higher in developing countries compared to developed countries due to socio-economic factors. The incidence of preterm delivery in the developed countries is 5-11% (Wenb et al, 2004). According to Theron (2003) there was evidence of a high incidence of preterm delivery in the Western Cape when the outcome of a survey done in an urban area in public hospitals and clinics suggest an incidence of 20% for both preterm delivery and low birth weight < 2500 grams. The incidence of birth weight < 2500 grams in the UK was 6%. In this study the overall incidence of preterm delivery was 10% (Table 4.24). The incidence for the HIV infected group was 13% and for the uninfected group was 8%. There was no significant difference in the incidence of preterm delivery

between the two groups (p = 0.43). This study suggests that a positive HIV status of women does not increase the risk for preterm delivery. Many studies have suggested that there is an increased risk of preterm delivery and low birth weight for symptomatic HIV infected women with CD4 T-lymphocyte count < 200 cells / mm^3 (Brocklehurst & French, 1998; Coley et al, 2001; Weng et al, 1998). This study showed no significant difference (p = 0.4) in the incidence of preterm delivery in the HIV infected group with CD4 T-lymphocyte count < 200 cells / mm^3 (19%) compared to those with CD4 T-lymphocyte > 200 cells / mm^3 (13%) (Table 4.31). Because of the small sample size for low CD4 T-lymphocyte count < 200 cells / mm^3 (n = 21) the validity of the results could be questioned. It is recommended that a larger sample size is needed to confirm an association between HIV infected women with low CD4 T-lymphocyte count and preterm delivery and low birth weight.

5.11 CAESAREAN SECTION

The incidence of caesarean section in South African public hospitals is 15 – 20% (Hofmeyer, 2003). The incidence of caesarean section in this study was 23%. The incidence in the HIV infected group was 29% and in the uninfected group was 18%. The difference between the two groups was not significant (p = 0.1) as seen in Table 4.25. Caesarean section performed in this study was due to placenta praevia, cephalopelvic disproportion, fetal distress, breech presentation, prolonged labour and previous caesarean section. Elective caesarean section for HIV infected women to reduce MTCT is not an option in South African public hospitals. Although it is practiced in developed countries there is no scientific evidence that caesarean section does reduce MTCT (Hofmeyer, 2003). Coovadia & Coutsoudis (2000) suggest that elective caesarean section is not a good option for South Africa because of the financial

constraints and the risk of post-operative complications in HIV infected women who have a higher mortality rate than uninfected women.

5.12 PERINATAL COMPLICATIONS

Perinatal complications occurred in 42% of all women in the study (Table 4.26). The perinatal complications found in the study are listed in Table 4.27. There was no significant difference ($p = 0.1$) in the number of perinatal complications between the two groups (Table 4.26). The prevalence of perinatal complications in the HIV infected group was 46% and that for the uninfected group was 40%.

In the group of women with perinatal complications ($n = 123$) the most common type found was gestational hypertensive disease (31.7%). The National Committee on Confidential Enquiries into Maternal Deaths (NCCEMD) published a report (Department of Health, 1998) on the primary causes of maternal death in South Africa. The report states that 23.2% of maternal deaths were caused by gestational hypertension disease. The incidence of hypertension in this study was 13.4% (Table 4.27). Hypertension in the obstetric patient remains a challenge in South Africa.

Fetal distress was 29.2% of the total number of perinatal complications found in the study (Table 4.27). Fetal distress is a common but serious complication that is an indication of fetal hypoxia and is often associated with placental insufficiency (Beck, 1993). If fetal distress is not managed appropriately it can result in fetal brain damage or intrauterine death (Odendaal, 2003). The prevalence of fetal distress in the study was 12.3% (Table 4.27).

Low birth weight was also one of the more common complications found in the study (16.2% of the total number of perinatal complications). The prevalence of low birth weight in the

study was 6.8%, much lower than suggested by Theron (2003) citing the results of a survey done in public hospitals in an urban area in the Western Cape where low birth weight was 20% (Theron, 2003).

Preterm delivery occurred in 24.3% of the total number of complications in the study. The prevalence of preterm delivery was 10.3% in the study, also much lower than the prevalence of 20% found in the survey (Theron, 2003).

Seven (2.4%) of the 123 perinatal complications were diagnosed IUGR (Table 4.27). The other main complications associated with IUGR were low birth weight ($n = 5$) and gestational hypertensive disease ($n = 3$). One IUGR case delivered prematurely (Table 4.28).

5.13 CD4 T-LYMPHOCYTE COUNT

In the HIV infected group the number of perinatal complications and preterm deliveries were not significantly different in women with CD4 T-lymphocyte count < 200 cells / mm^3 compared to those with CD4 T-lymphocyte > 200 cells / mm^3 ($p = 0.22$ and $p = 0.4$ respectively) (Tables 4.30 and 4.31). However the number of women with low CD4 count < 200 cells / mm^3 was small ($n = 21$). A larger study with a bigger sample may yield different results.

6. CONCLUSION

The primary objective of the study was to establish whether there was a significant difference in fetal growth in HIV infected women compared to uninfected women at Khayelitsha MOU and Gugulethu MOU. The results of the antenatal ultrasound findings showed no significant difference when comparing fetal biometry (BPD, HC, AC and FL), EFW, placental thickness and Amniotic Fluid Index of the HIV infected and uninfected groups. The null hypothesis "*there is no significant difference in the fetal growth in HIV infected pregnant women compared to uninfected pregnant women at Khayelitsha MOU and Gugulethu MOU*" was therefore accepted ($p = 0.05$). This finding that HIV infection had no significant effect on fetal growth supports the suggestions by many authors that MTCT of HIV more often occurs during labour and delivery. The outcome of this study suggests that there is no need to adapt clinical monitoring of fetal growth specifically for HIV infected women attending these two MOU's.

A secondary objective of the study was to establish whether there was an association between a low CD4 T-lymphocyte count < 200 cells / mm^3 and IUGR in HIV infected pregnant women at Khayelitsha MOU and Gugulethu MOU. The results were statistically inconclusive because of the small number of IUGR cases ($n = 7$); 4 were HIV infected; one of the 4 had a low CD4 < 200 cells / mm^3.

The study did however show no significant difference in the prevalence of maternal complications including preterm delivery in HIV infected women with a low CD4 < 200 cells / mm^3 compared to those with CD4 > 200 cells / mm^3. The sample ($n = 123$) was however small and it is recommended that further research is needed to assess the impact of low CD4 T-lymphocyte count < 200 cells / mm^3 on fetal well-being.

The results of the postnatal findings showed no significant difference in the number of perinatal complications, birth weight, incidence of preterm delivery and low birth weight of the HIV infected and uninfected groups.

Other interesting findings in the study were the discrepancy between the average birth weight in the study and normal average birth weight suggested by Cilliers (2003); and the discrepancy between the mean EFW in the study compared to EFW reference chart by Benson and Doubilet (1998). These findings raise the question about the suitability of the fetal reference charts used for the Western Cape population. Recent studies have suggested that there is a variation in fetal growth and birth weight not only in different populations but also within populations with different ethnic groups (Gardosi, 1995; Wilcox et al, 1993; Spencer et al, 1995). It is recommended that further research be done developing reference charts (fetal growth and birth weight) for the diverse population in the Western Cape.

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8. APPENDICES

APPENDIX I: English Consent

PENINSULA TECHNIKON/GROOTE SCHUUR HOSPITAL
OBSTETRIC ULTRASOUND RESEARCH PROJECT

INFORMED CONSENT

Patient No.: _____

GROUP (✔): G1 ☐ G2 ☐ K1 ☐ K2 ☐

I, _____ agree to participate in the above-mentioned research project.

It has been explained to me that:

1. The aim of the project is to establish whether the Human Immunodeficiency Virus (HIV) has any significant effect on the growth of the fetus. Fetal biometry of HIV infected women will be compared to that of uninfected women to see if there is a significant difference.
2. Blood will be taken from all HIV infected women to check their immune system.
3. It will be necessary to have a minimum of 4 ultrasound examinations at 4 of the routine visits to the MOU i.e. ± 22 weeks, 28 weeks, 32 weeks AND 36 weeks gestational age.
4. The ultrasound examination is a non-invasive procedure and will have no adverse effects on the fetus or mother.
5. Any information obtained in this project will be kept strictly confidential, but the results may be used in a thesis or publication.
6. Any participant can withdraw from the project at any time.
7. No pressure will be put on anyone to participate in this study.

SIGNED: _____ DATE: _____

WITNESS 1: _____ WITNESS 2: _____

APPENDIX II: Xhosa Consent

PENINSULA TECHNIKON/GROOTE SCHUUR HOSPITAL
UPHONONONGO LOKYBELEKISA: UPHONDO NGALE PROJEKTI

IMVUME EYAZISIWEYO (INFORMED CONSENT)

Inombolo Yesigulana: _____

IQELA (✔): G1 ☐ G2 ☐ K1 ☐ K2 ☐

Mna, _____ ndiyavuma ukuthatha inxaxheba kuleprojekti.

Icacisiwe kum into yokokuba, injongo yale projekti kukuphanda ukuba ingaba:

1. Umntwana ongekazalwa uyasulelwa na yintsholongwane yesifo sikagawulayo asele enayo umama wakhe.
2. Igazi liyakutsalwa kubo bonke abafazi abosulelekileyo yintsholongwane ka gawulayo ukukhangela ukomelela komzimba wakhe ekulweni izifo.
3. Kuyakufuneka ubuncinana ndixilongwe ngogesi ka-4 kwimijikelo emithathu ndihambela isibhedlela sokubelekela ngoko ke Iveki ye – 22, iveki ye – 28, neveki ye 32 kunye neveki ye – 36 ngexesha ndisakhulelwe.
4. Uxilongi ngogesi ngegesha ukhulelwe aluchukumisi nto kwisiqhamo kwaye lungenamivuka kwinto leyo ephethweyo nakumama ngokwakhe.
5. Yonke inkcazelo efunyenweyo iyakugcinwa isekhusini okanye ilihlebo elingasokuze lithiwe pahaha, kodwa iziphumo zinokupapashwa kwizophando nezenzululwazi.
6. Ubani othabatha inxaxheba usenako ukurhoxa naninina xa efuna.
7. Akukho mntu unyanzelwayo ngokuthabatha inxaxheba.

SAYINA: _____ DATE: _____

INGQINA 1: _____

INGCINA 2: _____

APPENDIX III: Protocol for HIV-US Study

PENINSULA TECHNIKON: OBSTETRIC ULTRASOUND STUDY PROTOCOL

A. CRITERIA for BOOKING

1. Age: 18-32 years
2. Weight: < 95 kg
3. Gestation age: 20 - 24 weeks
4. Exclude women with the following history:
   - 4.1 Cigarette smoking, alcohol and drug abuse
   - 4.2 History of obstetric complications (IUGR, PTD)
   - 4.3 Fetal abnormality in current pregnancy
   - 4.4 Grand multipara pregnancy
   - 4.5 Multiple pregnancy
   - 4.6 Maternal vascular disease (severe diabetes, chronic hypertension, renal disease, cardiac disease, collagen disease e.g. systemic lupus erythematosus)

Please try to book 10 women who have tested HIV positive and 10 women who have tested HIV negative per week. A total of 100 HIV positive and 100 HIV negative women need to be recruited at this MOU.

B. 20 – 24 Week Scan

On the day of the first scan at 20 – 24 weeks gestation the following must be done for each participant:

1. Weight and height
2. Blood taken for CD4 cell count

C. Book for Follow-Up Scans

1. 28 weeks
2. 32 weeks
3. 36 weeks

Thank you for your assistance.

Researcher: Ferial Isaacs
Tel: 0842255110 Email: isaacsf@cput.ac.za

APPENDIX IV: Data Form

PENINSULA TECHNIKON

GROOTE SCHUUR HOSPITAL

OBSTETRIC ULTRASOUND RESEARCH PROJECT

ULTRASOUND REPORT

Patient No.		DOB:	
Group: G1 / G2 / K1 / K2		At first visit:	
		Weight / Height / BMI	
CD4 count (only if HIV+): Date:		Parity	
LMP: EDD(LPM):		EDD(US):	

	Scan 1(20-22 weeks)	Scan 2(28 weeks)	Scan 3(32weeks)	Scan 4(36weeks)	FOLLOW UP
Date					Birth date
Gestation Age (US)					Birth weight
Fetal heart					Gestation age
Lie/Presentation					Alive [yes / no]
Placenta					Still birth [yes / no]
AFI					Placenta
Biometry: BPD &HC					APGAR
AC					Complications
FL					
EFW					
Fetal structures:					
Stomach					
Kidneys & Bladder					
Head					
Spine					
Doppler (RI)					

COMMENT:

APPENDIX V

Amniotic Fluid Index (mm) and Gestational Age: Normal Values

Gestational Age	5th Percentile	50th Percentile	95th percentile
16	79	121	185
20	93	141	212
24	98	147	219
28	94	146	228
32	86	144	242
36	77	138	249
38	73	132	239
40	71	123	214
42	69	110	175

Jaffe & Abramowicz, 1997: 196

APPENDIX VI

(a) Birth Weight and EFW Correlation

		Birth weight (grams)	EFW4
Birth weight	Pearson Correlation	1	.579(**)
	Sig. (2-tailed)	.	.000
	N	290	197
EFW4	Pearson Correlation	.579(**)	1
	Sig. (2-tailed)	.000	.
	N	197	251

** Correlation is significant at 0.01 level (2-tailed).

(b) Comparison of Study: EFW and Doubilet et al: EFW

Gestational Age	**Study: EFW (grams)**	**Doubilet et al: EFW (grams) 95th percentile (10th – 90th)**
28 weeks	1219 (SD: 102.05)	1005 (765 – 1322)
32 weeks	1905 (SD: 219.25)	1702 (1338-2167)
36 weeks	2729 (SD: 226.99)	2622 (2129-3230)

Doubilet et al, 1997