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# Review of invasive prenatal testing at Rahima Moosa Mother and Child Hospital

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# REVIEW OF INVASIVE PRENATAL TESTING AT RAHIMA MOOSA MOTHER AND CHILD HOSPITAL

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A Dissertation submitted to the Faculty of Health Sciences, University f the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Obstetrics and Gynaecology

Johannesburg, 2016

## DECLARATION

I, Chrysanthi Georgiou, declare that this M.Med is my own, unaided work. It is being submitted for the Degree of Master of Medicine in Obstetrics and Gynaecology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

.....

.....day of .....2017 in.....

To my parents,

who taught me the art of medicine and the importance of women's healthcare.

Thank you for your support, guidance and teaching, always.

## PUBLICATIONS AND PRESENTATIONS

Presentation at Academic Meeting, Rahima Moosa Mother and Child Hospital, December 2016

Poster presentation at the Royal College of Obstetricians and Gynaecologists World Congress, Cape Town 2017

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Presentation will be presented at the BIT 5th International Congress of Gynaecology and Obstetrics, China, Nov 2017

# ABSTRACT

## Introduction

Invasive prenatal testing is the gold standard of prenatal diagnosis of chromosomal abnormalities. Outcomes of invasive prenatal procedures have been studied previously, however it has not been looked at in a resource poor, tertiary setting in South Africa.

## Aim and objectives

The aim of this study is to review the outcome of invasive prenatal testing at Rahima Moosa Mother and Child Hospital (RMMCH) from January 2014 to May 2016. The main objectives of the study were to evaluate invasive prenatal testing in terms of indications, ultrasound markers, cytogenetic diagnosis, complications and pregnancy outcome.

## Methods

The study took place at RMMCH, a regional academic hospital in Johannesburg which performs approximately 12 000 deliveries annually. Charts were reviewed retrospectively for patients who underwent invasive prenatal testing.

## Results

Ninety-seven patients were identified and 96 results obtained. The main indication for invasive prenatal testing was abnormal ultrasound findings followed by advanced maternal age. In total, 12,5% of test results were abnormal, including two patients with Trisomy 13, two with Trisomy 18, two with Trisomy 21, two with Klinefelter syndrome, one with a balanced translocation, one with cystic fibrosis, one with spinal muscular atrophy and one with Wolf-Hirschorn syndrome. The miscarriage rate was 1,5%. There were four terminations of pregnancy directly related to an abnormal invasive test result.

## Conclusion

It is expected that in a resource restricted area where biochemical screening is not available that advanced maternal age and ultrasound findings are the main reasons to lead to invasive prenatal testing. The rate of abnormalities found is higher than internationally quoted and the miscarriage rate higher than the internationally

accepted 0,5-1%, this is likely due to selection bias and sample size. The study shows that an invasive testing service can be successfully run in a resource restricted setting but ongoing education of the availability of the service in the public sector is needed.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ACOG | American College of Obstetricians and Gynaecologists |
| AMA | Advanced Maternal Age |
| ART | Antiretroviral Therapy |
| BHCG | Human Chorionic Gonadotropin |
| CD4 | Cluster of Differentian 4 |
| CVS | Chorionic Villous Sampling |
| DNA | Deoxyribonucleic Acid |
| FISH | Fluorescent In Situ Hybridisation |
| FMF | Fetal Medicine Foundation |
| Hb | Haemoglobin |
| HIV | Human Immunodeficiency Virus |
| IUFD | Intrauterine Fetal Death |
| LR | Likelihood Ratio |
| MA | Maternal Age |
| NB | Nasal Bone |
| NIPT | Non Invasive Prenatal Testing |
| NT | Nuchal Translucency |
| PAPP-A | Pregnancy Associated Plasma Protein A |
| PCR | Polymerase Chain Reaction |
| PIPP | Perinatal Problem Identification Program |
| QF-PCR | Quantitative Fluorescent Polymerase Chain Reaction |
| REDCAP | Research Electronic Data Capture |
| RCOG | Royal College of Obstetricians and Gynaecologists |
| RMMCH | Rahima Moosa Mother and Child Hospital |
| RPR | Rapid Plasma Reagin |
| SOGC | Society of Obstetricians and Gynaecologists of Canada |
| TOP | Termination of Pregnancy |
| VL | Viral Load |

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# 1 INTRODUCTION

## 1.1 BACKGROUND

In the modern era of medicine combined first trimester screening is the gold standard for screening women for chromosomal anomalies. If a patient is screened high risk – non invasive prenatal testing (NIPT) is the recommended second line screening that should be offered to these patients. Anatomical problems in a fetus can be identified by ultrasound, this combined with maternal serum markers can be used as screening to identify fetuses at risk of chromosomal abnormalities (1). Cell free deoxyribonucleic acid (DNA) can be obtained from maternal serum for genetic screening and one can perform genetic testing on preimplantation embryos but this is often not feasible or cost effective in a resource restricted setting. It must also be remembered that cell free DNA analysis is a screening test and not a diagnostic tool (2).

Prenatal screening has been defined as the identification, among apparently normal pregnancies, of those at sufficient risk for a specific fetal disorder to justify subsequent invasive and/or costly prenatal diagnostic tests or procedures (3). The prevalence of Trisomy 21 in live births is relatively high in the absence of prenatal screening and is said to be about 1 in 600 live births and Trisomy 18 is 1 in 4000 live births (4). The widespread implementation of prenatal screening combined with prenatal diagnosis and termination of pregnancy services has substantially reduced the expected number of infants born with Down Syndrome (5).

### 1.1.1 Serum Markers and Ultrasound screening tests

Multiple screening tests are available. The first trimester combined test includes sonographic nuchal translucency and biochemical markers. The biochemical markers used are free human chorionic gonadotropin (BHCG) and pregnancy associated plasma protein-A (PAPP-A). This test is done between 11-14 weeks. Based on these results, as well as the patient's age and other variables (for example body mass index and singleton or multiple pregnancy), the patient can be given an adjusted risk of whether she is possibly carrying a fetus with a

chromosomal abnormality (6). In pregnancies with Trisomy 21 the fetal nuchal translucency measurement is increased and free BHCG is higher and PAPP-A is lower than a chromosomally normal fetus (7,8).

During the first trimester ultrasound the measurement of nuchal translucency has been shown to identify 75% of fetuses affected by Trisomy 21 with a 5% false positive rate. Another marker of Trisomy 21 in the first trimester ultrasound is the absence of the nasal bone. When maternal age, nuchal translucency and nasal bone are combined, the detection rate is 90% with a false positive rate of 2%. Abnormalities of the ductus venosus flow are observed in up to 80% of fetuses affected by Trisomy 21. Studies have reported an association between fetal tachycardia with Trisomy 13 and Turner Syndrome and between fetal bradycardia with Trisomy 18 and Triploidy. (7,9,10).

In the second trimester, with the patient's age, the triple test measures the level of the biochemical markers alpha feto-protein, unconjugated estriol and human chorionic gonadotropin in maternal serum. The quadruple test has the added serum marker of Inhibin A. These tests are performed between 15-20 weeks (6). The quadruple test was the most commonly performed test for Down Syndrome screening in the United States of America in 2012 (8,11).

Ultrasound is a method of screening that can be used in association with other screening tests to give an associated risk for chromosomal abnormalities as previously discussed. Both first trimester and second trimester markers can be associated with chromosomal abnormalities (12).

Soft markers are ultrasound findings that could be a variant of normal but may indicate an increased risk of fetal aneuploidy (13). These markers in isolation do not refute or confirm a chromosomal abnormality and most fetuses (> 99%) with an isolated marker will not be affected (12).

Soft markers associated with chromosomal abnormalities are choroid plexus cysts, a thickened nuchal fold, an echogenic cardiac focus, echogenic bowel, renal pyelectasis, shortened humerus, shortened femur, single umbilical artery, mild ventriculomegaly, enlarged cisterna magna, nasal bone hypoplasia, brachycephaly, increased iliac angle, shortened ear length, shortened middle phalanx and an absent subclavian artery (12,13).

According to the Van der Hof et al. only five of the above mentioned soft markers should be used in a screening ultrasound namely an increased nuchal fold, echogenic bowel, mild ventriculomegaly, echogenic foci in the heart and choroid plexus cysts. Choroid plexus cysts are mainly associated with Trisomy 18. A single umbilical artery, an enlarged cisterna magna and pyelectasis do not have a well-established association with aneuploidy but might be important in the diagnosis of non chromosomal problems (13).

Each of these markers have an associated likelihood ratio associated with the occurrence of a certain aneuploidy. Multiple abnormalities seen on ultrasound thus increase the overall likelihood of an aneuploidy as each associated likelihood ratio is multiplied by a patient's background risk and risks found on other screening tests. Thus multiple markers have a cumulative effect and will increase a patient's individual risk (14).

Table 1.1 below shows the likelihood ratio for Trisomy 21 for each isolated soft marker (7,13)

**Table 1.1 Likelihood ratios for Trisomy 21 for each soft marker**

| Soft Marker | *LR for isolated marker* |
|---|---|
| **Nuchal fold** | 9 – 17 |
| **Nasal bone hypoplasia or absence** | 51 |
| **Short humerus** | 4 - 7.5 |
| **Short femur** | 1 - 2.7 |
| **Ventriculomeglay** | 9 |
| **Hydronephrosis** | 1.0 |
| **Echogenic cardiac focus** | 1 – 2 |
| **Echogenic bowel** | 3 – 6 |

So called hard markers are structural abnormalities found on ultrasound that represent major abnormalities and even if found in isolation should warrant invasive prenatal testing (9).

During the second trimester ultrasound a single abnormality found should prompt the search for other markers as certain clusters of markers are associated with certain aneuploidies. As discussed above each soft marker has a certain likelihood ratio of associated risk to the fetus. If only second trimester markers are used as a screening test it will have a detection rate of 75% with a false positive rate of up to 15% (9). In the absence of ultrasound soft markers, the background risk of chromosomal abnormalities is reduced. (14).

The risk of an aneuploidy increases with structural abnormalities found at ultrasound and is strongly associated with the number of structural abnormalities found. The more abnormalities found, the more likely the diagnosis of an aneuploidy at invasive testing (13).

It must be remembered that ultrasound like any screening test does not give a definitive diagnosis of a chromosomal abnormality and it must be weighed against a false positive result causing unnecessary anxiety and clinical intervention, in the same breath a false negative result might give reassurance to a women carrying a child with an abnormality (12).

If maternal age only is used as a screening test for chromosomal abnormality there is a 50% detection rate and a 15% false positive rate. The detection rate increases to 75% if maternal age and nuchal translucency are combined for the same false positive rate of 5%. A combination of the above with nasal bone further increases the detection rate to 90% while the false positive rate remains unchanged. In conclusion, in a resource-limited setting where serum markers might not be available, materal age specific risk should be combined with early ultrasound markers of aneuploidy for screening purposes (7).

In the South African setting the maternal age and background risk factors combined with ultrasound are often the only methods of screening available due to the cost of serum markers. In a local study conducted by Naidoo et al. at Chris Hani Baragwanath Academic Hospital nuchal translucency, maternal age and a first trimester anatomical survey at ultrasound were used as a screening test. They

found that the sensitivity was 92,9% and specificity was 88,6% in detecting structural and chromosomal abnormalities with their screening protocol without biochemical screening. They concluded that their protocols were equivalent to international standards (15).

In order to calculate an individual's risk, their background risk is taken into account which includes factors like maternal age and gestational age. Sequential screening is used and can be done by multiplying the background factors by several independent factors depending on serum and ultrasound findings. Every time a new test is performed it is multiplied by the background risk to calculate a new risk, which then becomes the background risk for the next test. (9).

The table below published by Nicolaides et al. illustrates the detection rate of Trisomy 21 and false positive rate of screening tests (7).

**Table 1.2: Detection rate of Trisomy 21 and false positive rate of screening tests**

| *Screening Test* | *Percentage detection rate* | *Percentage false positive rate* |
|---|---|---|
| **MA** | 50 | 15 |
| **MA + serum BhCG + PAPP-A at 11-14 weeks** | 60 | 5 |
| **MA + fetal NT at 11-14 weeks** | 70 | 2 |
| **MA + fetal NT + NB at 11-14 weeks** | 90 | 2 |
| **MA + fetal NT + serum BhCG + PAPP-A at 11-14 weeks** | 80 | 2 |
| **MA + fetal NT + fetal NB + serum BhCG + PAPP-A at 11-14 weeks** | 95 | 5 |
| **MA + serum biochemistry at 15-18 weeks** | 60-70 | 5 |
| **Ultrasound for fetal defects and markers at 16-23 weeks** | 75 | 10-15 |

### 1.1.2 Non invasive prenatal testing (NIPT)

The testing of cell free DNA, in maternal blood is another method of screening known as non invasive prenatal testing (NIPT) . The method screens mainly for the most common Trisomies and Sex Chromosome abnormalities (2). Other uses include fetal sexing and fetal Rh typing (16). It is used as a primary or secondary screening tool. These tests have a 99% sensitivity and a 99% specificity when screening for both Trisomy 18 and 21. Trisomy 13 and Sex Chromosome abnormalities have a sensitivity of between 80-90% and a specificity of 99% (2).

The American Colleges of Medical Genetics and Obstetricians and Gynaecologists guidelines state that NIPT should be done in the following patients as an effective screen for Down Syndrome: (17)

- Patients 35 years and older
- A mother with a fetus affected previously with Trisomy 13, 18 or 21
- A parental translocation involving chromosome 13 or 21
- Fetal ultrasound or other screening test that specifically increases the risk for Down Syndrome

Norton et al. provided support for offering this test to all woman as a form of screening. Over 15 000 woman presenting at 10 -14 weeks of gestation at 35 international centres underwent both standard screening (with measurement of nuchal translucency and biochemical markers) and cell free DNA testing regardless of their baseline risk of aneuploidy. For Trisomy 21, NIPT had a 100% sensitivity and had a positive predictive value of 80,9% while standard first trimester screening had sensitivity of 78,9% and a positive predictive value of 3,4%.Cell free DNA screening had a higher sensitivity for detection of Down Syndrome, a lower false positive rate and a higher positive predictive value (18). However the ACOG recommends given the performance of conventional screening methods, the limitations of cell free DNA screening performance, and the limited data on cost effectiveness in the low-risk population, conventional screening methods remain the most appropriate choice for first line screening for most women in the general population (2).

NIPT testing is available in South Africa but the genomic and bioinformatics information of samples needs to be done in other countries. This option is not available in the public sector in South Africa mainly due to the large cost associated with it, and is only available in the private sector for those who can afford the out of pocket payment or are members of certain medical aids (19).

If the patient has a screen positive test she can either undergo secondary screening or might want a definitive diagnostic test. It can be seen that an individualised risk assessment must be done for every patient including background risk and screening results before an invasive test is offered (14). A population can be divided into high risk (1:100 or lower), intermediate risk (1:101-1:2500) and low risk (<1:2500), NIPT

should be offered to patients in the intermediate risk category. Some studies suggest that NIPT should be offered in a patient with a risk of 1:101-1:2500 (20,21)

The Society of Obstetricians and Gynaecologists of Canada (SOGC) recommends that no definitive obstetric decision should be made in pregnancies with a positive non-invasive prenatal testing result without an invasive diagnostic test to confirm the diagnosis (22). It is estimated that around 5% of the pregnancy population in the United Kingdom are offered a choice of invasive prenatal diagnostic tests (23).

### 1.1.3 Invasive Prenatal Testing

#### 1.1.3.1 Introduction

Invasive prenatal testing is a definitive form of diagnosis used during pregnancy to detect among other things chromosomal abnormalities. There are three forms of invasive prenatal testing namely amniocentesis, CVS and cordocentesis.

Prenatal detection of chromosome abnormalities has been offered for more than 40 years, first by amniocentesis in the early 1970s and additionally by CVS in the 1980s (6). Prenatal testing decreases the burden of disease to the affected individual and their family and gives new parents time to prepare for the birth of their infant. The American College of Obstetricians and Gynaecologists recommend that all women should be offered aneuploidy screening before 20 weeks of gestation and all women should have the option of invasive testing, regardless of maternal age (24).

Diagnostic testing requires the harvesting of fetal cells derived from skin, mucous membranes, amnion, placenta or umbilical cord during pregnancy for subsequent karyotype and genetic analysis (25). The diagnostic accuracy of karyotyping cultured cells obtained by invasive testing has been found to be 97,5 to 99,8% (6).
Invasive prenatal testing remains the gold standard for conclusive prenatal genetic diagnosis.

### 1.1.3.2 Amniocentesis

Amniocentesis was first used for fetal karyotyping in 1966 (26).Today it is widely accepted as the method most easily performed among the prenatal invasive diagnostic methods, especially when carried out in the second trimester (27).

Amniocentesis is used as prenatal diagnostic procedure to obtain amniotic fluid for prenatal diagnosis. It is usually performed after 15 weeks gestation but can be performed in the first trimester (16,23,25).

Amniocentesis should be done under ultrasound guidance in order for the position of the placenta to be visualised and a suitable entry point to the mother's abdomen is found (23). Continuous visualisation of the needle with ultrasound guidance reduces bloodstaining from 2.4% to 0.8% (28), it also reduces serious fetal trauma or maternal bowel injury that can be caused by the needle (23).

Transplacental passage of the needle should be avoided unless this is the only way to access an adequate pool of liquor. If this approach is used the insertion of the cord must be avoided and the thinnest part of the placenta must be traversed (23). A prospective case control study done by Mungen et al. of 2068 women undergoing second trimester amniocentesis and their 2068 controls showed that there was no significant difference between the transplacental and non-transplacental approach (29).

Amniocentesis should be performed using a sterile technique, an amniocentesis needle and ultrasound guidance. A sterile technique is achieved by the use of abdominal antiseptic cleaning, sterile gloves, sterile drapes, sterile ultrasound gel and a sterile ultrasound cover (16). Generally a sample of 20-30 mls of amniotic fluid is obtained from a liquor pocket that is free of fetal parts and umbilical cord (24). The fluid obtained for testing is usually from fetal skin and bladder cells and is processed for chromosome, protein, biochemical and enzymatic analysis of the amniotic fluid supernatant (16). Amniocentesis has been shown to have a cytogenetic accuracy of more than 99% (24).

SOGC recommends decreased physical activity for 12-24 hours after the procedure but bed rest is not required. The use of prophylactic antibiotics is not required (16).

### 1.1.3.3 Chorionic villous sampling

The Royal College of Obstetricians and Gynaecologists (RCOG) recommend that CVS is performed between 11 weeks and 13 weeks and six days. It is a transabdominal or transcervical technique where placental villae are aspirated or biopsied (23).

It is recommended that both transabdominal and transcervical CVS be performed under continuous ultrasound guidance. The tip of a needle or specialised catheter is placed in the placenta without entering the amniotic sac. Negative pressure with a syringe is used to aspirate a small amount of placental villi (24). RCOG recommends that the technique used, whether aspiration by negative pressure syringe, negative pressure by vacuum aspirator or biopsy forceps, should be under the guidance of an experienced obstetrician (23).

The advantage that CVS has over amniocentesis is that the diagnosis can be given earlier in pregnancy and the expecting couple can make definitive treatment decisions earlier in pregnancy.

The success rate of cytogenetic diagnosis on CVS samples is reported to be 99.7% with 1.1% of patients requiring a further diagnostic test to interpret results (30).

### 1.1.3.4 Cordocentesis

Cordocentesis is a procedure done at later gestations and is generally done between 16 and 24 weeks and is used for both fetal diagnosis and therapy (31).

A 20-22 gauge needle is directed under continuous ultrasound guidance into the umbilical vein. Puncture of the umbilical artery might cause constriction and can lead to fetal cardiac abnormalities. The needle can be directed to various sites like the umbilical cord insertion site, fetal intrahepatic vein or a free loop of cord (16).

Cordocentesis is rarely performed with the availability of other invasive procedures like amniocentesis and CVS but can be very useful in a case of chromosomal mosaiscm found at amniocentesis or CVS (24).

### 1.1.4 Indications for invasive prenatal testing

Invasive prenatal testing is usually offered to a population in which a there is a predetermined risk of chromosomal abnormality as previously mentioned. The most frequent indication for invasive prenatal testing is:

- advanced maternal age
- diagnosis of diseases associated with DNA analysis
- enzyme analysis of metabolic disease
- determination of congenital infection
- abnormalities detected on ultrasound
- increased first and second trimester screening risk
- family history of chromosomal abnormalities
- patient request (32)

The ACOG recommend that diagnostic prenatal testing be offered to all pregnant women but especially patients with a previous pregnancy complicated by fetal Trisomy, at least one major or two minor fetal structural abnormalities on ultrasound in the current pregnancy, a chromosomal inversion, aneuploidy or translocation in the pregnant woman or her partner (24).

Chang et al. did a retrospective review of 30 years at the Tapei General Hospital, Taiwan looking at the indications and outcomes of amniocentesis. 16749 women were included between 1981 and 2010 who had undergone midtrimester amniocentesis. The main indications for amniocentesis were advanced maternal age, increasing risk maternal triple marker Trisomy 21 screening, history of previous birth of a baby with an abnormality, abnormal ultrasound findings, family history of chromosomal abnormality, abnormal parental karyotype, drug and radiation exposure, abnormal CVS and intrauterine fetal death. The most common indication was advanced maternal age that accounted for 65.5% (33).

Daniilides et al. found in a retrospective study in Greece over a four year period that the main indications for amniocentesis were an increased risk for Down Syndrome, maternal request, abnormal ultrasound findings and family history of chromosomal abnormality (25).

A study conducted in Korea by Cho et al. which included 2000 women, showed that the indications for amniocentesis were advanced maternal age, abnormal serum markers and abnormal ultrasound findings. The most common indication was advanced maternal age (34).

Kim et al. conducted a similar study in Korea which included 2942 patients and concluded that in this setting the most common indication for invasive testing was abnormal maternal serum markers (35). Pala et al. in a study conducted in Turkey in 2012 looked at 83 cases of amniocentesis performed also found the most common indication for was increased in serum markers. This study also found a 10% rate of maternal request for amniocentesis, maternal request is either a very small percentage in other studies or not mentioned at all (36).

#### 1.1.4.1 Advanced maternal age

Advanced maternal age is commonly defined as an age of 35 years or older (37). Delayed child bearing is an increasing trend in developing countries and this has led to an increased burden of prenatal testing (38). This trend has been attributed to contraception being available, advances in infertility medicine, delaying marriage, high rates of divorce and remarriage and women finding education and careers important (39).

Women of advanced maternal age are at increased risk of delivering an infant with a chromosomal or congenital abnormalities (39,40). Most chromosomal abnormalities originate from abnormalities in female meiosis (aberrations in meiotic spindle assembly in oocytes) (41,42). Sex chromosome abnormalities don't have an association with advanced maternal age (7). In a study done by Hook et al. which looked at chromosomal abnormality rates in women with only advanced maternal age as a risk factor it was found that the rate was 5/1000 at age 35 years, 15/1000 at age 40 years and 50/1000 at age 45 years (43).

From 1970 to 2000, live births among women aged 35 and older in the United States increased from 5% to 13% of live births (44). In South Africa 13, 4% of pregnant women fall into the advanced maternal age category which accounts for the high prevalence of Down Syndrome, documented to be 1,8 and 2,09 per 100 live births in urban and rural populations respectively (45,46).

Most of the studies mentioned above showed that advanced maternal age was the most common indication for invasive prenatal testing. Advanced maternal age was the main screening tool for invasive testing before the advent of serum and ultrasound screening tests. In a resource restricted setting maternal serum screening is often not possible and ultrasound screening might be limited thus advanced maternal age is still a mainstay of screening in a resource restricted setting like the South African public health service.

Antenatal care aims to provide the identification of avoidable factors to provide a normal pregnancy and delivery of a healthy infant with the least possible morbidity. Advanced maternal age is an easily recognisable risk factor that can be used in antenatal care to provide prenatal genetic counselling for invasive prenatal testing. In Johannesburg invasive prenatal testing has been free since 1994 and there is an open referral system to academic hospitals who provide the service (40).

In a local study by Kromberg et al. 55% of mothers of infants affected with Trisomy 21 were over the age of 35 but none of these women were offered prenatal testing. Kromberg et al. also found in their study that 73% of these women would have accepted prenatal diagnosis if it had been offered to them and 52% said that they would have considered termination of pregnancy (47). In other local studies Delport et al. found 52% of mothers of Trisomy 21 infants were over the age of 35 (48), while Venter et al. found 56% in theirs (49). Another showed that in the local population the overall acceptance rate of amniocentesis was 75.9% and of termination of pregnancy was 76,3% (50). These studies show that offering invasive prenatal testing will increase the rate of Trisomy 21 detection and give the patient options for further management. They further show that prenatal testing is acceptable to most patients.

1.1.4.2 Chromosomal abnormalities

Chromosomal abnormalities are major causes of perinatal death and childhood morbidity thus the detection of chromosomal abnormalities is a common indication of invasive prenatal testing (7).

The prevalence of chromosomal abnormalities if recognised in early pregnancy loss is greater than 50%. Fetuses with aneuploidy account for 6-11% of stillbirths and early neonatal deaths (24). Major fetal abnormalities occur in approximately 5% of all live births , 3% are identifiable prenatally and 2% at birth or during the first year of life (16).

In a developing country like South Africa issues of infectious disease and malnutrition are being brought under control and it can be expected that congenital abnormalities will assume a greater relative proportion of childhood causes of morbidity and mortality (48). According to under five mortality statistics in South Africa 3% of infant deaths between 0-11 months are because of congenital abnormalities (51). Data from the Perinatal Problem Identification Program (PIPP) in 2014 showed that congenital abnormalities is the third leading cause of early neonatal death after hypoxia and immaturity. With immaturity accounting for 40,6% of deaths, hypoxia 10% of deaths and congenital abnormalities 9,6% of deaths (52,53).

In a local study done by Delport et al. 17351 black infants were examined at birth in an urban hospital and the total congenital anomalies incidence were found to be 11,87/1000. This study also showed that Down Syndrome was the most common chromosomal abnormality diagnosed with an incidence of 1,33 per 1 000 live births or 1 in 752 babies delivered . The study concluded that the incidence of congenital anomalies in black South African neonates were comparable to that of First and Third world countries (48). Similarly, another local study by Venter et al. which took place in a rural community and included 7617 infants reported an incidence of congenital abnormalities of 14,97/1000 (49).

Several international studies found the following rate of chromosomal abnormalities. Chang et al. found the overall rate of abnormalities to be 2,72% (455/16 749 patients) and among these abnormalities 274 were chromosomal abnormalities and 181 were structural abnormalities (33). In the 73 cases in a study in Greece of

pregnant women undergoing amniocentesis between 2000 and 2005 Daniilides et al. found that 68 had normal karyotypes (25). Grether-Gonzalez et al. did a study in Mexico of 1500 women who underwent amniocentesis and found chromosomal abnormalities in 4,5% of patients (54).

The table below summarises the rates of chromosomal abnormalities found in several studies (25,33,34,36,54–58)

**Table 1.3: Rates of chromosomal abnormalities in various studies**

| Study | % Chromosomal abnormalities | Year Study Conducted | Country Study Conducted | Number of Patients | Invasive Prenatal Test Performed |
|---|---|---|---|---|---|
| **Wulff et al.** | 0.6 | 2010 | Denmark | 14 987 | CVS, Amniocentesis, Post natal diagnosis |
| **Han et al.** | 3.1 | 1994-2007 | Korea | 31 615 | Amniocentesis |
| **Chang et al.** | 2.7 | 1981- 2010 | Taiwan | 16 749 | Amniocentesis |
| **Tseng et al.** | 2.9 | 1995-2004 | Taiwan | 7 028 | Amniocentesis |
| **Hsieh et al.** | 3 | 1982-1990 | Taiwan | 2 975 | Amniocentesis |
| **Cho et al.** | 2.5 | 1984-1987 | Korea | 2 000 | Amniocentesis |
| **Grether-Gonzalez et al.** | 4.5 | 2010 | Mexico | 1 500 | Amniocentesis |
| **Pala et al.** | 10 | 2012 | Turkey | 83 | Amniocentesis |
| **Daniilides et al.** | 6.9 | 2002-2005 | Greece | 73 | Amniocentesis |

It can be seen from the above table and discussion that the rate of chromosomal abnormalities found in various studies range between two and ten percent.

Daniilides et al. found the majority of chromosomal abnormalities identified to be Trisomy for chromosomes 13, 18, 21 and Sex Chromosome aneuploidies (25). These abnormalities were also found to be the most common in an analysis by Grether-Conzalez et al. of 1500 women who underwent amniocentesis (54). In all the studies above, Trisomy 21 was the most common chromosomal abnormality found by invasive testing.

#### 1.1.4.3 Other indications for invasive prenatal testing

Advanced paternal age has been associated with an increased risk of single gene disorders and this pertains mainly due to mutations that occur during spermatogenesis (59). Although there is no consensus advanced paternal age is considered to be from 40-50 years of age and older (24).

Other risk factors for a positive invasive prenatal test result include: A couple carrying a chromosomal rearrangement. Carriers of balanced chromosome rearrangements can produce gametes with unbalanced chromosomes that result in a genetic abnormality in their children (60). Parental aneuploidy or aneuploidy mosaicism (61). In parents who are carriers of genetic disorders their offspring might be affected. In an autosomal dominant disorder the offspring has a 50% risk of having the disorder.

A couple who has had child with a structural birth defect has a 2-3% risk of the next child being affected but this varies in terms of the abnormality and sex of the affected child (24).

A previous child with an autosomal Trisomy or sex chromosome aneuploidy gives a higher risk of occurrence in the current pregnancy. This depends on the type of Trisomy, age of the patient in the current pregnancy and the age of the mother in the previous pregnancy and whether the previous abnormal pregnancy ended in spontaneous miscarriage (62,63). In a study of 2054 women who had a previous pregnancy with Down Syndrome it was found that the risk of recurrence in the subsequent pregnancy was 0.75% higher than the maternal and gestational age-

related risk. The risk of Trisomy 18 was also found to be 0.75% but it was found that the risk in these women were not increased for Down Syndrome and it was concluded that the risk of recurrence is specific to the chromosomal abnormality (7).

Knowledge of the indications of prenatal invasive testing is of paramount importance in prenatal counselling of the pregnant patient. Cho et al. found that maternal age, maternal serum markers and information on ultrasound should be included in prenatal counselling (34).

### 1.1.5 Laboratory technique in invasive prenatal testing

The type of test that the laboratory performs on a invasive test specimen is guided by the gestational age at which the test is performed and the indication for the test. Karyotyping is done for chromosomal disorders and DNA analysis for genetic disorders where a specific mutation is suspected. (24).

Karyotyping is the traditional test performed for the identification of aneupoloidies which include Trisomies and Sex Chromosome abnormalities. Karyotyping can also detect large rearrangements. Mosaicism in the fetus may not be detected by karyotype analysis if the specific fetal line of cells tested does not contain the mosaicism (24). Karyotyping has a diagnostic accuracy of more than 99% for aneuploidy and chromosomal aberrations of larger than 5-10 megabases(64).

Quantitative fluorescent polymerase chain reaction (QF-PCR) is a method that can be beneficial in a resource restricted setting as it takes away the need for fetal cell culture thus making it very cost effective. It is also characterised by a rapid turnover time and is fully automated. Specific DNA sequences are amplified by fluorescent primers. These DNA segments are then quantified as peak areas on DNA scanners. It has a very high detection rate of up to 98,6% for certain chromosomal abnormalities like Trisomy 21, 18, 13 and Sex Chromosome abnormalities X & Y. This method has the disadvantage that some abnormalities might be missed as the primers only test for the five chromosomal aberrations mentioned above (65).

If the aim of the test is to find smaller microdeletions and duplications fluorescent in situ hybridisation (FISH) can be considered as this method uses detection via chromosomal microarray. Fluorescent -labelled probes for a specific region of the

chromosome are used and find the number of those chromosome regions that are in the specimen. Many panels are available on special request but the one most commonly used is a panel including chromosome 13, 18, 21, X and Y. This method remains a screening test and a positive result must only be acted upon in the following cases: when there is a confirmatory karyotype or chromosomal microarray, clinical findings that support the FISH result or another positive screening test (66).

Chromosomal microarray analysis is a method that can detect both major aneuploidy and submicroscopic changes that cannot be detected by karyotyping (24). It is recommended that a patient who has structural abnormalities on ultrasound should be offered this test. In fetuses with a normal karyotype it has been shown to detect chromosomal abnormalities in 6% and 1,7% in patients with normal karyotypes and normal ultrasound examinations (67,68). ACOG recommends that this method be offered to any women undergoing prenatal invasive testing (24).

### 1.1.6 Complications of invasive prenatal testing

An invasive diagnostic test will give a definitive diagnosis regarding the presence of certain fetal abnormalities; however the risk of the test must be weighed against the need for a diagnosis.

#### 1.1.6.1 Risk of fetal loss

Miscarriage is the most widely quoted complication of invasive prenatal testing. In a landmark randomised control trial by Tabor et al. 4606 women at low risk of miscarriage aged 24-35 were randomised to have (or not to have) an amniocentesis. Most procedures were performed between 16 and 18 weeks of gestation. The amniocentesis group had a loss rate which exceeded the control group by 1%. This study also concluded that raised levels of alpha feto protein, tranplacental passage and retrieving discoloured amniotic fluid were associated with increased risk of spontaneous miscarriage (27). In a review done by Tabor et al. it was found that procedure related miscarriage rate for midtrimester amniocentesis was 0,5-1% (14). The Danish Fetal Medicine Study Group did a large study, with a cohort of 147 987 women, aiming to assess the risk of fetal loss with invasive testing following

combined first trimester screening for Trisomy 21. Risk was assessed by propensity score stratification. Risk of fetal loss following CVS over time points between three and 21 days after combined first trimester screening showed a range of 0.08-0.41% for miscarriage and for stillbirth a risk of 0.18-0.58%. Risk of fetal loss following amniocentesis was taken over time points of 28-42 days after combined first trimester screening. This showed a risk of fetal loss by miscarriage of between 0.42-0.56% and risk of stillbirth of 0.09-0.26%. The study thus found that the risk of fetal loss by miscarriage or stillbirth was not increased significantly in women undergoing CVS or amniocentesis compared to women not undergoing a invasive prenatal test. They thus concluded that the procedure related risk of invasive prenatal testing was very low.(58)

It has been shown that operators who perform procedures frequently have lower miscarriage rates (69), consequently single centre studies might have lower miscarriage rates because of very skilled operators but it must be emphasized that these rates cannot be used for counselling in general (14).

Early amniocentesis has higher rates of complications than that of late amniocentesis. It has been found that the pregnancy loss rate is significantly higher than that of midtrimeter amniocentesis. In a multicentre randomized trial, the spontaneous loss rate after early amniocentesis was 2.5% compared with 0.7% for midtrimester amniocentesis, significantly higher rates of amniotic fluid culture failures and talipes equinovarus were also found (70).

Meta analyses of all randomised control trials comparing CVS and second-trimester amniocentesis showed excess pregnancy loss following CVS (23). However a randomised control trial by Smidt-Jensen et al. showed similar pregnancy loss between transabdominal CVS and amniocentesis (71). A Cochrane review of amniocentesis and CVS concluded that the total pregnancy loss of transabdominal CVS is comparable to that of midtrimester amniocentesis, while transcervical CVS has a much higher rate of miscarriage than midtrimester amniocentesis (1). Some studies find the overall loss rate after CVS is greater than the rate after midtrimester amniocentesis. This can be explained by the increased background rate of spontaneous pregnancy loss between nine and sixteen weeks of gestation. Thus it

can be concluded that the procedure related loss rate is similar to that of midtrimester amniocentesis (24).

In a Cochrane review Alfirevic et al. concluded that second trimester amniocentesis is safer than early amniocentesis or trancervical CVS, and this is the procedure of choice for second trimester testing. If a diagnosis is needed earlier, transabdominal CVS is regarded as the procedure of choice over transcervical CVS and early amniocentesis (1).

Cordocentesis is associated with a pregnancy loss rate of 1,3% in fetuses with no fetal abnormalities and 1.3% to 25% in fetuses with single or multiple abnormalities or intrauterine growth restriction (16).Total miscarriage rate in fetuses with IUGR is up to 14% and in fetuses with hydrops fetalis up to 25% (72). Wilson et al. in a four year retrospective review showed that the procedural loss rate of cordocentesis was higher in fetuses where an abnormality was diagnosed (73).

#### 1.1.6.2 Other complications of amniocentesis

Amniocentesis is also associated with talipes equinovares, which is increased if early amniocentesis is done, and is mainly secondary to temporary or intermittent oligohydramnios (74). A Swedish national study which included 21748 women who had an amniocentesis and 47854 controls, showed an increased risk of musculoskeletal deformities when amniocentesis was done before 14 weeks of gestation (75).

Other minor complications caused by amniocentesis might include transient vaginal spotting and amniotic fluid leakage. Amniotic fluid leakage occurs in 1-2% of cases (70). The rate of amniotic fluid leakage increases the earlier the amniocentesis is performed (14). Bombard et al. suggests that there was less amniotic fluid flow from the puncture site with smaller gauge needles (76).The perinatal survival rate in cases of amniotic fluid leakage after midtrimester amniocentesis is greater than 90% (77). Culture failure occurs in 0.1% of samples (24). There may be an association with amniotic band syndrome and amniocentesis (78).

Midtrimester amniocentesis has been associated with neonatal respiratory distress syndrome and neonatal pneumonia. An animal study where primates underwent

midtrimester amniocentesis had structural changes in their lungs which were not found in controls (79).

#### 1.1.6.3 Other complications of chorionic villous sampling

CVS associated vaginal spotting can occur in up to 32% of patients and this is less in transabominal versus transvaginal CVS (80). ACOG postulates that the incidence of infection, culture failure or amniotic fluid leakage after CVS is less than 0,5% (24).

Chorionic villous sampling before ten weeks gestation has previously been shown to have an association with oromandibular limb hypoplasia and isolated limb disruption defects. This association was first reported in 1991 when a cluster of five babies with limb reduction defects was reported among a series of 289 women undergoing transabdominal CVS between eight and nine weeks (81). In an analysis by the World Health Organisation, an incidence of limb reduction defects after CVS was found to be 6/10000 which is not significantly greater than the incidence in the general population. After ten weeks there was no increased risk of limb reduction defects, while there might be an association with a CVS being performed before ten weeks of gestation (76,82). The RCOG recommend that CVS is not performed before ten weeks of gestation (23).

Although more studies are needed there has been an association between CVS and the development of preeclampsia which might be explained by the procedure causing placental disruption and consequently placental dysfunction (83).

Chromosomal mosaicsm is the presence of more than one cell line during cytogenetic analysis, it occurs in 0.25% of amniocentesis samples and 1% of chorionic villous samples. Confined placental mosaism is a condition where mosaicism is only present in the placenta, it is found in 1-2% of placentas. This condition can be found in fetuses which have a normal amount of chromosomes but may have a genetic abnormality such as uniparental disomy, this might impact the CVS result (16). Another case of mosaicism is maternal cell contamination of the fetal specimen. This can be minimised by discarding the first 1-2ml of fluid obtained at amniocentesis and by careful dissection of chorionic villi from maternal decidua (24). It must be mentioned at this point that there is an association with confined

placenta mosacism and a false positive results in non invasive prenatal testing (NIPT). This is due to the fact that cell free DNA is mainly derived from the placental trophoblast. As there is a known risk of false positive results with NIPT, each positive NIPT result should be confirmed with an invasive prenatal test before definitive management is instituted (84,85).

#### 1.1.6.4 Other complications of cordocentesis

Cordocentesis might be associated with continual bleeding from the cord with significant anaemia and hypotension in the fetus. Fetal bradycardia is another complication that might occur in 5-10% of fetuses. (16)

#### 1.1.6.5 Infective complications including HIV transmission

Severe sepsis including maternal death has been reported following invasive prenatal techniques. The risk is likely to be less than 1/1000. RCOG recommends the use of strict sterile techniques, as discussed previously, in all invasive prenatal tests (23).

Blood borne virus transmission from mother to fetus poses a potential risk during invasive testing. Davies et al. concluded that the risk of transmission of Hepatitis B is very low (86). Vertical transmission rate is not increased in women who have low viral load, whereas women with high viral loads might have a 21 fold increased rate of transmission and newborn infection (87).

There is no evidence that Hepatitis C is transmitted (88). In a study of 22 pregnant women who were hepatitis C positive and who underwent second trimester amniocentesis, only one woman had hepatitis C virus detected in the amniotic fluid and none of the infants born had the virus (88).

It is thought that transmission of HIV is not a risk provided that the viral load of the mother is low and that antiretroviral therapy has been instituted (23). Somigliana et al. demonstrated no difference in transmission rates between HIV positive women undergoing amniocentesis and those who did not and this was also shown by Mandelbort et al. (89,90). One study did show that transmission rate is high if there

was no treatment in place or mono or double therapy was being used (91). Data from the French Perinatal Cohort study included 81 HIV infected patients who had amniocentesis and were on combination antiretroviral therapy. There was no difference in the rate of vertical transmission in this group compared with controls who did not have an amniocentesis (90). A study done by Floridia et al. also concluded that HIV transmission is not increased if a patient is virally suppressed and undergoes invasive prenatal testing (92). The RCOG recommends that the viral load be undetectable before an invasive test is done (93).

South Africa has a large burden of HIV and more than 50% of the people living with HIV in South Africa are women and many of these are of child bearing age. The South African Department of Health showed in 2013 that 29,7% of pregnant women were infected with HIV (94,95). Clinical practice guidelines published in the South African Medical Journal in 2014 state that if a HIV positive woman requires an amniocentesis antiretroviral therapy should be initiated and that the procedure should be delayed until the viral load is suppressed (94). In 2012 Mnyani et al recommended a patient should be on antiretroviral therapy for four to six weeks to become adequately virally suppressed (96). The decision should be guided by the nature of the chromosomal abnormality suspected. If the abnormality is thought to be severe and might cause great morbidity, exposing the fetus to a small risk of HIV transmission will be outweighed by the benefit of a prenatal diagnosis (94).

It is further stated that third trimester amniocentesis, CVS and cordocentesis are not recommended in HIV positive woman. Mnyani et al. states that due to the increased technical difficulty CVS and cordocentesis procedures might have a significantly increased risk of transmission to the fetus even if the patient is on antiretroviral therapy (96). It is also advised that transplacental amiocentesis is avoided (97). There must be a clear indication for the use of an invasive prenatal test in a HIV positive woman and the use of screening tests are recommended (94,96). This is supported by Naidoo et al. in a local study that concluded that in a country with a very high rate of HIV that a first trimester screening programme was needed as it would lead to less invasive tests being needed (15).

It is advisable that a viral load is obtained in the woman before an invasive test is done and that this should form part of the counselling procedure. It is also good

practise to repeat an HIV test before an invasive prenatal procedure if the patient initially tested HIV- negative (96).

It is of upmost importance that a patient should be counselled on the risk of mother to child transmission of HIV as part of the informed consent process for the invasive prenatal testing (96).

### 1.1.7 Counselling for prenatal testing

Counselling of a patient and her family, whether she is undergoing a screening test or an invasive prenatal test, is probably the most important aspect of the procedure. A thorough knowledge of the procedure is needed by the clinician in order to do this and the approach of a multidisciplinary team including the use of genetic counsellors, clinical geneticists and support groups must be remembered.

Firstly the patient must understand that all screening and invasive testing is completely voluntary (98). ACOG recommends that prenatal genetic testing be discussed as early as the first antenatal visit and that a risk of aneuploidy be made as early as possible (24).

The RCOG recommend that the following should be included when written consent for an invasive prenatal test is obtained: (23)

- The indication for the procedure
- The type of cytogenetic result that might be obtained
- Risks nationally and locally pertaining to pregnancy loss
- Limitations of the laboratory tests that are available
- How the results will be communicated to the patient
- Possible outcomes of a test and available treatments and follow up for a positive result

Genetic prenatal counselling can often be difficult to the patient and the clinician. It must be remembered that non directive counselling should be given to the patient and her partner. Autonomy, one of the principles of medical ethics, dictates that the physician respects the patient's preferences towards her care (10).

Genetic counselling starts with a good patient history which must include a family pedigree and previous obstetric and genetic history. In certain circumstances parental karyotyping might be needed especially if a family history of genetic translocation risks is elicited. The patient should be counselled in a language she understands and at her level of literacy (16).

If a chromosomal abnormality is diagnosed the patient should be provided with information of the natural history of the disease and the decision of termination of pregnancy should also discussed. It must be remembered that invasive prenatal testing is not only done in order to aid a patient with the decision to terminate her pregnancy but may provide details to the patient and her medical team to plan the rest of her pregnancy, delivery and care in the neonatal period (24). According to the SOGC it is a common misconception that invasive testing is only offered to patient's who will undergo termination of pregnancy (98). It is pivotal that patient's understand although a decision of termination of pregnancy will be supported, if a decision is made to continue with the pregnancy the best possible care will be given. This care must include a discussion of intrapartum care, fetal monitoring, mode of delivery and care of the fetus at birth. It is often of value to include a neonatologist in this discussion (99).

Another important aspect to discuss in a positive test is the small possibility of a false positive result and the converse is also true in that a negative test might not mean that all abnormalities have been ruled out (98).

It is important to give a couple time to discuss the findings with their family and schedule follow up appointments with ample time for question asking.

## 1.2 PURPOSE OF STUDY

Invasive prenatal diagnosis is the gold standard of prenatal diagnosis of chromosomal abnormalities in the modern era of medicine. Although non-invasive screening tests should always be considered it is often not available in a resource restricted setting such as the public sector in South Africa. In the South African public sector biochemical screening and NIPT is not available, methods of screening mainly rely on advanced maternal age and antenatal ultrasound. Although outcome

of invasive prenatal procedures have been studied previously, it has not been looked at in a resource poor, tertiary setting in South Africa.

The aim of this study is to review the outcome of invasive prenatal testing at Rahima Moosa Mother and Child Hospital over a two year period and will include a review of patient selection, complications and diagnosis. It is important to conduct a review of a service provided by a centre and compare this to international standards as these findings can be used in counselling patients on the possible complications and outcomes of such a procedure.

## 1.3 SETTING

The study took place at Rahima Moosa Mother and Child Hospital, a tertiary hospital in Johannesburg which performs approximately 12 000 deliveries per annum. The hospital has a dedicated ultrasound department, genetics clinic and antenatal clinic. Invasive prenatal diagnostic testing is available. The patients are referred from consultants, registrars and sonographers to the specialists providing the service for consideration of further testing. Outside referrals from private doctors are also seen.

The dedicated ultrasound department does approximately 13 000 ultrasounds per annum. It should be noted that this includes ultrasounds for paediatric and gynaecological pathology. The team consists of one fetal medicine specialist, one fellow in fetal medicine, two consultants in obstetrics and gynaecology with a special interest in fetal medicine and three full time sonographers. There are four ultrasound machines available in rooms that are equipped for invasive procedures.

Three types of ultrasounds are done in the department. The first being a dating scan that is mainly opportunistic and can also be done by registrars in the obstetrics and gynaecology department at the time of a patient presenting for antenatal care. This scan may then be done at any gestation. The second is a nuchal translucency scan that is done between 11 and 13 weeks six days gestation. The third is a fetal anomaly scan that is done between 18 and 23 weeks gestation.

If a patient is booked or referred early enough she may be referred for a nuchal translucency scan and afterwards will be booked for a fetal anomaly scan. If any abnormalities are detected in any ultrasound the patient is referred to one of the

clinicians in the department and further follow up is organised as needed after the patient is seen by the clinicians. All invasive prenatal testing is performed by the aforementioned specialists.

The department is also supported by dedicated genetic counselling clinic which is available by appointment.

## 1.4 OBJECTIVES OF THE STUDY

The main aim of the study is to evaluate prenatal invasive testing, including amniocentesis, CVS and cordocentesis over the period from January 2014 to May 2016 in terms of

1. Describing ultrasound markers that lead to a prenatal test being offered to a patient
2. Cytogenetic diagnosis made
3. Complications and complication rates related to the procedure
4. Pregnancy outcome and final diagnosis where possible

# 2 MATERIALS AND METHODS

## 2.1 STUDY SAMPLE

### 2.1.1 Study population

The study included all patients who had had an invasive prenatal diagnostic tests, including amniocentesis, CVS and cordocentesis at Rahima Moosa Mother and Child Hospital .

### 2.1.2 Timing

The testing was carried out over the period January 2014 to May 2016. The patients had to have completed their pregnancy by the end of July 2016. There were 97 patients who had a test in this time period. One of these 97 patients was excluded as her invasive test result was not found on the laboratory data base.

A retrospective chart review was done on all the patients' files who underwent the above mentioned testing. The list of patients on whom an invasive prenatal test was performed was obtained from a data base kept within the ultrasound department. It was expected that some files would not be available in the hospital's record department as a certain number of patients would have continued their antenatal care in other facilities, provinces or countries and would have taken their maternal chart with them.

## 2.2 METHODS

Patients who underwent invasive prenatal testing including amniocentesis, CVS and cordocentesis, were identified using the data base that exists in the ultrasound department of Rahima Moosa Mother and Child Hospital. The patient name, hospital number, indication for invasive test, type of invasive test and gestation at which the test was performed of all patients undergoing prenatal invasive testing are recorded and kept in book form in the ultrasound department.

Charts of the patients identified from the above mentioned data base were obtained from the Rahima Moosa Mother and Child records department and information was

collated on a data sheet  and entered into the Redcap (research electronic data capture) program. In the Redcap program each patient had a unique code and all patient identifiers were removed to keep anonymity. Patient identifying data was only known to the researcher. (Appendix 1)

The result of the invasive prenatal testing was obtained from the National Health Laboratory Service system and reports of ultrasound examination during the patients' pregnancy were obtained from patients' files.

As outlined in the objectives complications related to the procedure were identified and a fetal loss that occurred within two weeks of the procedure was recorded as a complication associated with the procedure. Most miscarriages related to invasive prenatal testing occur within 72 hours of the procedure and previous studies in this field have defined the cut off for procedure related miscarriage to be two weeks (36,100). Pregnancy outcome, where available, was also identified and this included all known pregnancy losses including termination of pregnancy, spontaneous miscarriage, stillbirths and perinatal mortality. According to the South African Maternal Care guidelines of 2015 a miscarriage can be defined as a fetus of 28 weeks or less with no evidence of life at delivery (101). According to the South African PIPP guideline perinatal mortality can be defined as all babies who have demised after 22 weeks of gestation (500g or more) (52) thus in this study a miscarriage was defined as a fetus weighing less than 500g. Where a normal karyotype or PCR result was obtained the phenotypic appearance of the baby at birth as described in the patient delivery chart was recorded.

# 3 RESULTS

A total of 97 invasive prenatal tests were performed between January 2014 to May 2016, of these results 96 results were obtained. During this time Rahima Moosa Mother and Child Hospital had performed 29380 deliveries.

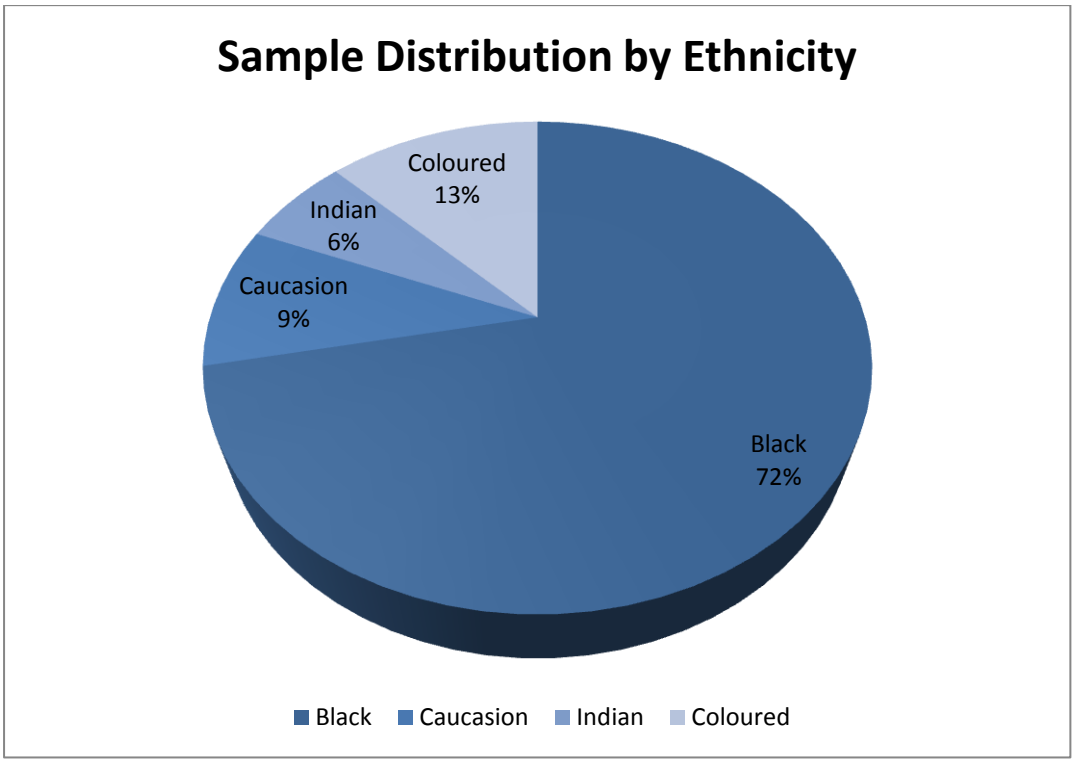
## 3.1 STUDY POPULATION DEMOGRAPHICS

### 3.1.1 Age

The mean age of women in the sample was 35 years. The oldest patient in the sample was 47 years old and the youngest patient was 19 years old.

### 3.1.2 Ethnicity

Among the patients evaluated 71, 9% were Black, 9,4% were Caucasian, 12,5% were from the Coloured community and 6,3% were from the Indian community.




**Graph 3.1: Sample distribution by Ethnicity**

There were three Black patients in the sample with infants with chromosomal abnormalities, three with fetuses with chromosomal and structural abnormalities and four patients with infants with normal karyotypes but structural abnormalities at birth.

In the Caucasian population there was one patient with a fetus affected by a chromosomal abnormality.

In the Coloured community there were two patients with fetuses affected by chromosomal abnormalities, two with fetuses with chromosomal and structural abnormalities and two patients with infants with normal karyotypes but structural abnormalities at birth.

In the Indian Community there was one patient with a fetus affected with a chromosomal and structural abnormality.

### 3.1.3 Parity

Parity of 69 (72%) patients was known. The mean parity was two live births, with a range from nought to seven live births.

### 3.1.4 Gravidity

Gravidity of 69 (72%) patients in the sample was known. The mean gravidity was three pregnancies, with a range from one to eight pregnancies.

### 3.1.5 Chronic illnesses and medication use

Of the 66 patients in which this information was known, 14 patients had hypertension with one not being on treatment. No chromosomal abnormalities were found in these patients. One patient had a fetus with structural abnormalities and is discussed later as patient 6.

Three patients had gestational diabetes. There were no patients with Type 1 or Type 2 diabetes. All the patients were managed with a diabetic diet and were not on medication. One of these patients had a infant with a balanced translocation and is discussed later as patient I.

There was one patient who is epileptic not on medication. She had an infant with a normal karyotype who was healthy at birth.

There was one patient with Tuberculosis on treatment. She had an infant with a normal karyotype who was healthy at birth.

There were three patients with asthma in the cohort who were all on treatment. Their infants had normal karyotypes. Two patients had normal infants at birth, one birth outcome is unknown.

There was one patient with hyperthyroidism on treatment who had a fetus with Trisomy 21, this patient is discussed later as patient B. One patient was known with thyroid cancer of the papillary type and was scheduled for surgery after the delivery of her infant. She had an infant with a normal karyotype who was healthy at birth.

There was one patient with major depressive disorder on treatment. She had an infant with a normal karyotype who was healthy at birth.

One patient had a previous deep vein thrombosis and was on anticoagulation in the studied pregnancy. She had an infant with a normal karyotype who was healthy at birth.

There was a patient in the sample with idiopathic intracranial hypertension on treatment. She had an infant with a normal karyotype who was healthy at birth.

There were no known cardiac patients in the sample.

There was one patient with a known genetic condition, namely neurofibromatosis. She had an infant with a normal karyotype who was healthy at birth.

### 3.1.6 Smoking, alcohol use and illicit drug use

There was one patient in the sample who smoked three cigarettes a day, the duration is not known. This patient also drank four to five glasses of alcohol a day, the type of alcohol and duration is not known. She had an infant with a normal karyotype who was healthy at birth.

There were no patients that used illicit drugs.

### 3.1.7 Booking status of patients

Booking status of 66 (69%) patients was known.

#### 3.1.7.1 Rhesus blood group

In the sample six patients (9%) were recorded as Rhesus blood group negative. There was no record in the patient's files of whether these patients received anti-D immunoglobulin after their invasive prenatal procedures. It is a standing departmental policy that Anti-D should be administered to all Rhesus negative women after an invasive procedure.

#### 3.1.7.2 HIV status

Seventeen patients were found to be HIV positive, sixteen of these patients were on antiretroviral therapy at the time of invasive prenatal diagnosis and one patient had defaulted her treatment. CD4 counts of fourteen of the patients were available; the range being 29cells/uL to 987cells/uL. Viral loads of five of the patients were known, with three being lower than detectable and the other two having viral loads of 1910 IU/ml and 862IU/ml respectively. In the patient who had a viral load (VL) of 1910IU/ml antiretroviral therapy was initiated before booking for her pregnancy, in the other patient the duration of antiretroviral treatment was not known. It is not known what the viral loads were at the time of the invasive procedure being done as the patient with a VL of 1910IU/ml had her VL done after the invasive procedure was done, there is no record of a VL before the procedure. In the patient with a VL of 862IU/ml it is not known when in relation to the invasive procedure the VL was done.

Of the sixteen patients on antiretroviral therapy, it is known that six started their antiretroviral treatment at the time of booking, this means that the minimum duration that the patients were on antiretroviral therapy before their invasive prenatal test ranged between one week and five weeks. Five patients started their antiretroviral therapy before booking but the exact duration is unknown. The duration of antiretroviral therapy taken is unknown in five patients.

3.1.7.3 RPR status

One patient was found to be RPR positive at time of invasive prenatal diagnosis and was adequately treated.

3.1.7.4 Haemoglobin

The haemoglobin (Hb) of 57 patients were known, the mean Hb was 12,1g/dL at the time of booking, with a range from 7,8g/dL to 14.8g/dL. There were eight patients with haemoglobin values below 11g/dL.

### 3.1.8 Gestational age at first booking

The gestational age of first booking was known in 62 patients. The average gestational age at booking was 16 weeks. The gestational age at first booking ranges from five weeks to 27 weeks.

## 3.2 PATIENT RESULTS

Between the study period of January 2014 to May 2016, a total of 97 invasive tests were performed. Results of 96 of these tests were obtained as one test result could not be retrieved from the National Health Laboratory Services system.

There were 94 (98%) singleton pregnancies and two (2%) multiple pregnancies, both being twin pregnancies. One twin pregnancy had one fetus affected by a chromosomal abnormality (Patient F below), the other twin pregnancy had no abnormalities found.

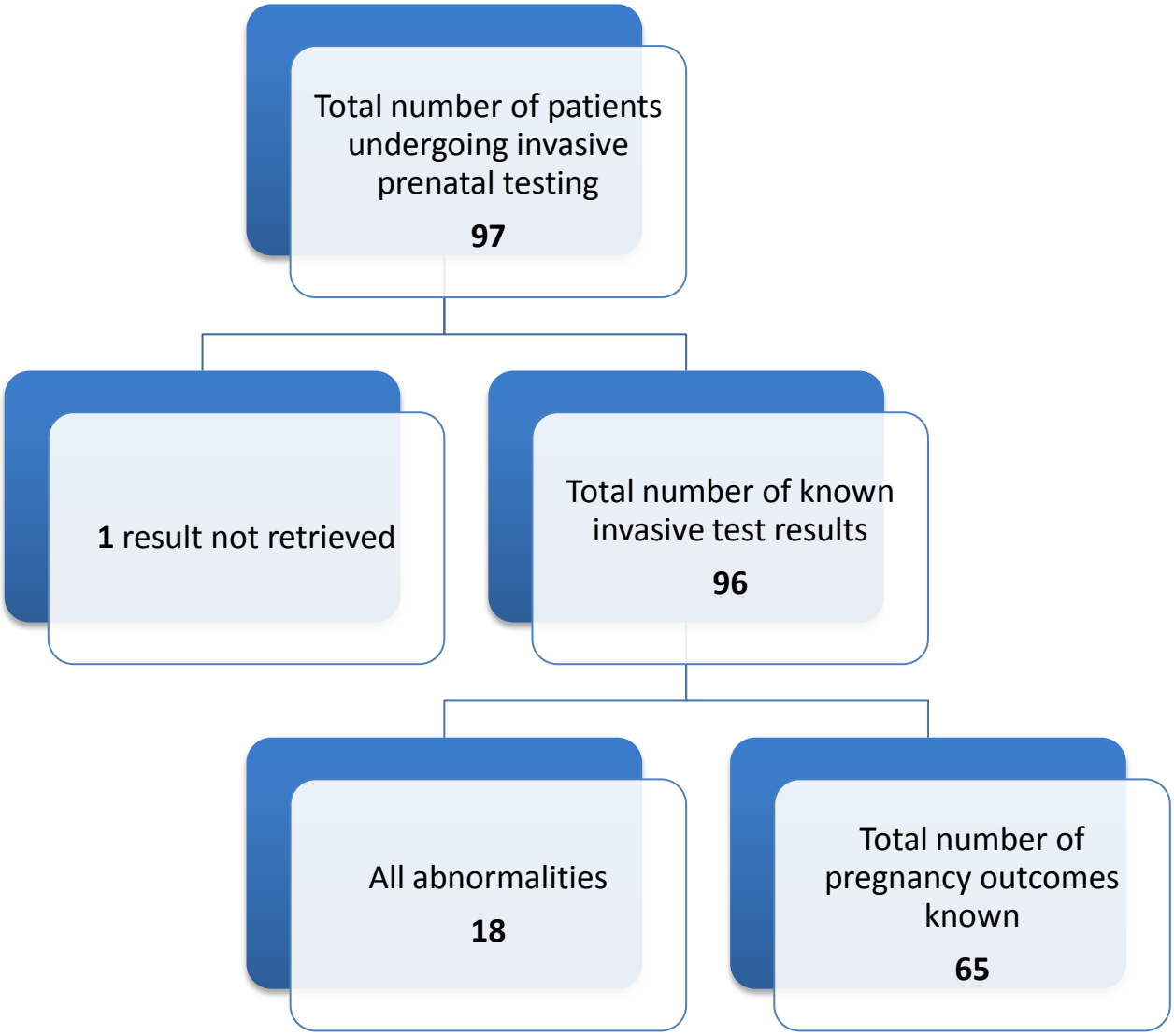



**Diagram 3.1: Flow diagram of results**

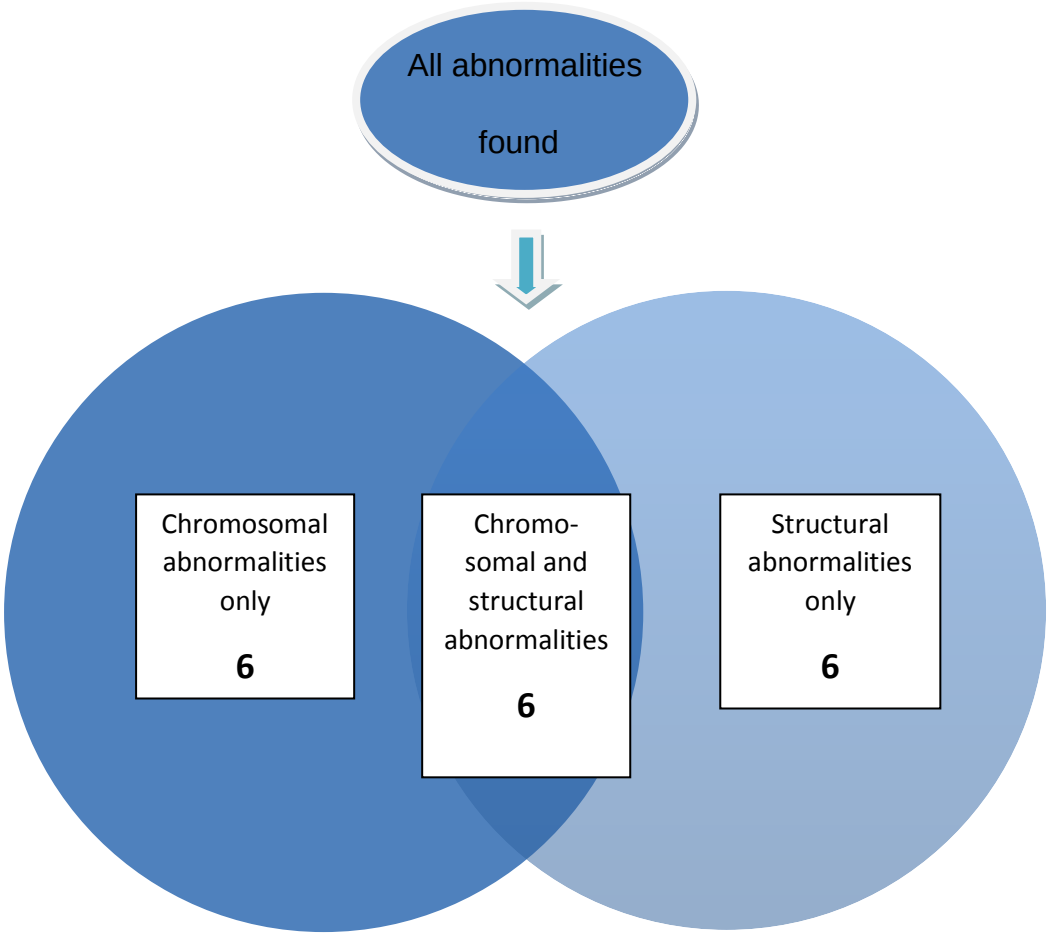
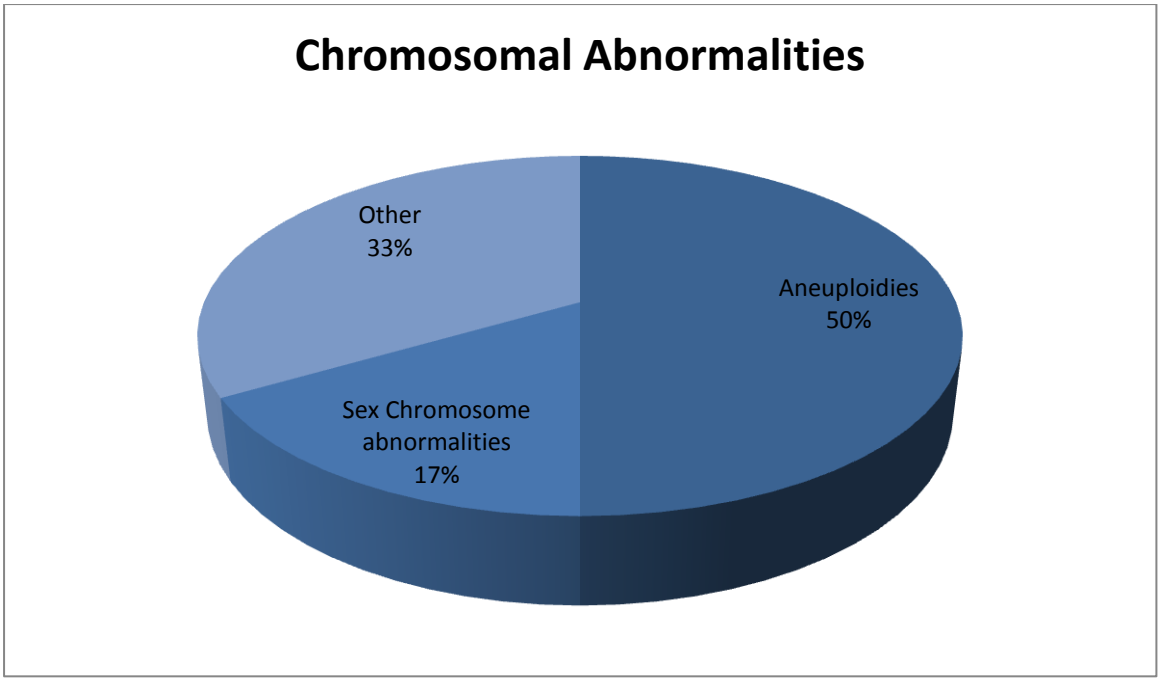



**Diagram 3.2: Relationship between chromosomal and structural abnormalities**

### 3.2.1 Chromosomal and genetic abnormalities found in the sample

A total of 12 (12, 5%) chromosomal abnormalities were found in the sample of 96 patients. Six of these patients had fetuses with Trisomies, two with Trisomy 21, two with Trisomy 13 and two fetuses with Trisomy 18. Two fetuses were affected by sex chromosome abnormalities in the form of Klinefelter Syndrome. One fetus had a balanced translocation [4;13 (q35;q14)], one a diagnosis of cystic fibrosis, one had spinal muscular atrophy and one had Wolf-Hirschorn syndrome.




**Graph 3.2: Chromosomal abnormalities in the sample**

3.2.1.1 Trisomy 21

Patient A

The first patient with a fetus with Trisomy 21 was a 38 year old P1G2 with no previous history of chromosomal abnormalities. She booked at 21 weeks where a fetal anomaly scan was done which found several markers namely thickened nuchal oedema and an echogenic focus in the left ventricle. A second trimester amniocentesis was offered to her and once the results of Trisomy 21 were communicated and counselling done, the patient opted for a termination of pregnancy at 24 weeks gestation. She delivered a 595g female fetus.

Patient B

The second patient with a fetus with Trisomy 21 was a 45 year old P3G7 known with hyperthyroidism and on treatment who booked in the first trimester and underwent a nuchal translucency scan at 13 weeks gestation that showed an increased nuchal translucency and an absent nasal bone. The patient underwent a CVS that

confirmed Trisomy 21. The patient opted for a termination of pregnancy in the first trimester.

#### 3.2.1.2 Trisomy 18

Patient C

The first patient with Trisomy 18 was a 42 year old P1G2 with a family history of a child affected with ectrodactyly and missing right hand metacarpals. She had an increased Trisomy 18 risk on her first trimester biochemical screening of 1 in 14. Second trimester amniocentesis confirmed Trisomy 18. A fetal anomaly scan showed choroid plexus cysts and a fetus with clenched fists and rocker bottom feet. The outcome of her pregnancy is unknown

Patient D

The second patient found to have an infant affected with Trisomy 18 was a 27 year old P3G4 with no previous history of chromosomal abnormalities. Her fetal anomaly scan showed multiple abnormalities namely a mono-ventricle with thalami fused, the cerebellum splayed with an absent vermis, a flattened forehead, cardiomegaly with a pericardial effusion, an echogenic focus in the left ventricle and polyhydramnios. A provisional diagnosis of holoprosencephaly was made. The patient underwent a second trimester amniocentesis. The patient opted not to have a termination of pregnancy or fetocide and delivered at 41 weeks by normal vaginal delivery. The neonatal team made a clinical diagnosis of Trisomy 13 rather than the prenatal diagnosis of Trisomy 18 but there was no karyotyping available after birth to confirm the diagnosis. Unfortunately the infant was lost to follow up.

#### 3.2.1.3 Trisomy 13

Patient E

The first patient with a fetus with Trisomy 13 was a 41 year old P7G8 who booked at 20 weeks gestation. She had no previous family or obstetric history of chromosomal

anomalies. She underwent an ultrasound at 25 weeks which found several ultrasound abnormalities namely brachycephaly, micrognathia, alobar holoprosencephaly, bilateral echogenic foci in the heart and an abnormal nose. A second trimester amniocentesis showed Trisomy 13. The patient declined termination of pregnancy and fetocide and delivered a fetus with features of Trisomy 13 at 32 weeks gestation by caesarean section. The patient's records state the indication for the caesarean section was grand multiparity. The baby passed away shortly after birth.

Patient F

The second patient to have fetus with Trisomy 13 was a 26 year old P1G2 who booked at 12 weeks gestation where a twin pregnancy was found. The patient underwent a fetal anomaly scan at 21 weeks where features of Trisomy 13 were found in the second twin namely a bulky thalamus, a distorted cerebellum, a brachycephalic head, a soft tissue mass protruding from the nasal area and a hypoplastic left ventricle. The first twin was sonographically normal. A second trimester amniocentesis confirmed a normal karyotype in the first twin and Trisomy 13 in the second twin. The patient underwent a caesarean section at 33 weeks gestation. The patient's records state that the indication for her caesarean section was a compound presentation with a cord presentation. The first infant was healthy at birth and the second twin had clinical features of Trisomy 13 and passed away shortly after birth.

3.2.1.4 Sex Chromosome Abnormalities

Patient G

The first patient found to have a fetus with Klinefelter Syndrome was a 25 year old female who underwent an anomaly scan at 23 weeks where an echogenic focus in the left ventricle was found and the fetal growth was on the 5$^{th}$ centile. The patient

was offered a second trimester amniocentesis that showed Klinefelter Syndrome. The outcome of her pregnancy is unknown.

Patient H

The second patient with a fetus with Klinefelter Syndrome was a 42 year old P1G2 with no history of previous chromosomal abnormalities in her family or other child. She had a normal fetal anomaly scan and underwent a second trimester amniocentesis. She delivered an infant at term by normal vaginal delivery with no abnormal features noted at delivery and is being followed up by paediatrics.

3.2.1.5 Other Chromosomal Abnormalities

Patient I

A 23 year old P1G2 underwent an amniocentesis at 21 weeks as her partner was known with a 4;13 and 8;10 translocation. She had also previously had an early neonatal death following her previous pregnancy. Facial and brain abnormalities were found in that infant. Her amniocentesis results showed that this fetus carried a translocation 4;13 (q35;q14) but not a translocation 8;10. A healthy looking infant was delivered at 40 weeks gestation by caesarean section.

3.2.1.6 Genetic abnormalities

Patient J

One case of cystic fibrosis was found in a 21 year old patient known with gestational diabetes who had previously had a daughter with cystic fibrosis. She underwent an amniocentesis at 18 weeks gestation which confirmed that her fetus was homozygous for the F508 mutation. The outcome of her pregnancy is unknown.

Patient K

A 38 year old patient underwent a second trimester amniocentesis at 19 weeks gestation as she had a son with spinal muscular atrophy who had a homozygous deletion SMN1 exon 7 (CGC1301363). The patient was tested and was found to be a carrier for spinal muscular atrophy. The second trimester amniocentesis revealed a fetus who was also a carrier of the disease. The outcome of her pregnancy is unknown.

Patient L

A 23 year old primigravida who had a fetal anomaly scan at 23 weeks gestation was found to have a fetus with Wolf-Hirshcorn disease. She had no previous family history of chromosomal abnormalities. The fetal anomaly scan showed multiple abnormalities namely an absent nasal bone, increased prenasal oedema, an hourglass posterior fossa, a thickened nuchal translucency, short femur and humerus, clinodactyly, a probable membranous venrticuloseptal defect, a clubfoot, rocker bottom feet and a single umbilical artery. The patient opted for a fetocide at 30 weeks gestation and delivered a 1000g infant by normal vaginal delivery. The postnatal diagnosis was compatible with the antenatal diagnosis.

**Table 3.1: Chromosomal abnormalities found in sample**

| Patient | Chromosomal abnormality |
|---|---|
| ***Aneuploidy*** | |
| **Patient A** | Trisomy 21 |
| **Patient B** | Trisomy 21 |
| **Patient C** | Trisomy 18 |
| **Patient D** | Trisomy 18 |
| **Patient E** | Trisomy 13 |
| **Patient F** | Trisomy 13 |
| ***Sex Chromosome Abnormalities*** | |
| **Patient G** | Klinefelter Syndrome |
| **Patient H** | Klinefelter Syndrome |
| ***Translocation*** | |
| **Patient I** | Balanced Translocation 4;13 (q35;q14) |
| ***Genetic Abnormalities*** | |
| **Patient J** | Cystic Fibrosis |
| **Patient K** | Spinal Muscular Atrophy |
| **Patient L** | Wolf-Hirshcorn Disease |

In summary there were six patients (50%) who were of advanced maternal age in the sample of patients who had chromosomal abnormalities. Two patients (17%)

underwent invasive prenatal testing due to a previous history of chromosomal abnormalities. In the sample eight patients (67%) had abnormal ultrasound findings. There were three patients (25%) with both risk factors of advanced maternal age and abnormal ultrasound findings. Of the twelve patients, eleven had singleton pregnancies and one patient had a twin pregnancy. The invasive tests included ten second trimester amniocenteses, one third trimester amniocentesis and one CVS.

Outcome of eight pregnancies (67%) are known and of these two patients (17%) underwent termination of pregnancy and one patient (8%) underwent fetocide. There was one instance (Patient D) where neonatal findings did not agree with invasive test results but this finding was not confirmed.

**Table 3.2: Outcome of pregnancies with fetuses affected by chromosomal abnormalities**

| Abnormality | Continued pregnancy | TOP | Fetocide | Unknown |
|---|---|---|---|---|
| **Trisomy 21** | | 2 | | |
| **Trisomy 18** | 1 | | | 1 |
| **Trisomy 13** | 2 | | | |
| **Klinefelter Syndrome** | 1 | | | 1 |
| **Cystic fibrosis** | | | | 1 |
| **Spinal muscular atrophy** | 1 | | | |
| **Wolf-Hirschorn Syndrome** | | | 1 | |
| **Translocation** | 1 | | | |
| **Total** | 6 | 2 | 1 | 3 |

### 3.2.2 Structural abnormalities in the sample

In the sample of 97 patients, there were thirteen patients with major structural anomalies at ultrasound alluding to possible outcome of structural abnormality at birth. Two of these patients had normal infants at birth. Six patients had fetuses with chromosomal abnormalities confirmed at karyotyping and are discussed above (Patients A, B, D, E, F and L) .

Below the remaining five patients with major structural anomalies on ultrasound and structural abnormalities in their infants confirmed at birth are discussed. One patient is also discussed where only soft markers were found in her infant at ultrasound but structural abnormalities at birth.

Patient 1

The first patient who had a fetus with structural abnormality diagnosed on ultrasound was 34 year of P2G4. A large exomphalos with liver involvement was found at a 21 week fetal anomaly scan. The patient had no previous history of chromosomal abnormalities and her second trimester amniocentesis had a normal karyotype. The patient delivered at 32 weeks gestation by caesarean section due to cord prolapse. The antenatal findings were confirmed by the neonatal team and the infant was admitted into their care.

Patient 2

The second patient was a 23 year old P0G1 who underwent an ultrasound at 31 weeks gestation that found the fetus had an increased biparietal diameter, hypomineralisation of the fetal skull bones, strawberry sign head, frontal bossing, a small mandible and mouth, a short humerus, an enlarged cardiothoracic ratio, polyhydramnios, an absent stomach bubble alluding to a trachea-eosophageal fistula, an omphalocoele involving small bowel, a bowed femur, bowed tibia and fibula and rocker bottom feet. It must be assumed that this late scan was done opportunistically at time of referral. The patient underwent a third trimester amniocentesis which showed a normal karyotype. Although the patient had booked at eight weeks gestation she was referred to Rahima Moosa Mother and Child Hospital in her third trimester. She underwent a caesarean section at 37 weeks gestation and the antenatal findings were confirmed by the neonatal team. Her infant demised shortly after birth.

Patient 3

The third patient was a 37 year old P3G4 who had a previous early neonatal death. The only record made of this death is that the infant was found to have a short umbilical cord at birth. The patient underwent a fetal anomaly scan at 20 weeks gestation that showed holoprosencephaly, this finding was confirmed on an antenatal MRI. The patient had a cordocentesis at 30 weeks gestation with a normal karyotype. The patient opted for fetocide at 30 weeks gestation and underwent a caesarean section as induction of labour had failed.

Patient 4

The fourth patient was a 23 year old P1G2 with an unremarkable antenatal history. The patient underwent an opportunistic ultrasound at 28 weeks gestation which showed polyhydramnios, cerebellar vermis agenesis, an abnormal four chamber heart view, cardiomegaly and axis deviation, a small stomach bubble, a single kidney, bilateral cleft lip and palate, a single umbilical artery and flat facial profile. The patient underwent a second trimester amniocentesis which found a normal karyotype. She presented with an IUFD at 37 weeks gestation and delivered by normal vaginal delivery. No notes were made of the postnatal findings of the infant at birth. Placental histology showed features of uteroplacental underperfusion.

Patient 5

The fifth patient was a 24 year old who underwent an anomaly scan which showed holoprosencephaly, a second trimester amniocentesis showed a normal karyotype in her fetus. Due to this patient's record being lost no further information about the outcome of her pregnancy is known.

Patient 6

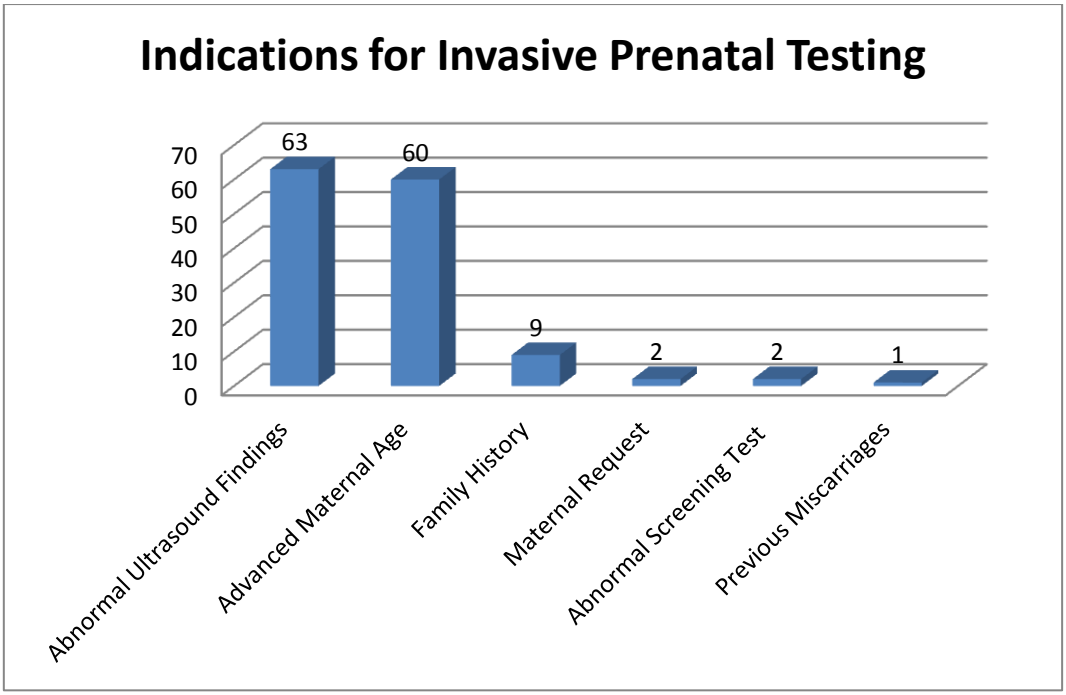
The sixth patient was a 23 year old primigravida who had a fetus with several soft markers on ultrasound namely a ventriculomegaly, echogenic bowel and a lemon shaped head. The patient had a second trimester amniocentesis with the fetus

having a normal karyotype. At birth this infant was found to have a cleft lip and palate, low set ears, elongated anterior fontanelles, flat nasal bridge and upfolding epicanthic folds. The infant is being followed up by paediatrics and has multiple cardiac abnormalities including a patent ductus arteriosus, a ventriculoseptal defect and neonatal dilated cardiomyopathy. The infant also developed epilepsy and is neurodevelopmentally delayed, a specific genetic diagnosis has not been made but follow up is ongoing.

### 3.3 INDICATIONS FOR INVASIVE PRENATAL TESTING

**Table 3.3: Indications for invasive prenatal testing**

| Indication | Number | Percentage (%) |
|---|---|---|
| **Abnormal Ultrasound Findings** | 60 | 63 |
| **Advanced Maternal Age** | 58 | 60 |
| **Family History** | 9 | 9 |
| **Maternal Request** | 2 | 2 |
| **Abnormal Screening Test** | 2 | 2 |
| **Recurrent first and second trimester miscarriages** | 1 | 1 |




**Graph 3.3: Indications for invasive prenatal testing**

As can be seen from the above mentioned table and chart there were some patients who had more than one indication for invasive prenatal testing. The most common being abnormal ultrasound findings and advanced maternal age. There were three patients (25%) with both risk factors of advanced maternal age and abnormal ultrasound findings in the group with chromosomal abnormalities and one patient with both risks in the group with structural abnormalities.

### 3.3.1 Abnormal Ultrasound Findings (Objective 1)

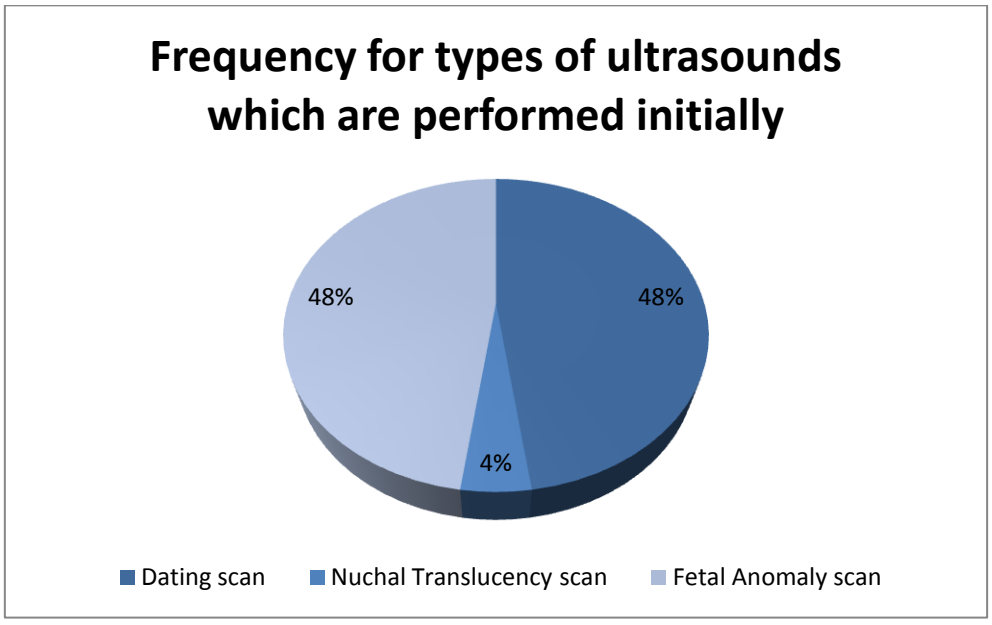
As per objective one, in 90 women it is known when their first ultrasound was done, the mean gestational age of first ultrasound was 19 weeks, the range was from six to 34 weeks.

A dating scan was performed in 43 patients (47.8%) with one of these patients going on to have a nuchal translucency scan as her dating scan was done at six weeks gestation. Fifteen of these patients went on to have an anomaly scans. Nuchal translucency scans were done in four patients (4,4%), one having a dating scan

initially as aforementioned. These three patients went on to have a fetal anomaly scan.

Fetal anomaly scans were done in 43 (47,8%) patients as their initial scan. Thus 61 patients underwent anomaly scans either initially or after other types of ultrasounds.

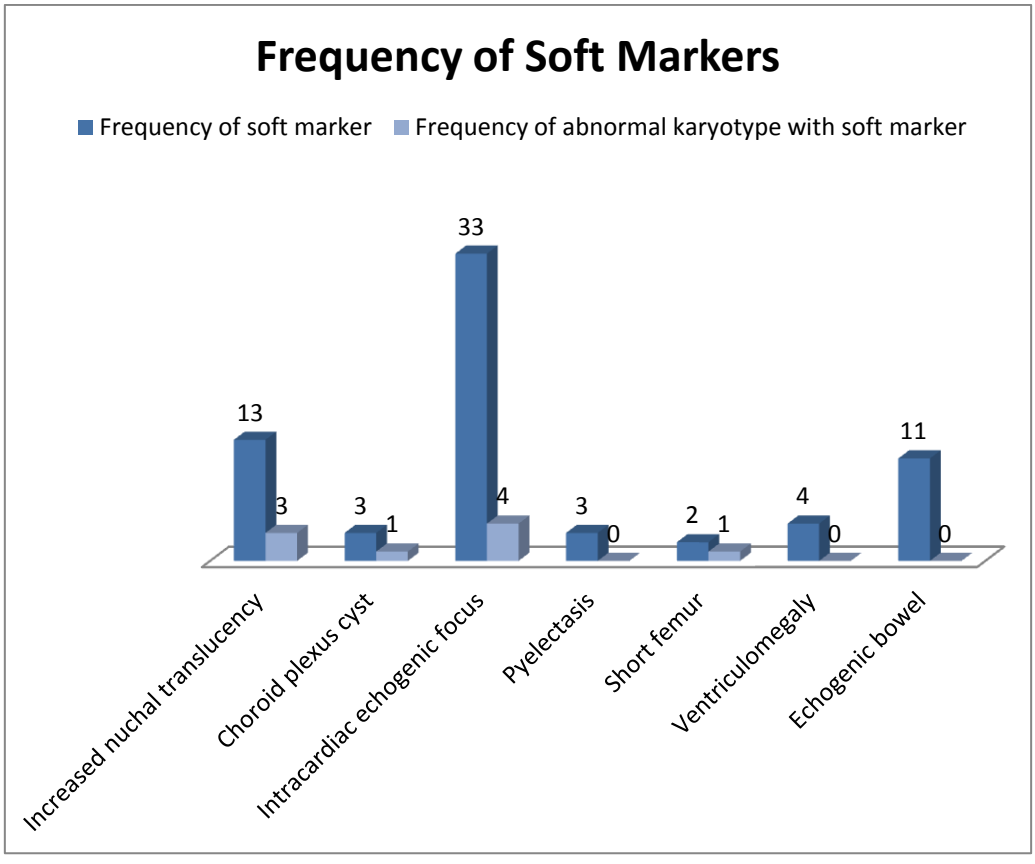
Of the four patients who underwent nuchal translucency scans, three went on to have anomaly scans. One of the patients with an increased nuchal translucency had a CVS that showed Trisomy 21 (Patient B). Of the remaining three patients, one had a normal nuchal translucency and anomaly scan and the indication for invasive prenatal testing was based on advanced maternal age. The second patient had an abnormal nuchal translucency scan with an increased nuchal thickness and subsequently had an amniocentesis at 16 weeks gestation due to this finding. The amniocentesis showed a normal karyotype in the fetus. She had a previous infant affected with pyelectasis. An anomaly scan later in the studied pregnancy did show pyelectasis but she  had a healthy infant at birth. The third patient had an abnormal nuchal translucency scan at 13 weeks and two days and underwent a amniocentesis because of this finding at 16 weeks gestation. The result of the amniocentesis was normal.




**Graph 3.4: Frequency for types of ultrasounds performed initially**

In 60 patients (63%) the abnormal ultrasound findings led to an invasive prenatal test being performed.

The most common soft marker found of all the ultrasounds performed was an echogenic cardiac focus which was found in 33 patients (34,7%). The following distribution of frequency of soft markers were found; intracardiac echogenic focus in 33 patients, increased nuchal fold thickness in thirteen patients, echogenic bowel in eleven patients, ventriculomegaly in four patients, choroid plexus cysts in three patients, pyelectasis in three patients and a short femur in two patients. Thirteen patients had more than one soft marker. In the patients where soft markers were found seven patients had an abnormal karyotype (Patients A, B, C, D, E, G and L) thus 11,6% had abnormal karyotypes. All these patients had multiple soft markers accept patient G who had only one soft marker.




**Graph 3.5: Frequency of Soft Markers**

There were thirteen patients in which structural abnormalities were found in the fetus at ultrasound. A range of major structural anomalies were found on ultrasound namely exomphalos, holoprosencephaly, duodenal atresia, omphalocoele, tracheoesphageal fistula, absent nasal bone, cerebellar vermis agenesis, cleft lip and palate. The most common major structural anomaly found in fetuses was holoprosencephaly followed by duodenal atresia. In five patients both hard and soft markers were found in the fetuses. Nine patients had antenatal major structural anomaly findings in the fetuses confirmed at the birth. Of these eight patients, six patients were also found to have fetuses with abnormal karyotypes with chromosomal abnormalities (Patients A,B,D,E,F and L), one patient with an infant with exomphalos was admitted into neonatal care (Patient 1), one patient with a baby with an omphalocoele and a tracheoesphageal fistula underwent a caesarean section and the infant passed away shortly after birth (Patient 2). One patient with a fetus affected with holoprocencepaly opted for fetocide (Patient 3), one patient with a fetus affected by cerebellar vermis agenesis presented with an IUFD (Patient 4). One of the twelve patients with a fetus affected by the major structural anomaly of holoprosencephaly was lost to follow up. Two of the patients who were thought to have fetuses affected with duodenal atresia antenatally had structurally normal infants at birth. One patient had multiple soft markers on ultrasound but structural abnormalities were found at birth (Patient 6).

### 3.3.2 Advanced Maternal Age

In the sample of 96 women, 58 were of advanced maternal age. The mean age of women in the sample was 35 years. The oldest patient in the sample was 47 years old and the youngest patient was 19 years old. Advanced maternal age was found in six (50%) of the women with chromosomal abnormalities. One patient with structural abnormalities and a normal karyotype was of advanced maternal age. Of the total of 58 advanced maternal women, seven abnormalities were found (chromosomal and structural), thus 12%. The chromosomal abnormalities found were Trisomies most commonly Trisomy 21. Abnormal ultrasound markers were found in 24 (25%) women of advanced maternal age. Six patients of advanced maternal age had a previous history of a chromosomal or structural abnormality, two having a previous infant born

with Trisomy 21 and one with a previous infant born with Trisomy 13. These three patients had normal karyotypes in the pregnancy studied.

There were 2334 patients of advanced maternal age who attended Rahima Moosa Mother and Child Hospital antenatal clinic over the period studied which means 7,9% of the patients who delivered over the study period was of advanced maternal age. Therefore 58 out of 2334 patients underwent invasive testing meaning 2.5% of advanced maternal age mothers were offered invasive prenatal testing. (E Bera 2016, Principal Specialist at RMMCH, oral communication, 30 September 2016)

### 3.3.3 Family History of Chromosomal and Genetic Abnormalities

Twelve patients underwent invasive prenatal testing due to a family or personal history of chromosomal, genetic or structural abnormality. Of these abnormalities three were previous infants with Trisomy 21, one with a previous infant with Trisomy 13, one with a previous infant with pyelectasis, one patient with a previous child with cystic fibrosis (Patient J), one patient with neurofibromatosis, one patient with a previous infant with brain and facial abnormalities as well as a known balanced translocation in her partner (Patient I), one patient with an infant death due to congenital heart disease, one with a previous infant with spinal muscular atrophy (Patient K), one patient with an unexplained stillbirth at term but also known with gestational diabetes and one with a sister with an infant with ectrodactyly and missing right hand metacarpals (Patient C).

Seven of the twelve patients were of advanced maternal age and five of the twelve patients had abnormal ultrasound findings.

Four of patients had  fetuses with abnormal karyotypes namely patients C, I, J and K. Of these patients only patient C had abnormal ultrasound findings.

### 3.3.4 Positive Biochemical Screening Tests

Two patients were transferred from the private sector for invasive prenatal testing due to an abnormal first trimester screening test. This was a 36 year old P4G5 with an increased first trimester Trisomy 21 risk. She had no previous history of

abnormalities and a normal ultrasound. The patient underwent a second trimester amniocentesis that was found to be normal. She delivered a healthy infant at 31 weeks gestation via normal vaginal delivery. The reason for her premature labour is unknown.

The second patient was Patient C as discussed above (pg. 45)

### 3.3.5 Other Indications for Invasive Prenatal Testing

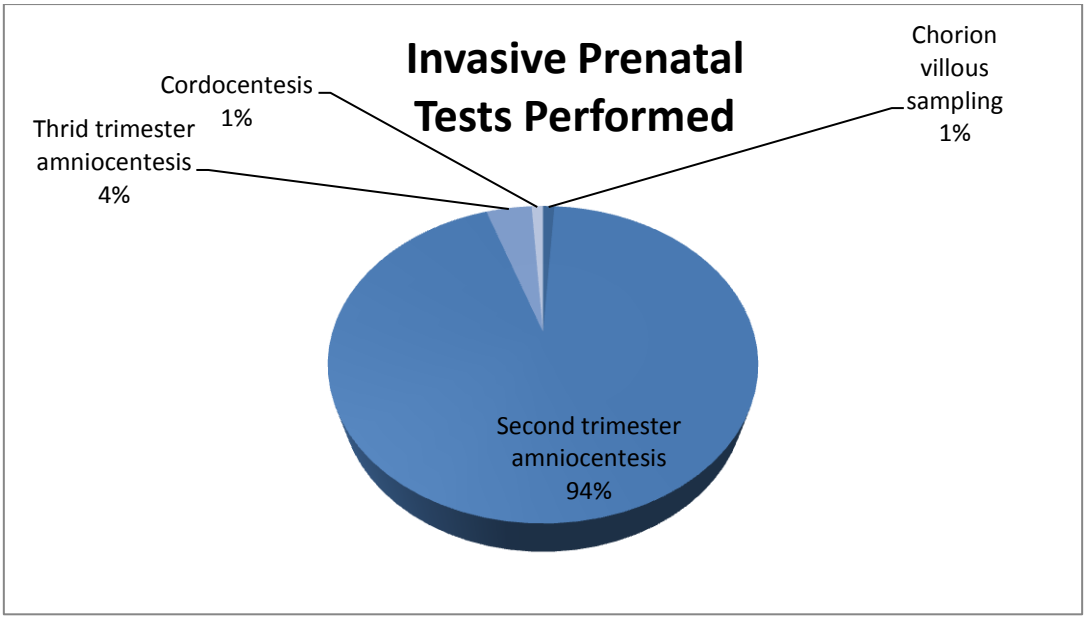
A 26 year old patient underwent second trimester amniocentesis as she had suffered several first trimester and second trimester miscarriages as well as an IUFD. On ultrasound severe intrauterine growth restriction was found. Her amniocentesis result was normal but unfortunately the outcome of her pregnancy is unknown as she was lost to follow up.

There were two patients who requested invasive prenatal testing. These patients were Patient I and J as discussed above.

## 3.4 INVASIVE PRENATAL TESTS PERFORMED

There were 97 invasive prenatal tests performed with 96 results being obtained. One second trimester amniocentesis result was lost and cannot be found on the National Health Laboratory Service system. CVS was done in one patient in the first trimester, second trimester amniocenteses was done in 90 patients, four patients underwent third trimester amniocentesis and one patient underwent cordocentesis at 30 weeks gestation. No first trimester amniocentesis were done.

The gestation at which second trimester amniocentesis was done is known in 86 patients with a mean gestation of second trimester amniocentesis is 21 weeks, and a range from 16 weeks to 27 weeks.




**Graph 3.6: Invasive Prenatal Tests Performed**

The CVS was done in a 45 year old P3G7 who booked in the first trimester and underwent a nuchal translucency scan at 13 weeks gestation that showed an increased nuchal translucency and an absent nasal bone. The patient underwent a CVS that confirmed Trisomy 21. The patient opted for a termination of pregnancy in the first trimester (Patient B).

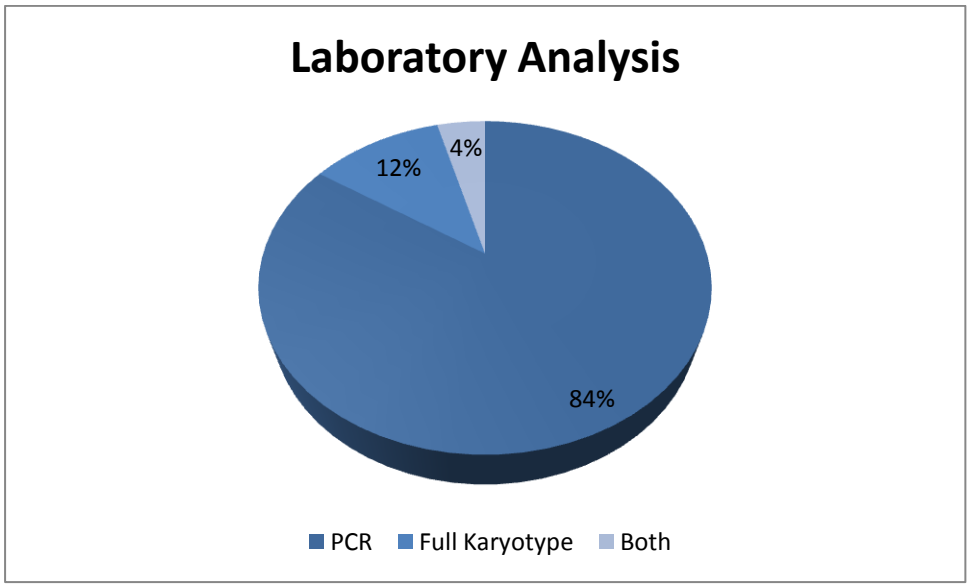
Third trimester amniocentesis was done in four patients. The first patient had a dating scan at 32 weeks gestation that showed several soft and major structural anomalies, she had a normal karyotype subsequently. The second patient was a 24 year old P1G2 who had multiple abnormalities found on fetal anomaly scan and underwent an amniocentesis at 27 weeks gestation, the fetus consequently had a normal karyotype. The third patient who underwent a third trimester amniocentesis was a 21 year of P0G1 who underwent an amniocentesis at 34 weeks gestation due to suspected duodenal atresia found on ultrasound. Her fetus had a normal karyotype and she delivered a normal infant at birth. The fourth patient with a third trimester amniocentesis was a 23 year old P0G1 who had the procedure at 31 weeks gestation due to multiple structural abnormalities seen on ultrasound. Her fetus had a normal karyotype, her infant was born confirming the antenatal structural abnormalities passing away shortly after birth (Patient 2).

A cordocentesis was done for Patient 3 as discussed previously. (pg 43)

### 3.5 CYTOGENETIC DIAGNOSIS MADE (Objective 2)

#### 3.5.1 Laboratory Analysis of Results

As per objective two, laboratory analysis of the 96 known invasive tests included 81(84%) PCR results, 11(12%) full karyotypes and four (4%) instances where both PCR and full karyotyping was done.




**Graph 3.7: Laboratory Analysis of Results**

#### 3.5.2 Sexing Errors and Culture Errors

There were two invasive prenatal test results that did not include sexing. One was done for a diagnosis of cystic fibrosis where an Elucigene CF30 kit was used for genetic diagnosis and not conventional karyotyping or PCR. The other for a diagnosis of spinal muscular atrophy where a multiplex ligation-dependent probe amplification analysis was used which tested for spinal muscular atrophy. Neonatal outcomes of 65 patients in the study are known and no sexing errors were found. No instances of culture failure could be found. No cases of mosaicism or pseudomosaicism were found.

### 3.6 COMPLICATIONS OF INVASIVE PRENATAL TESTING (Objective 3)

As per objective three, only one case of miscarriage after a second trimester amniocentesis was found. This patient was a 36 year old patient who had had two previous second trimester miscarriages that were thought to be due to cervical incompetence. She underwent a amniocentesis at 22 weeks due to her advanced maternal age and an echogenic intracardiac focus found at ultrasound. She had a spontaneous complete miscarriage of a 550g infant two days after the amniocentesis was done. The amniocentesis procedure was uneventful and showed a normal karyotype.

The outcome of the pregnancy in 65 patients (68%) was known, thus the miscarriage rate is 1,5%.

No other complications due to the procedure were found in the study.

### 3.7 PREGNANCY OUTCOMES (Objective 4)

As per objective four, in the sample of 97, the outcomes of 65 (68%) of the pregnancies are known.

Included are two cases of termination of pregnancy that were directly related to the result of the invasive prenatal test. Both terminations of pregnancy were done for confirmed Trisomy 21 fetuses, one in the first trimester and one in the second trimester (Patients A and B)

There was one case of elective fetocide directly related to prenatal testing. A case of a fetus affected with Wolf-Hirschorn syndrome where a fetocide was done at 30 weeks gestation (Patient L). There was another case of fetocide of a infant affected by holoprosencephaly where a fetocide was done at 30 weeks gestation, this was done as a direct result of antenatal ultrasound diagnosis and not prenatal testing. (Patient 3).

There was one case of intrauterine fetal death. This was a case of a woman where multiple ultrasound abnormalities were found including agenesis of the cerebellar vermis and cleft lip and palate. She presented with an IUFD at 37 weeks gestation and underwent an induction of labour (Patient 4).

After excluding chromosomal and structural abnormalities, 84 pregnancies with normal karyotypes were traced. Pregnancy outcome of 65 of the cohort of 96 patients are known, 52 of these pregnancies had infants with normal phenotypic appearances at birth, eight patients had features of chromosomal abnormalities as diagnosed prenatally and five patients had features of structural abnormalities that were diagnosed prenatally.

# 4. DISCUSSION

## 4.1 INTRODUCTION

Invasive prenatal testing has been available in the public sector in South Africa since 1994 (40). The review of this service at Rahima Moosa Mother and Child hospital has highlighted several successes and shortcomings of such a service being run in a resource restricted setting.

## 4.2 PATIENT POPULATON DEMOGRAPHICS

### 4.2.1 Antenatal care - Booking and referrals

Antenatal care aims to provide the identification of avoidable factors to provide a normal pregnancy and delivery of a healthy infant with the least possible morbidity (40). Patients tend to book late in their pregnancies, the average gestational age of booking in the study was 16 weeks, with 61 of the 62 patients in which this information is known booking before 24 weeks gestation. Myer et al. showed in a study done in rural South Africa that women generally do not perceive antenatal care as important as pregnancy is not perceived to carry risk but showed that there is a perception that labour and delivery carries the risk. They found that women mainly went for one antenatal clinic visit to receive an antenatal card to present at delivery (102). Peripheral clinics are also referring patients late, this was also found by Watcham et al (40). This creates a situation where the opportunity for invasive prenatal screening and testing especially in the first trimester is missed.

In the government sector ultrasound is one of the methods available for screening due to the cost related to other screening options. The literature has shown that this is a method of screening that can be used in association with other screening tests to give an associated risk and both first trimester and second trimester markers can be associated with chromosomal abnormalities (12). During the first trimester ultrasound the measurement of nuchal translucency has been shown to identify 75% of fetuses affected by Trisomy 21 with a 5% false positive rate. Another marker of Trisomy 21 in the first trimester ultrasound is the absence of the nasal bone. When

maternal age, nuchal translucency and nasal bone measurement are combined it has a detection rate of 95% with a false positive rate of 5% (7,9,10). By booking late or being referred late the patient also misses the opportunity of a screening ultrasound.

All of this highlights the lack of understanding and education in the community of the importance of antenatal care and the lack of knowledge by referring healthcare professionals of the importance of prenatal diagnosis in a high risk population. As the average age of booking was found to be 16 weeks and all the patients except one booked before 24 weeks there is potentially a missed opportunity as all patients could not have a fetal anomaly scan. The gestation at booking for all the patients who delivered in this time period has not been examined.

### 4.2.2 Ethnicity

There were chromosomal abnormalities found in all ethnic groups in the sample with the most being found in the Black community. Most structural abnormalities with normal karyotypes were found in the Black community. This may be explained by the fact the Black community comprised the largest component of the sample at 71,9% Venter et al. concluded in their study that the incidence of congenital anomalies in black South African neonates were comparable to the incidence in that of other First and Third world countries (49).

Inferences of incidence of congenital anomalies in different ethnic groups cannot be drawn in this study cohort due to the small study sample, bigger population based studies are needed.

### 4.2.3 HIV and invasive prenatal testing

South Africa has a large burden of HIV and 29,7% of pregnant women were infected with HIV in 2013 (94,95). In the study cohort seventeen patients were found to be HIV positive at the time of an invasive prenatal test being performed.

HIV transmission during invasive prenatal testing is thought not to be a risk provided that the mother is on antiretroviral therapy and has a low viral load (86–89,91)

One patient was not on antiretroviral therapy as she had defaulted her treatment. It is advisable that a patient be on antiretroviral therapy for 4-6weeks before an invasive procedure is performed as this will allow for adequate viral suppression (96). Of the sixteen patients on antiretroviral therapy it is known that six started their antiretroviral treatment at the time of booking, this means that the minimum duration that the patients were on antiretroviral therapy before their invasive prenatal test ranged between one week to five week. Five patients started their antiretroviral therapy before booking. This indicates that patients might not have had a adequate duration of antiretroviral treatment before their invasive prenatal tests were done.

Of the patients with known viral loads three had lower than detectable viral loads and two had viral loads of 1910IU/ml and 862IU/ml. The RCOG recommends that the viral load of a patient be undetectable before and invasive procedure is done (93). In the last two patients mentioned it is not known what the viral loads were at the time of invasive testing as the VL test was performed after the invasive procedure in one patient and it is not known when the viral load was done in relation to the amniocentesis in the second patient. Guidelines state that viral load of a patient should be done before the invasive test is done and that this is a part of counselling for the invasive test (96).

The implementation of first trimester screening can limit the amount of invasive tests needed in a country like South Africa where the burden of HIV is high and resources for VL testing might not be available. This is also supported by Naidoo et al. in their local study as well as Constantatos et al. (15,94).

In summary HIV transmission must be taken into consideration when an invasive prenatal test is performed. The patient must be on antiretroviral therapy and have a known suppressed viral load. Most importantly the patient must be counselled on the risk of transmission as part of informed consent for the procedure (96). We live in an era where HIV testing and antiretroviral therapy is freely available and every patient has the opportunity to be virally suppressed and undergo an invasive prenatal test safely. Serious ethical and medico-legal issues can be the consequence if there is no attention paid to a patient's HIV status.

## 4.3 INDICATIONS FOR PRENATAL TESTING

Different settings in the world have different indications for invasive prenatal testing, notably in resource restricted settings and third world countries advanced maternal age remains the mainstay of screening where in the first world abnormal serum markers and abnormal ultrasound findings are much more common indications for invasive testing (25,33,35,36). In the setting of this study abnormal ultrasound findings (63%) was the most common indication for invasive testing with advanced maternal age (60%) being a close second.

As mentioned above the greatest indications for invasive prenatal testing are abnormal ultrasound findings and advanced maternal age. In the modern era of fetal medicine there are many advances in non invasive prenatal testing and biochemical markers to detect chromosomal abnormalities. It has been shown that a combination of maternal age, nuchal translucency, nasal bone an biochemical markers in the first trimester have a detection rate of 95% and a 5% false positive rate for Trisomy 21 (7).

In the study population only two patients underwent biochemical screening that led to invasive prenatal testing. Both these patients were referred from the private sector and had had positive first trimester screening tests. This highlights the outdated system of risk assessment and screening of patients needing invasive prenatal testing, which is mainly based on advanced maternal age and ultrasound findings. It would greatly benefit the pregnant population in the public sector in South Africa if either combined first trimester screening service by accredited personnel or second trimester screening was made available to them. As resources are limited the cost to benefit ratio must be taken into consideration before such a service is implemented. Naidoo et al. did however show that the use of nuchal translucency and maternal age as a screening protocol were equivalent to international standards (15). This protocol of course has the caveat that patients need to book early enough to have a nuchal translucency scan.

A very encouraging finding in the study is the that there were eleven patients who underwent invasive prenatal testing due to a family history of chromosomal or genetic abnormalities and that there were two patients who requested invasive prenatal testing (both having a previous history of a chromosomal abnormality). In

the literature Pala et al. found a 10% rate of maternal request in their study but in most studies maternal request is either a very small component of the indications for invasive testing or not mentioned at all (36).

Although these numbers are small it is encouraging that the community and the referring healthcare workers show understanding and knowledge for other indications for invasive prenatal testing.

Non invasive prenatal testing (NIPT) was not done in any patients in the cohort as this service is not available in the public sector in South Africa (19). The ACOG do currently recommend that conventional screening methods remain the most appropriate choice for most women in the general population but does recommend it being done in certain high risk populations (2,17). A population can be divided into high risk (1:100 or lower), intermediate risk (1:101-1:2500) and low risk (<1:2500), NIPT should be offered to patients in the intermediate risk category. Some studies suggest that NIPT should be offered in a patient with a risk of 1:101-1:2500 (20,21).

### 4.3.1 Abnormal Ultrasound Findings

As previously discussed the most common indication for invasive prenatal testing was abnormalities found on ultrasound. The most common ultrasound that was performed initially was a dating scan, dating scans are generally opportunistic ultrasounds done when patients present to RMMCH antenatal clinic and may be performed by registrars in the department or by the ultrasound department. If the dating scan is early enough an anomaly scan will be booked. Anomaly scans were done in 59 patients and this can be explained by the mean gestational age at first ultrasound being late, at nineteen weeks gestation. This advance gestation at ultrasound is most probably a combination of late booking by patients and late referral by surrounding clinics. But due to the average age of booking being 16 weeks gestation it may be that late referral plays a bigger role as well as availability of trained staff to do anomaly scans. In seven of the patients where soft markers were found there was also a chromosomal abnormality found. Six of these patients had multiple soft makers, the literature supports this in that multiple soft markers have a cumulative effect and will increase a patient's risk of having an aneuploidy (14).

Thirteen of the patients in the cohort had so called major structural anomalies that alluded to possible structural abnormalities at birth, ten of these patients had structural abnormalities confirmed at birth (six having a concomitant chromosomal abnormality). This is supported by the literature where the risk of aneuploidy increases with structural abnormalities found (13).

### 4.3.2 Advanced Maternal Age

Women with advanced maternal age are at increased risk of delivering an infant with a chromosomal or congenital abnormalities (40,103). In South Africa 13,4% of pregnant women fall into the advanced maternal age category (46). The study showed 60% of women were of advanced maternal age. Of the patients with fetuses with either chromosomal and/or structural abnormalities 7 were found to be of advanced maternal age. It is well known from the literature that this is a important risk factor that needs to be taken into account when screening is done (39,40,43). But it is inadequate to use it as an independent risk factor with the advances in fetal medicine and other screening methods. It is still an important component of prenatal testing but independently advanced maternal age has a 50% detection rate for Trisomy 21 with a 15% false positive rate which shows it needs to be used in combination with other screening tests (7). The high number of AMA patients is one of the reasons the results are biased towards an increased rate of abnormalities.

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### 4.3.3 Chromosomal abnormalities

In the study cohort twelve patients (12,5%) were identified with chromosomal abnormalities in comparison to international studies which range from 2-10% (25,33,36,54). The high rate is most probably due to the fact that the study population was small and biased towards more high risk individuals.

The Perinatal Problem Identification Program (PIPP) in 2014 showed that congenital abnormalities is the third leading cause of early neonatal death in South Africa (52), thus highlighting the importance detecting chromosomal abnormalities prenatally. In most studies the most common chromosomal abnormality detected was Trisomy 21 (25,48,54). In this study Trisomies were the most common chromosomal abnormality

found with Trisomy 21, Trisomy 18 and Trisomy 13 found in equal measures (six patients were found to have fetuses affected by Trisomies, two with fetuses affected by Trisomy 21, two with Trisomy 18 and two with Trisomy 13). In the patients who were of advanced maternal age with fetuses affected by chromosomal abnormalites two had fetuses affected with Trisomy 21, one fetus was affected with Trisomy 18 and one fetus with Trisomy 13.

Klinefelter syndrome was found in two fetuses of mothers who underwent invasive prenatal testing. One test was done due to advanced maternal age with no abnormalities on ultrasound and one test was done due to an echogenic focus in the left ventricle and fetal growth being on the 5$^{th}$ centile on ultrasound . Both infants were healthy looking at birth. This diagnosis is usually incidental and not due to ultrasound abnormalities or abnormalities found at delivery of the infant. Gruchy et al. showed that making the diagnosis is important and can provide information to the expecting couple in terms of treatment options for the infant. These patients usually present with infertility thus infertility treatments can be offered earlier to the affected individual. Delayed language development can also be seen and can be corrected by early speech therapy. Their study also showed that providing adequate counselling to the couple at the time of diagnosis and treatment options decreased the rate of termination of pregnancy and that although it is mostly an incidental diagnosis it is an important one to make (104).

It has been shown that the widespread implementation of prenatal screening combined with prenatal diagnosis and termination of pregnancy services substantially reduced the expected number of infants born with Trisomy 21 (5). Delport, Kromberg and Christianson et al. showed that Trisomy 21 is a important condition in South Africa. (47,48,50).

## 4.4 INVASIVE PRENATAL TESTS PERFORMED

Second trimester amniocentesis was the most common invasive prenatal test performed in the study population and it can be concluded that the trend of late booking and the high number of second trimester fetal anomaly scans led to this. The average gestation at which ultrasound was performed was 19 weeks. Second trimester amniocentesis is accepted in the literature as the method most easily

performed among the invasive diagnostic methods (27). Third trimester amniocentesis was done in four patients where structural abnormalities were found at late gestations. It can be concluded that in view of the severity of the ultrasound findings an invasive test was a reasonable offer despite the advanced gestation . This might have aided patients to assist in counselling on prognosis and to help in the future when determining risk of recurrence.

Only one patient in the cohort underwent a CVS and this was due to abnormalities found on a nuchal translucency scan. There were two instances that abnormalities were found on a nuchal translucency scan but a second trimester amniocentesis was opted for later on during the patients' pregnancies. There may be several reasons for this, both these patients underwent their nuchal translucency scan at thirteen weeks gestation which may have necessitated a second trimester amniocentesis due appointment for invasive testing only being available in their second trimester. It may also have been due to the patients choice of waiting for a second trimester ultrasound and the spontaneous miscarriage risk of CVS being higher than that of second trimester amniocentesis (1), or that there were no personnel skilled in CVS available at the time of their abnormal nuchal translucency scan.

There was one case where a cordocentesis was performed in a patient who had a late diagnosis of an fetus affected by holoprosencephaly on ultrasound. Literature dictates that cordocentesis is rarely performed with the availability of other invasive prenatal testing techniques that are easier to perform and safer (16,24). This patient also underwent a fetocide at the same time as her cordocentesis and this might be the reason this type of test was opted for.

### 4.5 CYTOGENETIC TESTS PERFORMED

The most common test performed for cytogenetic diagnosis was a PCR, this might be explained by this test overcoming the need for culture of fetal cells, making it more cost effective with a rapid turn over time. It is also fully automated (65). In a resource limited setting a cost effective automated test is of great value.

## 4.6 COMPLICATIONS OF INVASIVE PRENATAL TESTING

Spontaneous abortion as a complication of invasive prenatal procedure was found in one patient who underwent a second trimester amniocentesis. Thus a spontaneous abortion rate of 1.5% was found in the cohort, the patient however did have cervical incompetence which might have been a confounding factor. The rate of 1,5% is higher than the 0.5-1% quoted by Tabor et al (14). While the Danish Fetal Medicine Study Group found that CVS and amniocentesis do not significantly increase miscarriage rates and the fetal loss rate due to invasive procedure should be very low.(58) This information is helpful in terms of counselling as one can provide an institutionally specific rate for invasive prenatal procedures at RMMCH, however the sample size is small and there is missing data.

## 4.7 PREGNANCY OUTCOMES

It can be seen from the results that management options were conservative in terms of an abnormal invasive test result and that respect for patient choices exists as there are several instances of termination of pregnancy and fetocide but also several instances where termination of pregnancy was declined or where fetocide was declined. In these instances the Rahima Moosa Mother and Child team continued the care of the mother and fetus and provided paediatric support where indicated at birth. This is of upmost importance in a setting where many different cultures and beliefs come into play.

The SOGC showed that it is a common misconception that termination of pregnancy is only offered if a patient will undergo termination of pregnancy (98). Clark et al. showed in their study that the decision of declining termination of pregnancy must be supported once the patient has been adequately counselled and a multidisciplinary team approach must be followed (99). Counselling for the patient to make an informed decision about their pregnancy once a result of an abnormal invasive test is given remains one of the most important aspects of antenatal care in the setting of prenatal testing (24) .

## 4.8 RECORD KEEPING AND LOSS TO FOLLOW UP

Record keeping was probably the greatest flaw uncovered by this review. The ultrasound department kept a very good record of the invasive prenatal tests performed and the National Health Laboratory Service had only one patient where the result of the invasive prenatal test was lost. The problem arises from the records department where of the 97 patients who underwent invasive prenatal testing only 65 (67%) patient charts could be located. This led to only 67% of pregnancy outcomes being known and several outcomes of babies where abnormalities were identified were lost to follow up. Many pieces of vital information including gestational age at booking and booking bloods could thus not be included in the study. It must be noted that some of the patients might have been from other provinces and countries and might have travelled home for further antenatal care and delivery, thus their data would not have been available. Furthermore the ultrasound department does not keep a duplicate record of all the ultrasounds performed for a patient as these records are kept within the patient's chart. It can thus be seen if the patient's chart is missing that this vital information pertaining to their ultrasound findings will also be missing. It must be commended that most indications leading to invasive prenatal testing as well as the most important findings at ultrasound were indicated in the invasive prenatal procedure book.

## 4.9 INVASIVE PRENATAL TESTING SERVICE

It is difficult to ascertain whether this service is adequately provided to the patient population that Rahima Moosa Mother and Child Hospital serves as not all patients who deliver at the hospital attend the hospital's antenatal clinic. There were 29380 deliveries during the study period but only 97 invasive procedures were done. Because not all of these patient underwent antenatal care at the institution it is difficult to deduce whether the number of invasive prenatal tests is reasonable for the number of deliveries done.

Of the 2334 advanced maternal age women who attended Rahima Moosa Mother and Child hospital's antenatal clinic only 58 (2,5%) underwent invasive prenatal testing. This finding is very concerning and may be due to patients not being counselled and referred appropriately to have invasive prenatal testing. This may be

due to lack of primary healthcare clinics referring patients of advanced maternal age, late booking of patients thus missing the opportunity of genetic counselling and prenatal testing and lack of knowledge and education by healthcare professionals in the primary and academic settings for the need of prenatal testing. This finding is similar to the that of Watchman et al. where it was shown that patients were not being offered invasive prenatal testing although the service is available. They mainly found that it was a lack of referral by primary healthcare clinics to academic institutions and lack of academic institutions acting on referrals in a timely manner that lead to advanced maternal age women not being offered genetic counselling and invasive prenatal testing (40).

Another factor that has to be taken into consideration is the increasing amount of medico-legal issues that surround pregnancy and prenatal screening. It is thus of great importance to have such a service in the public sector and to increase its capacity. Although the ACOG recommend that all women should have the option of invasive testing, regardless of maternal age (24), this is not feasible in a resource restricted setting as RMMCH.

### 4.10 LIMITATIONS

The small sample size of 97 patients is limiting in that it is a small number from which important rates such as spontaneous miscarriage rate need to be worked out. This number is further compounded by the loss to follow up of patients due to poor record keeping. There were 32 patients (33%) lost to follow up due to files being lost or patients delivering elsewhere and vital data thus being lost. This may lead to an under or overestimation of various results including the complication rate.

An example of the above discussion is the abortion rate of 1,5%, this is possibly an overestimation looking at the 33% loss to follow up rate.

The results in terms of rates of abnormalities are not generlisable to the entire antenatal population, due to the afore-mentioned selection bias of higher risk individuals.

## 4.11 RECOMMENDATIONS

Late booking of patients decreases the rate of early detection of abnormalities on sonar and referral for other indications for invasive prenatal testing thus decreasing the use of CVS which is the first choice for first trimester invasive prenatal testing. It also limits the adequate use of genetic counselling services to prepare patients for invasive prenatal testing and the possible diagnosis of an aneuploidy in the fetus. Education on referral of high risk patients and referral protocols must be put in place. Emphasis must also be placed on early referral of patients at risk of chromosomal abnormalities as this will allow time for first trimester ultrasound, planning of invasive prenatal testing and adequate genetic counselling. Better orientation of staff at all levels to services like antenatal ultrasound, genetic counselling clinic and invasive prenatal testing being available is needed.

As previously discussed patients of advanced maternal age are not adequately referred for risk assessment and counselling regarding the option of invasive testing at Rahima Moosa Mother and Child Hospital. Further education is needed on the indications for invasive testing and that invasive prenatal testing does not only test for common Trisomies. Indications like families affected by previous chromosomal abnormalities must be highlighted.

Records should be kept of the patients undergoing invasive prenatal testing where there is a copy of their patient chart with the outcome of their pregnancy within the ultrasound department as this will ease the audit of the invasive prenatal testing services in the future. It is also recommended that the ultrasound department keep a separate record of all ultrasounds performed in the event that a patient's chart is lost.

Some of the outcomes of pregnancy were possibly not known in this cohort due to patients delivering in other facilities, provinces and countries. The hand held antenatal card goes with the patient wherever she undergoes antenatal care and delivery. A digital system recording the patient's antenatal care and pregnancy outcome which can be accessed nationally would greatly help the continuity of care of patients.

It must be brought up at a provincial and national level that there are many advances in fetal medicine. It would greatly benefit the pregnant population in the public sector in South Africa if either combined first trimester screening by accredited

personnel or second trimester screening was made available to them. Currently screening is opportunistic and not universal due to the inequitable distribution of resources. Better referral systems and increasing capacitance at health facilities is required as well as investment in skills training and equipment.

It is of great importance that HIV positive women undergoing invasive prenatal testing be well informed about the risk of transmission, the woman should be on antiretroviral therapy for an adequate duration and should be virally suppressed. Viral load results must be known before the procedure is done.

Rhesus negative women must be given anti-D after their invasive prenatal procedure, although there were six patients who were Rhesus negative and underwent invasive prenatal testing there were no notes in their records of anti-D being given.

## 4.12 CONCLUSION

The review of invasive prenatal testing at Rahima Moosa Mother and Child Hospital is of value as it has highlighted several recommendations that will better the service in the future.

There was a 12,5% rate of chromosomal abnormalities diagnosed with a spontaneous miscarriage rate of 1,5%. This shows that the procedure is beneficial in assisting the healthcare workers to better manage the patient and her pregnancy. Both for those with an abnormal finding and reassuring those with normal results. Although the miscarriage rate is higher than internationally quoted due to the small sample size; it translates to only one spontaneous miscarriage occurring in the cohort. The service appears safe in the hands of the Rahima Moosa Mother and Child team. The most common indication for invasive prenatal testing was abnormalities found at ultrasound followed by advanced maternal age. This highlights that a good ultrasound service is available at the hospital. Also that although advanced maternal age is a known risk factor for chromosomal abnormalities it is seen as an independent risk factor and this needs further education of healthcare professionals. Unfortunately there was a 33% loss to follow

up in the sample. This loss to follow up challenges the strength of any conclusions that might be made by the study.

It can be argued that in a resource limited setting where malnutrition and infectious disease play such a big role and take precedence, an invasive prenatal testing service should not be at the forefront of the services. However it must be remembered that the birth of a child with abnormalities can place tremendous burden on an already impoverished family. Thus giving such a family a choice of knowing prenatally of a child with abnormalities is vital..

Invasive prenatal testing remains the gold standard for conclusive prenatal diagnosis and it decreases the burden of disease to the affected individual and their family. As well as giving new parents time to prepare for the birth of their infant. The study shows that an invasive testing service can be successfully run in a resource restricted setting but ongoing education of the availability of the service in the public sector is needed. It is important to better the services provided in the government sector to match the international progression in fetal medicine and invasive prenatal diagnosis as all our patients deserve optimal care during their pregnancies for their unborn infants.

## APPENDIX A

**Data collection sheet**

Study number:_____

***Baseline information***

Age:_____Ethnicity:_____

Parity:_____Gravidity:_____

Chronic illnesses:

| Diabetes | Hypertension | Epilepsy | Cardiac disease | TB | Thyroid Disease | Other |
|---|---|---|---|---|---|---|
| | | | | | | |

Medication use:

| Yes | No | Unknown | If yes, type of medication: |
|---|---|---|---|
| | | | |

Smoking :

| Yes | No | Unknown |
|---|---|---|
| | | |

Alcohol use:

| Yes | No | Unknown |
|---|---|---|
| | | |

Illicit drug use:

| Yes | No | If yes, type of drug: |
|---|---|---|
| | | |

***Current pregnancy: (pregnancy in which amniocentesis was done)***

Rh:_____(antibodies if negative:_____RPR:_____Hb:_____

RVD:_____CD4:_____VL:_____Treatment:_____Duration:_____

Gestational age at booking:_____

***Previous pregnancies:***

Previous pregnancy outcomes:

| YEAR | MODE | GA AT DELIVERY | SEX | LIVE BIRTH, STILLBIRTH, MISCARRIAGE OR TOP | COMPLICATIONS |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
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| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

Previous diagnosis of chromosomal or structural abnormalities:

| YEAR | DIAGOSIS |
|---|---|
| | |
| | |
| | |
| | |
| | |

Family History of chromosomal, genetic or structural abnormalities:

| Yes | No | Unknown/not recorded | If yes, type of abnormality: |
|---|---|---|---|
| | | | |

***Ultrasound findings:***

Gestation at first ultrasound :_____

Singleton/Multiple gestation

Type of ultrasound:

| DATING | NT | FETAL ANOMALY | REVIEW SCAN |
|---|---|---|---|
| | | | |

Detail of scans:

Gestation at ultrasound:_____

Details of findings:_____
_____
_____
_____
_____
_____
_____

Gestation at ultrasound:_____

Details of findings:_____
_____
_____
_____
_____
_____
_____

Gestation at ultrasound:_____

Details of findings:_____
_____
_____
_____
_____
_____
_____

Did ultrasound finding prompt prenatal invasive test:

| Yes | No | If yes, details of findings: |
|---|---|---|
| | | |

**Invasive prenatal test:**

| | | |
|---|---|---|
| Chorionic villous sampling | Amniocentesis | Cordocentesis |

Gestation:_____

Indication:

| | | | | |
|---|---|---|---|---|
| Advanced maternal age | Abnormal ultrasound finding | Family History | Maternal request | |

Karyotype result:

| |
|---|
| |

**Complications related to procedure:**

| | | | | |
|---|---|---|---|---|
| Misciarriage | Amniotic fluid leak | Preterm labour | infection | Other |

***Pregnancy outcome:***

Misciarriage:

| | | |
|---|---|---|
| Yes | No | If yes, gestation |

TOP:

| Yes | No | If yes, gestation and reason |
|---|---|---|
| | | |

At Birth

| Date | Mode | Weight | Sex | Gestational age | Apgars | Complications |
|---|---|---|---|---|---|---|
| | | | | | | |

If abnormalities detected at birth, genetic follow up:_____

# APPENDIX B

## Approval letter from the University of the Witwatersrand Human Resources Ethics Committee

R14/49 Dr Chrysanthi Georgiou

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)**

**CLEARANCE CERTIFICATE NO. M160828**

| | |
|---|---|
| **NAME: (Principal Investigator)** | Dr Chrysanthi Georgiou |
| **DEPARTMENT:** | Obstetrics and Gynaecology<br>Rahima Moosa Mother and Child Hospital |
| **PROJECT TITLE:** | A Review of Invasive Prenatal Testing at Rahima Moosa Mother and Child Hospital |
| **DATE CONSIDERED:** | 26/08/2016 |
| **DECISION:** | Approved unconditionally |
| **CONDITIONS:** | |
| **SUPERVISOR:** | Prof Hendrik Lombaard and Dr Amy Wise |
| **APPROVED BY:** | Professor P Cleaton-Jones, Chairperson, HREC (Medical) |
| **DATE OF APPROVAL:** | 19/08/2016 |

**This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.**

**DECLARATION OF INVESTIGATORS**

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/2nd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was ir..tially reviewed in August and will therefore be due in the month of August each year.

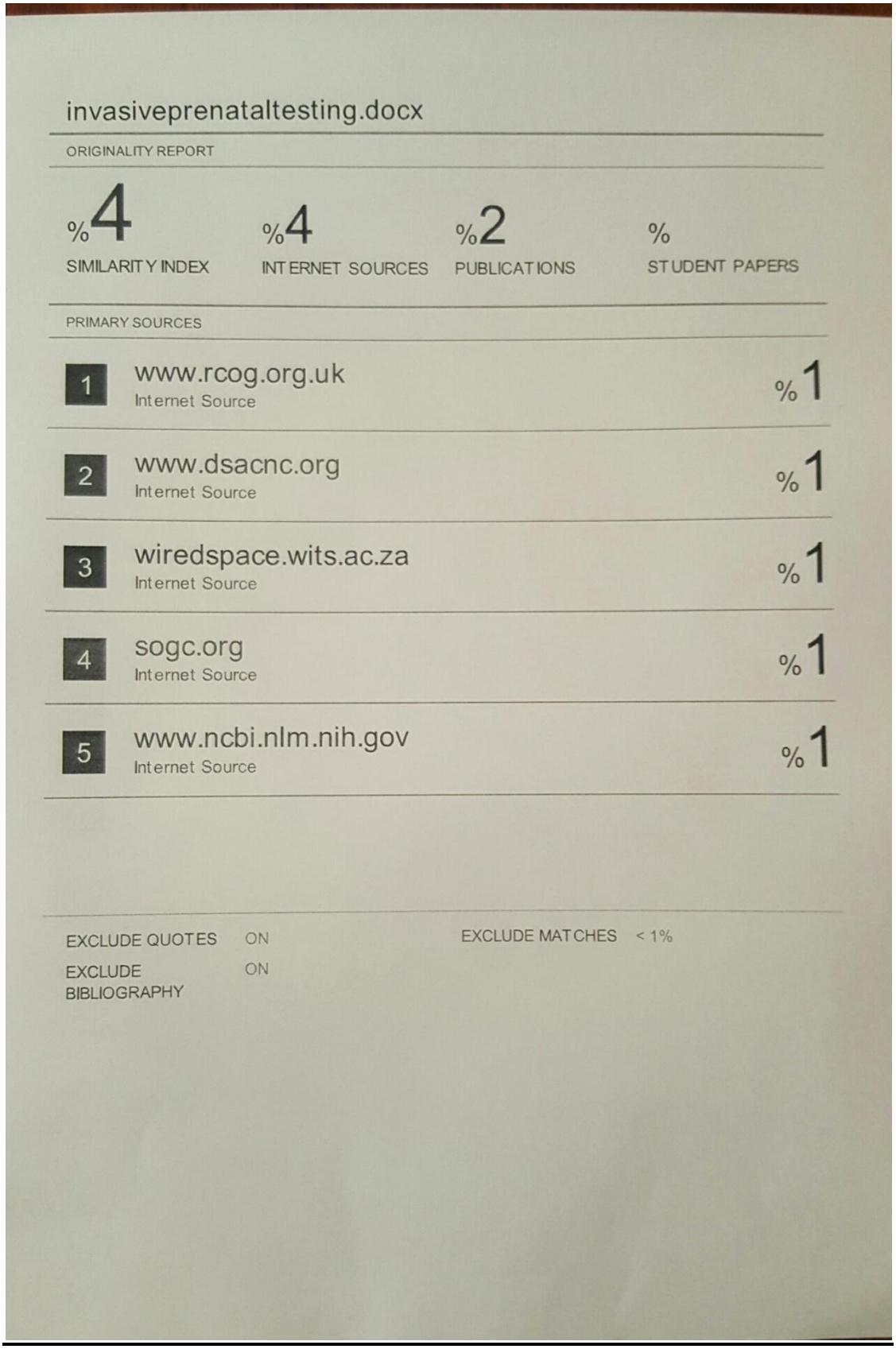
Principal Investigator Signature

Date 16/9/2016

**PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES**

## APPENDIX C

### Turnitin Report



invasiveprenataltesting.docx

ORIGINALITY REPORT

| %4 | %4 | %2 | % |
|---|---|---|---|
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | www.rcog.org.uk<br>Internet Source | %1 |
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## REFERENCES


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