Uptake rate of prenatal genetic screening and invasive testing in pregnant women over the age of 37 in Tygerberg Hospital and factors influencing this.

Thesis presented in fulfilment of the requirements for the Degree of Master of Medicine: Obstetrics & Gynaecology in the Faculty of Health Sciences, at Stellenbosch University.

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Declaration:

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December 2021

Abstract:

Introduction

Prenatal screening in pregnant women of advanced maternal age has been shown to be ineffective in Cape Town. This study aims to determine the uptake rate of prenatal screening and invasive testing by pregnant women over the age of 37 at conception referred to the Tygerberg Hospital fetal medicine unit in 2016, as well as factors which influence this.

Methods

A retrospective audit was done of prospectively collected data from women over 37. According to our protocol, invasive testing was offered for either age over than 40 years at conception, a high ultrasound-based risk or a fetal anomaly on ultrasound. Termination of pregnancy was offered for severe structural anomalies or confirmed genetic disorders.

Results

Of 1196 older women, 645 (54%) received formal genetic counselling and 640 (53.5%) were offered invasive testing. Only 114 (9.5% of all) underwent invasive genetic testing for an overall prenatal testing uptake rate of 17.8%. An additional 10 patients opted for termination of pregnancy without invasive testing but 80% of women who were offered invasive genetic testing (according to our protocol) declined this. Women older than 40 years at conception (75.8 vs 59.6%), those who had previous first trimester losses and those who received pre-screen counselling (21.2% vs 7.9%) were more likely to decline invasive testing. A higher adjusted risk and a less favourable risk adjustment had a significant effect on the acceptance rate of testing in both first and second trimester assessments and acceptance were highest when a fetal anomaly was detected (54%). Risk reduction was most effective in the first trimester (92% becoming low risk) than after only second trimester assessment (64 % becoming low risk) but most assessments were done and most aneuploidies and anomalies were detected in the second trimester. Diagnostic yield for chromosomal abnormalities was 15.8% (18/114).

Conclusions

Uptake of invasive testing in this study was low (17.8%), and lower than seen in previous years. Uptake rate was somewhat influenced by maternal characteristics (age, previous miscarriage) and somewhat by the ultrasound-based risk result, but more so by the offer of pre-screen counselling and predominantly by the women's pre-existing attitude towards the service, Trisomy 21 or the option of termination of pregnancy.

Efforts should focus on pre-screen counselling and first trimester ultrasound screening to improve the cost effectiveness of our prenatal genetic screening program for women of advanced maternal age, but the second trimester ultrasound screening remains paramount as it still provides sensitive aneuploidy screening (although less effective than in the first trimester) for women who initiate antenatal care or are referred after the first trimester and allows the detection of more fetal anomalies than in the first trimester. Pre-screen counselling and first trimester ultrasound assessment should be improved to dramatically improve the prenatal genetic screening service.

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List of abbreviations:

AMA	Advance maternal age
CI	Confidence interval
CNS	Central nervous system
CVS	Chorionic villous sample
ISUOG	International society of ultrasound in obstetrics and gynaecology
N	Number
NT	Nuchal translucency
OR	Odds ratio
Qf-PCR	Quantitative fluorescence polymerase chain reaction
SD	Standard deviation
T1-3	Trimester 1-3
T21	Trisomy 21
TOP	Termination of pregnancy

Introduction:

Chromosomal abnormalities, like trisomy 21 (Down Syndrome), are an important cause of perinatal death and childhood disability. The Down syndrome phenotype was first described by Dr John Langdon Down in 1866.(1) Lejeune et al(2) and Jacobs et al(3) established in 1959 that Down Syndrome is the result of an extra chromosome 21. In 1966, 100 years after the original description by JL Down, the first prenatal diagnosis of trisomy 21 was confirmed using karyotyping of cultured amniotic fluid cells.(4)

At present the most common reason for amniocentesis, or any other form of invasive prenatal testing, is for the diagnosis of chromosomal abnormalities. Invasive testing is not without risk, therefore it should be reserved for those who are at high risk of having an affected fetus.(5)
The big question is how do we determine who is at risk? With prenatal screening we aim to identify those patients at high risk of carrying an affected fetus. Over the past decades, prenatal screening for Down syndrome and other chromosomal aneuploidies has evolved significantly. In the 1980s there was a dramatic increase followed by a plateau in the use of amniocentesis.(6) During this time prenatal genetic testing was only available to mainly white South Africans in some urban areas, and the uptake of testing was high in this population.(6,7)

Despite the significant scientific advances of prenatal screening globally, there are still some challenges in the South African system. The focus of this dissertation will be on prenatal screening in Tygerberg hospital.

Literature review:

The live-birth prevalence of Trisomy 21 (T21) in South Africa is high: 1.33-1.8 per 1000 live births in two urban areas and 2.1 per 1000 in a rural area(8), while in the developed world it is much lower (USA 1.3; North England 1.08 and Paris 0.7 per 1000 live births)(9–11) . In the South African public health sector, screening for chromosomal abnormalities has been available for more than 3 decades. Due to resource constraints affecting the availability of modalities such as ultrasound screening and serum tests, the main screening strategy is still based on advanced maternal age (AMA).(12) Urban et al (2011) has shown that the prenatal screening and diagnosis based on AMA is done ineffectively in the Cape Town health district. This can probably be extrapolated to the rest of South Africa(13) as the overall prenatal diagnosis of T21 in the public sector in 2008 was only 7%.(14)

Evolution of prenatal screening

A screening test is implemented to discriminate between individuals who have a high or low risk of being affected. A good screening test should have a high detection rate and low false positive rate. The detection rate is the ability of a test to give a positive result in individuals who have the condition being screened for (sensitivity). The false positive rate is the proportion of unaffected individuals who screens positive (=1/specificity, specificity being the proportion of unaffected individuals who have a negative screening result).

In 1970, prenatal screening was introduced based on an observation that the risk of having an infant with Trisomy 21 increases with maternal age. All women 40 years and older were considered high risk and offered invasive testing. As amniocentesis was more readily available and the risk of miscarriage was found to be low, the cut-off for screening in the UK was changed to 35 years and older. This constituted 5% of pregnant women and included 30% of affected fetuses in the 1970's.(15) Due to the change in age cut off (from 40 years to 35-38 years of age) the number of amniocentesis increased, but the diagnostic yield decreased over time. (6% in1978 to 3.2% in 1989). (6,16) The problem when using maternal age alone is a low positive predictive value (2.2% for all aneuploidies and 1.9% for autosomal trisomies at the age cut-off of 37 years) and other chromosomal abnormalities which are not associated with maternal age will be missed.(14) The proportion of older pregnant women has however increased dramatically over the past decades, especially in developed countries(17) but also in SA.

In the 1980's maternal serum biomarkers (alpha-fetoprotein, unconjugated estriol, free β-human chorionic gonadotropin and inhibin A) and detailed ultrasonographic examination in the second trimester were used in combination with maternal age to determine the risk. Using a risk cut-off of 1:300, the detection rate for T21 improved from 30% with maternal age alone to 60-75% with maternal age combined with second trimester serum biochemistry, with a similar false positive rate of 5%.(17) The second trimester screening ultrasound is a genetic sonogram done between 18 and 22 weeks, where the presence or absence of aneuploidy markers is used in an algorithm to calculate an individualised risk for T21. The added advantage of second trimester screening includes the detection of other structural abnormalities not specifically associated with chromosomal abnormalities.(18) Due to financial constraints, serum tests are not performed in the public sector in South Africa.

The focus shifted to the first trimester in the 1990's, when it was found that the detection rate of major aneuploidies increases to 85-95% with the use of a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A. Additional ultrasonographic markers (nasal bone length, ductus venosus flow and regurgitation over the tricuspid valve) increased the detection rate even further to 93-96%, and decreased the false positive rate to 2.5%.(17) Wray et al (2005) showed that the availability of first trimester screening increased the number of AMA woman receiving early prenatal genetic counselling and decreased the number of women choosing invasive testing.(19)

Lo et al demonstrated the presence of cell-free fetal DNA in maternal blood plasma in 1997.(20) This opened the door to highly effective non-invasive prenatal screening by analysing cell-free DNA in maternal blood. Cell-free fetal DNA analysis improved the detection rate of Trisomy 21 to 99% with a reduction of the false positive rate to 0.1% but remains a screening test and requires confirmation.(9) Mahri et al(5) advised that cell-free DNA should be used in combination with combined first trimester screening and reserved for an intermediate risk group. Intermediate risk being an individual's risk assessment of between 1:101 and 1:2500. Currently cell-free DNA analysis is available in South Africa but very expensive and only available in the private sector.

Diagnostic tests

Invasive testing is a diagnostic test as a genetic abnormality is confirmed or excluded. A sample is obtained either by chorionic venous sampling (CVS), amniocentesis or cordocentesis. Chorionic villus sampling is performed between 10-12 weeks and has a 1% procedure-related risk of miscarriage, with a specificity of 99.8% and sensitivity of 98.9–99.6% for detecting aneuploidies (i.e. incorrect number of chromosomes) or large chromosomal unbalances. Amniocentesis is performed after 15 weeks and has a procedure-related risk of miscarriage of 0.5%, with a specificity of 99.6% and sensitivity of 99.3%

for detecting similar chromosomal abnormalities.(23) In the public health care sector in Cape Town metropole and surrounding rural areas, all invasive genetic testing is done at a tertiary level facility.

The sample can then be sent for full karyotyping or quantitative fluorescence PCR (qf-PCR). QF-PCR is faster and less expensive than full karyotyping, with comparable accuracy for trisomy 21. Balanced and unbalanced translocations and inversions could be missed with qf-PCR because it does not produce a full photographic display of all chromosome pairs.(22) In some laboratories in the South African public sector they now use the qf-PCR as primary test in cases where Down Syndrome is expected, in order to reduce cost. Microarray techniques is a fast growing field, especially to diagnose single gene disorders. Although the use of molecular cytogenetics are used in research, it is not used for routine cytogenetic diagnosis in South Africa.(14)

A retrospective review done in Cape Town over 30 years found that the indication for amniocentesis has changed significantly over the years. Advanced maternal age has always been the most common indication but more recently the detection of fetal anomalies on an ultrasound and high risk ultrasound screening have increased as indication for testing. The uptake of testing is also much higher for these indications, as seen in data for 2008-2009 of the Cape Town Metropole West. (Uptake per indication: Maternal age ≥37 years:31%; Fetal anomaly on scan: 66%; Ultrasound screen (first trimester nuchal translucency or second trimester soft markers): 54%)(14)

All medical staff rendering an antenatal care service should have a clear understanding of the difference between screening and diagnostic testing for chromosomal abnormalities as this will form the basis of any counselling session. In contrast to diagnostic tests, screening tests merely assess the risk of an abnormality without confirming whether it is indeed present or not.

Pre-screening counselling

Internationally, prenatal genetic screening has become an integral part of antenatal care and is mostly perceived as a routine procedure. The introduction of prenatal genetic screening has however created a new and complex ethical and social dilemma and should be combined with comprehensive pre-screen counselling focussed on informed, preference-based screening decisions.

The key principle in the counselling of expecting parents regarding prenatal screening for chromosomal abnormalities is that screening is voluntary. Counselling should be provided in a clear, understandable and non-directive way, enabling the parents to make an informed preference-based decision. The following aspects should be discussed: differences between screening and diagnostic tests, potential

consequences of prenatal screening, what high and low risk results mean, a description of how the screening and diagnostic tests are done, the option of a diagnostic test instead of screening, the procedure-related risk of diagnostic tests, the waiting time for results for each test, the implications of having a child with trisomy 21, the possibility of detecting other chromosomal abnormalities besides trisomy 21 and the implications of those findings, information on the option of continuing with the pregnancy and information on the option of terminating the pregnancy, information on delivery and paediatric care for a baby with trisomy 21 as well as information on possible support structures for families with an affected child.(18) All this information needs to be provided, taking into account each patient's own cultural and social views. Ried et al (2009) published a meta-synthesis of pregnant women's decision-making process regarding antenatal screening for Down syndrome and identified 12 themes that were combined into five core concepts:

a) **Destination unknown:** Screening is either anxiety relieving or anxiety provoking. Every parent wants to be reassured about the health and well-being of their baby, but what about the stress while waiting for the result, or stress caused by difficult decisions if the result comes back positive. Another contributing factor is a patient's view on abortion. Many women will not consider abortion and therefore perceive screening as pointless. The patient's perceptions of having a child with Down syndrome are deeply intertwined with their decision-making regarding screening. The way a patient perceives the burden or delight a child with Down Syndrome brings to the family and society will inadvertently influence her views on screening. The routinization of prenatal screening may lead to patients receiving information that is unwanted or misconstrued.
b) **To choose or not to choose:** Most women view screening as routine antenatal care. This is further reinforced by the trust women have in the expert opinion of the health care provider. The routine nature of the test and the woman's complete trust in the health care provider limits her ability to choose.
c) **Risk is rarely pure and never simple:** To the expert, risk is a reliable, clear-cut expression of a fact. Most women don't find risk categories (high or low risk) useful and would prefer individualized answers that take their context and culture into account. The possibility of the screening test being inaccurate complicates the risk categories even more as there is still no clear guide to govern decision making. Knowing there is an increased risk also changes the rest of the pregnancy for the parents, from a state of waiting to a path of multiple difficult decisions, such as diagnostic testing, termination or having a baby with Down Syndrome.
d) **Treading on dreams:** The technological advances in screening combined with the expectation of responsible motherhood has impacted greatly on the decision making of pregnant women in the quest to have a 'perfect baby'. Screening can hasten the bonding of the mother with the

perfect person growing in her womb, or it can cause uncertainty if the screening result is positive. This can cause the mother to rather keep her distance and not bond with the baby until the screening test is done, resulting in a 'tentative pregnancy'. Some women may perceive screening as reducing a fetus to a consumer object, subject to quality control based on future potential.

e) **Betwixt and between:** Women's perceptions rest upon the risk of not having information as opposed to the risk posed by information. Women would like to have all the information to feel in control over future motherhood. What makes this decision difficult is knowing that this information can also cause emotional distress and lead to even more difficult decision making.

These five core concepts point to the complexity of women's decision-making processes with regards to antenatal screening for Down Syndrome.(24) Implementing a service where you offer extensive pre-test counselling should not place an extra load on an already overburdened system. Kuppermann et al (2014) found in a randomized trial that the use of a decision support guide empowered women to make more informed decisions and fewer women opted for invasive testing.(25)

This is just an example of how providing comprehensive prenatal testing information and the opportunity to explicitly consider their own values and preferences influenced women's uptake of invasive testing. Darnes et al (2011) also found that pre-screen genetic counselling may be an influential factor in the patient's decision regarding prenatal diagnostic testing.(26)

Guidelines in South Africa

Health care in South Africa is provided by two parallel sectors, namely the private and public sector. There are great inequalities within this system with marked discrimination on economical basis.(27)
The private sector accounts for roughly 20% of deliveries. Services are generally rendered by obstetricians, general practitioners and to a lesser extend registered midwifes. Most patients present early, attend regular health care visits and often receive multiple ultrasound assessments. In the South African private sector prenatal genetic screening is part of every patient's antenatal care plan, this usually include biochemical screening for most patients who present early and/or ultrasound based screening in the first or second trimester. There is no uniform screening strategy, although clear guidelines are provided by organizations such as ISUOG. Other problems identified within the private sector first trimester screening include unaccredited operators performing ultrasound assessments (lack of quality control) and incorrect information provided to testing laboratories, resulting in inaccurate risk calculations.

The uptake of invasive testing after positive serum screen is very low (less than 50%) in the SA private sector and the reason for this is unclear. Fetal anomalies as indication for invasive testing is also very low in this sector, and this could be attributed to the fact that very few patients in the private sector have access to dedicated fetal medicine services.(14) Only 39% of Down syndrome cases in the private sector are diagnosed prenatally.(14) This is lower than expected when using an effective screening policy (like first trimester combined screening or the triple test). In the public sector serum screening is not practical as very few patients present in the first trimester, accurate pre-screen ultrasound dating is seldom possible and tracing of patients for follow up of results is very difficult. In the public sector the national policy states that all women between the ages of 35 and 40 years of age (age limit determined by province), who commence antenatal care before 20 weeks gestation, should be offered referral to a centre that offers prenatal diagnosis of Down Syndrome. A retrospective review done in Cape town showed that only 13% of Down Syndrome cases were diagnosed prenatally between 2001 and 2005 and a nationwide survey indicated that only 7% of Down syndrome diagnoses are made prenatally in the SA public sector.(14)

Limitations in the public sector with regard to prenatal diagnostic services include late or no initiation of antenatal care, inadequate referral and limited resources. Socioeconomic factors (cost of transport and challenges with child care), inaccessible and hostile health care services and ignorance of the importance of antenatal care are suggested as possible reasons for late initiation of antenatal care.(28,29) A study by Haddad et al (2015) highlighted that women were less likely to present earlier if the pregnancy was unplanned or if they had contemplated induced abortion earlier. Cultural concerns such as bewitchment and psychological stress caused by a positive HIV test result also plays a part in late initiation of antenatal care.(19) In 2007 only 27% of pregnant women initiated antenatal care in the public sector before 20 weeks gestation.(30)

Other than late initiation of antenatal care, low referral rates remain an obstacle in prenatal diagnostic services. An audit done in Cape Town in 2008 showed that only 16% of women of advanced maternal age from a well-run primary care facility received genetic counselling, considering only 23% qualified for referral. After interviewing the staff at the facility it was found that they misunderstood the age cut-off, they did not think that referral for maternal age alone was important and did not provide patients with enough information about the reason for referral. They were more likely to refer patients with a fetal anomaly on ultrasound.(14) In Gauteng it was found that more than 50% of patients of advanced maternal age who presented to health care services within the first trimester were not referred for genetic counselling, most of these opportunities were missed by other primary care services (70%), and the private sector (60%), and not by the antenatal clinics.(32) Currently in the

Metro East district of the Western Cape province, age- and ultrasound-based screening is offered to all pregnant women older than 37 years at the time of conception. If women of age 37-39 screen high risk (adjusted risk of 1:200 or a structural fetal anomaly is found), they receive formal genetic counselling and are offered invasive testing. Women who were 40 years or older at the time of conception and English or Afrikaans speaking, receive pre-screening counselling and are offered a choice between screening or invasive testing. For women who are not fluent in these languages, this counselling takes place with the assistance of a translator after the ultrasound examination.

Termination of pregnancy for a confirmed fetal aneuploidy is offered according to the Choice on Termination of Pregnancy Act of 1996. This act is considerably more liberal than the former Abortion and Sterilization Act of 1975. According to the act a pregnancy may be terminated:

a) upon request of a woman during the first 12 weeks of the gestation period in her pregnancy
b) from the 13th up to and including the 20th week of the gestation period if a medical practitioner, after consultation with the pregnant woman, is of the opinion that –
    i. the continued pregnancy would pose a risk of injury to the woman's physical or mental health; or
    ii. there exists a substantial risk that the fetus would suffer from a severe physical or mental abnormality; or
    iii. the pregnancy resulted from rape or incest; or
    iv. the continued pregnancy would significantly affect the social or economic circumstances of the woman; or
c) after the 20th week of the gestation period if a medical practitioner, after consultation with and medical practitioner or a registered midwife, is of the opinion that the continued pregnancy -
    i. would endanger the life of the women
    ii. would result in severe malformations to the fetus; or
    iii. would pose a risk of injury to the fetus

Conclusion

A patient's values, interests and goals should be the foundation of an informed decision making process regarding prenatal genetic screening. When we provide prenatal screening to patients without pre-screening counselling, we are impeding their ability to make an informed decision before starting on the screening pathway and we could be placing a burden on the system by screening women who would have declined this if they were given the option. Investigating the uptake rate of screening and

invasive testing at Tygerberg hospital and factors influencing this will help design a counselling and screening guideline which is both effective, efficient and patient orientated.

Aim and objectives of the study

The primary aim of this audit was to determine the uptake rate of prenatal genetic screening and invasive testing after screening in pregnant woman referred to Tygerberg Hospital ultrasound unit in 2016 due to their advanced maternal age (over the age of 37 at conception).

The secondary aim was to identify the factors influencing this. The parameters that were assessed in this regard included:

a) Demographics: age, gravidity, parity, previous obstetric history and residence.
b) Genetic counselling: whether genetic counselling was indicated as per current protocol, whether genetic counselling was provided and whether it was pre-screen or not, the indication for genetic counselling, who was counselled (patient, partner, family), was risk assessment accepted or not (i.e. acceptance of screening).
c) Risk assessment: background risk, adjusted risk and degree of risk adjustment after screening.
d) Invasive testing: was invasive testing offered and was it accepted or not, the reason why testing was declined, the type of fetal sample obtained and the result thereof.
e) Termination of pregnancy (TOP): was TOP offered and the reason why, was TOP not offered and the reason why, was TOP accepted or not, and the gestational age at TOP.

Methodology

Study design

This was a retrospective audit of all women of advanced maternal age seen at the Tygerberg Hospital Fetal Medicine unit in 2016. The audit was predominantly based on prospectively collected data in the Astraia™ database.

Setting and study population

This research was conducted in the setting of a public sector, academic hospital in Cape Town, South Africa, which serves half of the population of the Western Cape province with predominantly low-income communities. Tygerberg hospital serves as a level 3 referral centre for the West coast, Overberg and Cape wine lands district (rural) and as a level 2 referral centre for Eastern and Khayelitsha sub districts (urban). In 2016, advanced prenatal diagnostic services (including aneuploidy screening and invasive testing) were limited to the level 3 platform.

All woman aged 37 years and older at conception, who were seen at the Tygerberg Hospital ultrasound unit between 1 January 2016 and 31 December 2016 were considered.

For this audit, the following women were excluded:

a) Women who had screening and testing in the private sector.
b) Woman who had assisted reproduction with donor oocytes, not of advanced age.

Sample size

The number of women included in this analysis was 1196.

Data collection and management

Ethical approval was obtained from the Stellenbosch University Health and Research Ethics Committee (HREC), nr: S18/05/101. All data was collected from TBH OpenText ECM system, Astraia™ and the genetic databases (the last two contain patient data collected prospectively at the time of the patient visit) and entered directly into a Microsoft™ Excel 365 spread sheet by the principal investigator under supervision of the study supervisor.

The Excel spreadsheet was designed to ensure all important fields must be captured. Numerical data such as age, gravidity, parity was entered directly. Categorical data was entered using drop-down menus. Excel's Data validation feature was used to reduce the risk of entering inappropriate data. The

data was cleaned after entry to ensure that there are no missing values and that values are in permitted ranges and consistent with other variables.

Data analysis plan

The data was analysed using Statistica Software (Version13.5.0.17, 1984-2018, TIBCO Software Inc.). Continuous data with a normal distribution were expressed as means and standard deviations with ninety five percent (95%) confidence intervals for the population. The non-normally distributed data or categorical data were expressed as medians and interquartile ranges.

Secondary outcomes included a comparison between women younger or older than 40 at conception, a comparison according to timing at which women accessed ultrasound screening, a comparison between women accepting or declining invasive testing and a comparison between women opting for TOP or declining TOP. Inferential statistics was used for the secondary outcomes. Chi-squared tests was used for comparison of two or more categorical variables. Fischer's exact test was used with small numbers. Student t-test was used to compare normally distributed continuous data. A p-value of <0.05 was regarded as significant.

Results

Patient and pregnancy characteristics

Table 1: Descriptive data of all women of advanced age seen in a one year period, according to age category (40 or older at conception or not), and expressed in number (%), mean ± standard deviation or median (range).

	All	Age ≥ 40 years	Age < 40 years	P-value
Number (%)	**1196**	**534 (44.6%)**	**662 (55.4%)**	
Urban	849 (70.9%)	352 (65.9%)	497 (75.1%)	0.0005
Rural	347 (29%)	182 (34.1%)	165 (24.9%)	
Age	39.9 ± 1.97	41.7 ± 1.4	38.5 ± 0.9	< 0.001
Gravidity	4 (1-10)	4 (1-10)	4 (1-10)	0.005
Gravida 1	37 (3.1%)	16 (3.0%)	21 (3.1%)	0.9
Parity	2 (0-8)	2 (0-8)	2 (0-7)	0.0002
Para 0	65 (5.4%)	27 (5.1%)	38 (5.7%)	0.6
Previous Obstetric History				
Neonatal death	23 (1.9%)	8 (1.5%)	15 (2.3%)	0.5
Term loss	47 (3.9%)	18 (3.4%)	29 (4.4%)	0.5
T3 loss	40 (3.4%)	12 (2.3%)	28 (4.2%)	0.06
T2 loss	80 (6.7%)	28 (5.3%)	52 (7.9%)	0.1
T1 loss	289 (24.2%)	131 (24.6%)	158 (23.9%)	0.6
Ectopic	23 (1.9%)	9 (1.7%)	14 (2.1%)	0.8
T1 TOP	37 (3.1%)	16 (3%)	21 (3.2%)	1.0
T2 TOP	9 (0.4%)	1 (0.2%)	8 (1.2%)	0.2
First trimester assessment				
Number (%)	455 (38.0%)	186 (34.8%)	269 (40.6%)	0.04
Gestational Age (days)	90.3 ± 5.0	89.9 ± 5.6	90.6 ± 4.4	0.4
	91 (55-104)	91 (55-102)	91 (75-104)	0.4
1: Background Risk	86 (9-183)	56 (9-78)	114 (70-183)	< 0.001
1: Adjusted Risk	1064 (2-3256)	643 (2-1556)	1607 (114-91)	< 0.001
Risk Adjustment	17.1 (0.02-20.7)	19.3 (0.03-20.7)	15.7 (0.02-20.1)	0.7
Risk calculated	436/1196 (36.5%)	204/534 (38.2%)	232/662 (35.0%)	0.3

	All	Age ≥ 40 years	Age < 40 years	P-value
Low Risk (< 1:165)	382/436 (87.6%)	179/204 (87.7%)	203/232 (87.5%)	0.9
High Risk (≥ 1:165)	48 (11%)	23 (11.3%)	25 (10.8%)	
High Risk with Anomaly	6 (1.4%)	2 (1.0%)	4 (1.7%)	
"Abnormal" result	54 (4.5%)	25 (4.7%)	29 (4.4%)	0.8
Second trimester assessment				
Number (%)	933 (78%)	481 (90.1%)	552 (83.4%)	0.0008
Gestational Age (days)	151.3 ± 20.0	151.4 ± 20.3	151.2 ± 19.7	0.5
	146 (114-269)	146 (114-264)	145 (115-269)	1.0
1: Background Risk	124 (2-3228)	73 (2-1556)	154 (2-3228)	< 0.001
1: Adjusted Risk	654 (2-20000)	423 (2-13335)	999 (2-2000)	< 0.001
Risk Adjustment	7.5 (0.02-9.5)	7.6 (0.002-9.5)	7.6 (0.01-9.4)	0.2
Risk calculated	855/1196 (71.5%)	371/534 (69.5%)	484/662 (73.1%)	0.2
Low Risk (<1:200)	579/855 (67.7%)	255/371 (68.7%)	324/484 (66.9%)	0.6
High Risk (≥1:200)	220 (25.7%)	92 (24.8%)	128 (26.4%)	
Low Risk with Anomaly	10 (1.2%)	6 (1.6%)	4 (0.8%)	
High Risk with Anomaly	46 (5.4%)	18 (4.9%)	28 (5.8%)	
"Abnormal" result	276 (23.1%)	116 (21.7%)	160 (24.2%)	0.3
Any trimester				
Ever High Risk result	318 (30.5%)	159 (35.2%)	159 (26.9%)	0.02
Ever Fetal Anomaly	66 (5.5%)	34 (6.4%)	32 (4.8%)	0.2
Genetic counseling				
Indicated	656 (54.8%)	472 (88.4%)	184 (27.8%)	< 0.001
Provided	645	465	180	
Of all	(54.0%)	(87.1%)	(27.2%)	< 0.001
Of indicated	(98.3%)	(98.5%)	(97.8%)	0.5
Who received counseling	644	465	179	0.7
Woman	500 (77.6%)	359 (77.2%)	141 (78.8%)	
Couple	128 (19.9%)	93 (20.0%)	35 (19.6%)	
Woman with other	16 (2.5%)	13 (2.8%)	3 (1.7%)	
Indication	659	472	187	< 0.001
Low Risk	275 (41.7%)	269 (57%)	6 (3.2%)	
High Risk	270 (41.0%)	123 (26.1%)	147 (78.6%)	
Previous History	4 (0.6%)	0	4 (2.1%)	

	All	Age ≥ 40 years	Age < 40 years	P-value
Fetal Anomaly	59 (9%)	29 (6.1%)	30 (16.0%)	
Age alone	51 (7.7%)	51 (10.8%)	0	
Pre-screen counseling	93 (7.8%)	93 (18%)	0	
Accepts Risk Assessment	42/93 (45%)	42 (45%)	0	
(+ accepts invasive test)	(45) (48.4%)	(45) (48.4%)		
Invasive testing				
Invasive test offered	640 (53.5%)	464 (86.9%)	176 (26.6%)	< 0.001
Invasive test accepted	114	68	46	
Of all	9.5%	12.7%	6.9%	0.0007
Of those offered	17.8%	14.6%	26.1%	0.0007
(including TOP)	124	73	51	
Of all	10.4%	13.7%	7.7%	0.0008
Of those offered	19.4%	15.7%	29%	0.0001
Reason to decline	487/526 (92.6%)	362/396 (91.4%)	125/130 (96.2%)	0.06
Would not terminate	362 (74.3%)	275 (76.0%)	87 (69.6%)	0.002
Fear of miscarriage	50 (10.3%)	33 (9.1%)	17 (13.6%)	
Risk acceptable	30 (6.2%)	28 (7.7%)	2 (1.6%)	
TOP (without test)	10 (2.1%)	5 (1.4%)	5 (4%)	
Need partner's opinion	35 (7.2%)	21 (5.8%)	14 (11.2%)	
Fetal sample obtained	114 (9.5%)	68 (12.7%)	46 (6.9%)	0.4
Amniotic Fluid	95 (82.6%)	56 (82.4%)	39 (83.0%)	
Fetal Blood	9 (7.9%)	5 (7.4%)	4 (8.5%)	
Chorionic Villus	10 (8.7%)	7 (10.3%)	3 (6.4%)	
Genetic diagnosis	20 (1.7%)	11 (2.1%)	9 (1.4%)	0.3
Abnormal karyotype	18	10	8	
Of all	/1195 (1.5%)	/534 (1.9%)	/662 (1.2%)	0.3
Of tests (yield)	/115 (15.7%)	/68 (14.7%)	/47 (17.0%)	0.7
Termination of pregnancy				
TOP offered/All	38 (3.2%)	19 (3.6%)	19 (2.9%)	0.5
Reason TOP offered				0.1
Genetic syndrome	18 (47.4%)	9 (47.4%)	9 (47.4%)	
Multiple Anomalies	7 (18.4%)	6 (31.6%)	1 (5.3%)	
Central nervous system	7 (18.4%)	3 (15.8%)	4 (21.1%)	

	All	Age ≥ 40 years	Age < 40 years	P-value
Cardiac defect	2 (5.3%)	0	2 (10.2%)	
Renal	2 (5.3%)	0	2 (10.2%)	
Skeletal	1 (2.6%)	1 (5.3%)	0	
Maternal	1 (2.6%)	0	1 (5.3%)	
Reason TOP not offered	1158/1196 (96.8%)	515 (96.4%)	643 (97.1%)	
Not indicated	1155 (99.7%)	513 (99.6%)	642 (99.8%)	1.0
Too late	1 (0.1%)	0	1 (0.2%)	
Would not terminate	1 (0.1%)	1 (0.2%)	0	
Unknown	1 (0.1%)	1 (0.2%)	0	
TOP done	25 (2.1%)	14 (2.6%)	11 (1.7%)	0.2
TOP uptake rate	25/38 (65.8%)	14/19 (73.7%)	11/19 (57.9%)	0.3

T1-3: Trimester 1-3; TOP: Termination of pregnancy

In 2016, at total of 4995 women were scanned in the ultrasound unit (totalling 13101 visits) and 1196 of these (24%) were older than 37 at conception (1492 visits, 11.4%), and 534 (44.6% of these) were older than 40 years. Their mean age was 39.9 ± 2.0 years, median gravidity 4 (1-10) and parity 2 (0-8) and only 5.4% were nulliparous. The older group of women (≥ 40) had higher gravidity and parity with no other differences in previous obstetric history (pregnancy losses). Significantly more women were seen from the urban sub-districts than from the rural areas (71% vs 29%) and this indicates a significant underrepresentation of the rural patients ($p < 0.00001$) compared to the distribution within the province (in 2016 59% of deliveries occurred in the urban sub-district). A higher proportion of rural referrals was seen for women over 40 (p 0.00005).

First trimester risk assessment was performed in 455 women (38%) at a mean gestational age of 12w6d (90.3 ± 5days), similar between the two age-groups. The median background risk was 1:86 (9-183) and the mean adjusted risk was 1:1064, both being significantly higher in the older age group. The median risk adjustment was a 17.1-fold reduction (0.015-20.7), with no difference between the age groups. Most patients received a low risk result (87%), similar for both age groups, and a structural anomaly was detected in 6 fetuses (1.4%).

Second trimester risk assessment was performed in 933 women (78%) at a mean gestational age of 21w6d (151.3 ± 20 days), similar between the two age groups. The median background risk was 1:124 (2-3228) and the mean adjusted risk was 1:654, both significantly higher in the older women (P <

0.001). The median risk adjustment was a 7.5-fold reduction (0.015-9.48) and similar for both groups but significantly lower than in the first trimester. In the second trimester, only two thirds of patients (579/855, 67.7%) had a low risk result, with no significant difference between the groups. A fetal anomaly was detected in 56 cases (6.5%).

Genetic counselling was indicated as per protocol (i.e. either age over 40 or ultrasound based risk for T21 > 1:200 or other genetic risk factors before 24 weeks of gestation or fetal anomaly on scan) in 656 women (54.8%) and 645 of these (54% of all included patients) received genetic counselling, most often unaccompanied (77%). These proportions were significantly higher for the older group of women (88.4 and 87.1% compared to 27.8 and 27.2%, p<0.001) but the proportion who received indicated genetic counselling was the same (p=0.5). The indications for genetic counseling differed significantly (p < 0.001), in line with the protocol. Only 93 women (7.8% of all women, 18% of women over 40) received pre-screen genetic counselling and less than half of these (45.2%) accepted ultrasound-based risk assessment while 3 requested invasive testing and the remaining 48 (51.6%) declined any screening or testing for T21. Across the two trimesters, 30.5% of women received a high-risk result for trisomy 21, significantly more likely for the older age group (35.2 versus 26.9%, p 0.00008).

Invasive diagnostic testing was offered to 640 women (53.5%), significantly more so for the women over 40 (86.9 versus 26.6% p < 0.001). Only 114 women (17.8% of those who were offered) accepted invasive testing, with a higher acceptance rate by the younger women (26.1% versus 14.6%, p 0.00007). These findings persisted when including TOPs performed without genetic testing.

Reasons for declining invasive testing were recorded for 487 of the 526 women who declined and differed between the groups. Most (74.3%) declined invasive testing because they would accept a baby with Down syndrome and therefore would not consider termination of pregnancy. Thirty women (6.2%) found the adjusted risk acceptably low, 50 women (10.3%) found the risk of miscarriage unacceptable and 35 (7.2%) declined after discussing it with their partners.

Invasive genetic testing was performed in 114 women (9.5%), mostly by amniocentesis (82.6%), and 18 abnormal karyotype results were found (yield of 15.7%, similar for both age groups, p=0.7).

Termination of pregnancy was offered to 38 women (3.2%), mostly due to genetic abnormalities (47.4%), followed by CNS anomalies and multiple congenital anomalies (18.4% each). Of these 38 women, 25 (65.8%) accepted the termination of pregnancy, with no difference between the age groups.

Comparison according to timing of accessing ultrasound screening and genetic counselling services.

Table 2. Comparison according to timing at which women accessed ultrasound screening and genetic counselling services, expressed in number (%), mean ± SD or median (range).

	T1	P 1-1+2	T1 + T2	P 1+2-2	P any 1-2	T2	P T2-late	Too late
Number (% of 1196)	159 (13.3%)		296 (24.7%)			637 (53.2%)		104 (8.6%)
Urban **Rural**	81 (50.9%) 78 (49.1%)	0.0000	234 (79.1%) 62 (20.9%)	0.009	0.6	452 (71.0%) 185 (29.0%)	0.1	82 (78.8%) 22 (21.2%)
Age	39.5 ± 1.9	0.003	40.4 ± 2.0	0.9	0.1	40.0 ± 1.9	0.6	40.1 ± 2.1
≥ 40 years	49 (30.8%)	0.001	137 (46.3%)	0.9	0.05	298 (46.8%)	0.8	50 (48.1%)
Gravidity	4 (1-8)	0.2	4 (1-10)	0.2	0.7	4 (1-9)	0.1	4 (1-8)
Parity	2 (0-6)	0.7	2 (0-6)	0.5	0.7	2 (0-8)	0.008	3 (0-7)
Previous obstetric history								
Neonatal death	1 (0.6%)	0.2	7 (2.4%)	0.5	1.0	11 (1.7%)	0.2	4 (3.8%)
Term loss	2 (1.2%)	0.07	13 (4.4%)	0.9	0.3	29 (4.5%)	0.4	3 (2.9%)
T3 loss	3 (1.8%)	0.1	14 (4.7%)	0.4	0.8	22 (3.4%)	0.8	3 (2.9%)
T2 loss	8 (5%)	0.08	29 (9.9%)	0.04	0.3	38 (6%)	0.6	5 (4.8%)
T1 loss	34 (21%)	0.01	96 (32.4%)	0.002	0.03	144 (22.6%)	0.06	15 (14.4%)
Ectopic	4 (2.4%)	0.9	8 (2.7%)	0.2	0.2	10 (1.6%)	0.6	1 (1%)

	T1	P 1-1+2	T1 + T2	P 1+2-2	P any 1-2	T2	P T2-late	Too late
T1 TOP	5 (3%)	0.9	10 (3.4%)	0.8	0.8	19 (3%)	1.0	3 (2.9%)
T2 TOP	0	0.3	2 (0.7%)	0.7	0.3	6 (0.9%)	1.0	1 (1%)
First Trimester assessment								
Gestational age (days)	90.7 ± 6.1	0.005	90.1 ± 4.2	-	-	-	-	-
1: Background Risk	97.3 ± 37.9	0.002	86.0 ± 39.1	-	-	-	-	-
1: Adjusted Risk	1370.5 ± 926.2	0.002	1076.1 ± 838.1	-	-	-	-	-
Risk Adjustment	13.5 ± 7.4	0.4	12.8 ± 7.7	-	-	-	-	-
Risk calculated	148/159 (93.1%)	0.2	286/296 (96.6%)	-	-	-	-	-
Low Risk (< 1:165)	136/148 (91.9%)		246/286 (86.0%)					
High Risk (≥ 1:165)	10 (6.8%)		38 (13.2%)					
High Risk + Anomaly	2 (1.4%)		2 (0.7%)					
Second trimester assessment								
Gestational age (days)		-	143.9 ± 5.6	0.000	0.000	146.4 ± 8.3	0.000	202.0 ± 23.9
1: Background Risk		-	1064.4 ± 844.7	0.000	0.000	106.4 ± 78.9	0.4	109.1 ± 53.1
1: Adjusted Risk		-	3835.5 ± 6854.4	0.000	0.000	558.0 ± 585.3	-	-
Risk Adjustment		-	5.6 ± 3.4	0.03	0.09	5.1 ± 3.6	-	-
Risk calculated		-	214/296 (72.3%)	0.04	0.03	447/637 (70.2%)	0.2	74/104 (71.2%)
Low Risk (< 1:200)			155/214 (72.4%)			287/447 (64.2%)		53/74 (71.6%)
High Risk (≥ 1:200)			45 (21.0%)			132 (29.5%)		18 (24.3%)

	T1	P 1-1+2	T1 + T2	P 1+2-2	P any 1-2	T2	P T2-late	Too late
Low Risk + Anomaly			3 (1.4%)			6 (1.3%)		1 (1.4%)
High Risk + Anomaly			11 (5.1%)			22 (4.9%)		2 (2.7%)
Any trimester								
Ever High Risk	14/159 (8.8%)	0.009	69/296 (23.3%)	0.000	0.000	226/637 (35.5%)	0.000	9/104 (8.7%)
Ever Anomaly	4/159 (2.5%)	0.1	17/296 (5.7%)	0.9	0.3	38/637 (6.0%)	0.8	7/104 (6.7%)
Genetic counselling								
Indicated	55 (34.6%)	1.0	181 (61.1%)	0.3	1.0	413 (64.8%)	1.0	7 (6.7%)
Provided	55 (34.6%)	1.0	178 (60.1%)	0.3	1.0	405 (63.6%)	1.0	7 (6.7%)
Indication		0.2		0.003	0.0002		0.000	
≥ 40 Low Risk	31 (56.4%)		93 (51.4%)			146 (36.0%)		
≥ 40 High Risk	5 (9.1%)		25 (13.8%)			92 (22.7%)		
≥ 40 Fetal Anomaly	3 (5.5%)		1 (0.6%)			21 (5.2%)		3 (42.9%)
< 40 High Risk	5 (9.1%)		30 (16.6%)			106 (26.2%)		
< 40 Fetal Anomaly	1 (1.8%)		10 (5.5%)			16 (4.0%)		4 (57.1%)
< 40 Low Risk						1 (0.2%)		
Previous history	1 (1.8%)		2 (1.1%)			1 (0.2%)		
Age alone	9 (16.4%)		20 (11.0%)			22 (5.4%)		
Pre-screen counselling	18/159 (11.3%)	0.001	30/296 (10.1%)	0.9	0.05	46/637 (7.2%)	-	-
Accepts Risk Assessment	8/18 (44.4%)	0.08	10/30 (33.3%)	0.07	0.3	24/46 (52.2%)	-	-

	T1	P 1-1+2	T1 + T2	P 1+2-2	P any 1-2	T2	P T2-late	Too late
(+ chooses invasive testing)	(+0) (44.4%)		(+5) (50%)	0.7	0.5	(+0) (52.2%)		
Who received counselling	55/159 (34.6%)	0.1	178/296 (60.1%)	0.6	0.2	405/637 (63.6%)	0.08	6/104 (5.8%)
Woman	37/55 (67.3%)		138/178 (77.5%)			322/405 (79.5%)		3/6 (50%)
Couple	16 (29.1%)		36 (20.2%)			74 (18.3%)		2 (33.3%)
Woman with other	2 (3.6%)		4 (2.2%)			9 (2.2%)		1 (16.7%)
Invasive testing								
Invasive test offered	54/159 (34.0%)	1.0	176/296 (59.5%)	1.0	1.0	404/637 (63.4%)	1.0	6/104 (5.8%)
Invasive test uptake	7+4/54 (20.4%)	0.02	16/176 (9.1%)	0.0002	0.001	88/404 (21.8%)	0.01	3+1/6 (66.7%)
(+ TOP)		0.5		0.0002	0.001		0.004	
Invasive test done	7+4/159 (6.9%)		16/296 (5.4%)			88/637 (13.8%)		3+1/104 (3.8%)
Reason declined	36/159 (22.6%)	0.7	137/296 (46.3%)	0.5	0.5	268/637 (42.1%)	0.05	2/104 (1.0%)
Would not terminate	29/36 (61.1%)		109/137 (79.6%)			223/268 (83.2%)		1 (50%)
Fear of miscarriage	3 (8.3%)		17 (12.4%)			30 (13.5%)		
Risk acceptable	4 (11.1%)		11 (8.0%)			15 (6.7%)		1 (50%)
Fetal sample	7	0.007	16	0.000	0.000	88	1.0	3
Amniotic Fluid	1 (14.3%)		12 (75%)			81 (91.0%)		1 (33.3%)
Fetal Blood	0		0			7 (8.0%)		2 (66.7%)
Chorionic Villus Sample	6 (85.7%)		4 (25%)			0		
Genetic disorder	2/159 (1.3%)	0.6	3/296 (1.0%)	0.001	0.000	14/637 (2.2%)	0.002	1/104 (1.0%)

	T1	P 1-1+2	T1 + T2	P 1+2-2	P any 1-2	T2	P T2-late	Too late
Karyotype abnormality	2 (1.3%)		2 (0.7%)			13 (2.0%)		1 (1.0%)
Termination of pregnancy								
TOP offered	5/159 (3.1%)	0.8	8/296 (2.7%)	0.5	0.6	22/637 (3.5%)	0.8	3/104 (2.9%)
Reason TOP offered	5	0.4	8	0.4	0.1	22	0.1	3
Genetic	2 (40%)		3 (37.5%)			13 (59.1%)		1 (33.3%)
Multiple Anomalies			1 (12.5%)					
Central nervous system	3 (60%)		2 (25%)			4 (18.2%)		2 (66.7%)
Cardiac			2 (25%)			1 (4.5%)		
Renal						2 (9.1%)		
Skeletal						1 (4.5%)		
Maternal						1 (4.5%)		
TOP done	5 (100%)	0.2	4/8 (50%)	0.4	0.8	14/22 (63.6%)	0.9	2/3 (66.7%)

N: Number; T 1-3: trimester 1-3; TOP: Termination of pregnancy

Ultrasound screening and/or genetic counselling was offered to 1196 women, 159 (13.3%) had only a first trimester (T1) assessment, 296 (24.7%) had a first and second trimester (T1T2) assessment, 637 (53.3%) had only a second trimester (T2) assessment and 104 (8.6%) were seen after 24weeks and therefore too late for prenatal genetic screening (more likely for older women and less likely for women with a previous T1 loss). In total, 455 (38%) women were seen in the first trimester and 933 (78%) in the second trimester.

Overall, there was no difference in place of residence as to whether women were seen in the first or second trimester, but urban women were more likely to return for a second assessment after their first trimester scan. Women who did not return after T1 were least likely to have a high risk result or a fetal anomaly. Women having both assessments were more likely to be older, over 40, having had a previous early pregnancy loss and a higher adjusted risk on the first scan despite a similar risk adjustment, yet a lower uptake of invasive testing. On reassessment in the second trimester, the risk remained in the same category in 78.4% of cases in whom no anomaly was seen in the first trimester. Of the 242 with a low risk result, 192 remained low risk (79.3%, 2 (1%) opting for testing), 10 had an anomaly detected (4.1%; 3 (30%) tested) and 16 became high risk (6.6%; 2 (12.5%) tested). Of the 26 with a high risk result, 18 remained high risk (69.2%) but only 3 (16.7%) of these opted for testing, and 8 became low risk (one (12.5%) tested).

Women who missed the first trimester assessment did not differ in term of place of residence (p 0.05), gravidity or parity but were more likely to be over 40 (46.8 vs 40.7%, p 0.05) and less likely to have previous losses or receive pre-screen counselling, they had a much higher background and adjusted risk and were more likely to undergo invasive testing, resulting in most genetic diagnoses.
The indication to receive genetic counseling differed significantly, mostly in the older than 40 years group. The most common indication for genetic counselling in patients who received first trimester assessment was a low risk result in patients older than 40 years. While in patients who only had a second trimester assessment and over 40 years there was a bigger proportion who a high risk result.
There was no significant difference between the 3 groups undergoing screening with regards to who was counselled or whether invasive testing or termination of pregnancy was offered. There was no difference in acceptance of termination of pregnancy, but uptake of invasive testing was highest for those only having a second trimester assessment. The diagnostic yield for a trimester 1 assessment was 26%, compared to 15% in a second trimester assessment. Despite the difference, this is not statistically significant (p=0.2).

Comparison between women who accepted invasive testing and those who declined.

Table 3: Comparison between women who accepted invasive testing and those who declined among the 630 women who were offered invasive testing (excluding 10 who opted for TOP without testing) and expressed in number (%), mean ± SD or median (range).

	Declined	**Accepted**	**p-value**
N (% of those offered)	**516 (81.9%)**	**114 (18.1%)**	
Urban	351 (68%)	73 (64%)	0.4
Rural	165 (32%)	41 (36%)	
Age	40.9 ± 1.9	40.4 ± 1.9	0.02
≥ 40 years old	391 (75.8%)	68 (59.6%)	0.005
Gravidity	4 (1-10)	3 (1-9)	0.06
Parity	2 (0-7)	2 (0-6)	0.8
Previous obstetric history			
Neonatal death	9/513 (1.8%)	3/114 (2.6%)	0.9
Term loss	23 (4.5%)	4 (3.5%)	0.5
T3 loss	17 (3.3%)	1 (0.9%)	0.1
T2 loss	34 (6.6%)	4 (3.5%)	0.1
T1 loss	139 (27.1%)	20 (17.5%)	0.02
Ectopic	11 (2.1%)	0	0.08
T1 TOP	18 (3.5%)	3 (2.6%)	0.5
T2 TOP	4 (0.8%)	0	0.2
Genetic counseling			
Indicated	513 (99.4%)	114 (100%)	0.4
Pre-screen	84 (16.3%)	9 (7.9%)	0.02
Accepts Risk Assessment	39/84 (46.4%)	2 (22.2%)	0.2
+ (opts for invasive testing)	2/84 (2.4%)	5/9 (55.6%)	0.1
+ (opts for TOP)	1/84 (1.2%)	-	-
Accepts any test	41/83 (49.4%)	7/9 (77.8%)	0.7
Who received counseling			0.6
Woman	404 (78.3%)	87 (76.3%)	
Couple	99 (19.2%)	27 (23.7%)	
Woman with other	13 (2.5%)	8 (7%)	
First trimester assessment			

	Declined	Accepted	p-value
Number (%)	203 (39.3%)	23 (20.2%)	0.0001
Gestational age (days)	90.1 ± 4.9 91 (55-102)	91.0 ± 4.1 90 (86-99)	0.6
1: Background Risk	61 (12-159)	65 (25-150	0.1
1: Adjusted Risk	632 (2-2840)	178 (2-2854)	0.04
Risk Adjustment	15.4 (0.02-20.7)	3.8 (0.02-20.2)	0.006
Risk calculated	192 (37.2%)	23 (20.2%)	0.005
Low Risk (< 1:165)	152/192 (79.2%)	13/23 (56.5%)	0.04
High Risk (≥ 1:165)	38 (19.8%)	9 (39.2%)	
High Risk with Anomaly	2 (1.0%)	1 (4.3%)	
Second trimester assessment			
Number (%)	311 (60.2%)	88 (77.2%)	0.0007
Gestational age (days)	144.9 ± 7.5 144 (115-187)	147.0±13.0 146(114-228)	0.03
1: Background Risk	88 (2-1840)	88.5 (9-2854)	0.1
1: Adjusted Risk	354 (2-14525)	64 (2-1505)	< 0.001
Risk Adjustment	7.6 (0.0015-9.48)	1.3 (0.019-9.4)	0.02
Risk calculated	415 (80.4%)	94 (82.5%)	0.6
Low Risk (< 1:200)	230/415 (55.4%)	16/94 (17.0%)	<0.0001
High Risk (≥ 1:200)	162 (39.0%)	51 (54.3%)	
Low Risk with Anomaly	4 (1.0%)	5 (5.3%)	
High Risk with Anomaly	19 (4.6%)	22 (23.4%)	
Any trimester			
Ever High Risk or Anomaly	210 (40.7%)	86 (75.4%)	<0.0001
Prenatal aneuploidy	0	18 (15.8%)	<0.001
Termination of pregnancy			
TOP offered	6 (1.2%)	22 (19.3%)	<0.001
Reason TOP offered			0.004
Genetic	0	18 (81.8%)	
Multiple anomalies	1 (16.7%)	1 (4.5%)	
Central nervous system	1 (16.7%)	3 (13.6%)	
Cardiac defect	2 (33.3%)	0	
Renal	1 (16.7%)	0	

	Declined	Accepted	p-value
Skeletal	1 (16.7%)	0	
Reason TOP not offered	510	92	
Not indicated	507 (99.4%)	92 (100%)	0.5
Too late	1 (0.2%)	0	
Would not terminate	1 (0.2%)	0	
Unknown	1 (0.2%)	0	
TOP done	0	15 (13.2%)	<0.001
TOP uptake	0/6	15/22 (68.2%)	

TOP: termination of pregnancy; T 1-3: Trimester 1-3

Invasive testing was offered to 630 patients (excluding 10 women opting for TOP without invasive testing) and only 114 (18.1%) accepted. There were no significant differences between these groups with regard to gravidity, parity, place of residence (rural or urban) or who was counselled but women who declined were slightly older and more often older than 40 years (75.8% vs 59.6%) and more often had previous first trimester miscarriages and more likely to have received pre-screen counselling (16.3% vs 7.9%; p 0.02). The decision regarding invasive testing was not influenced by the GA or background risk at the first trimester assessment, but acceptance was significantly higher with a higher adjusted risk and less favourable risk adjustment as also seen in the second trimester. Women who declined invasive testing were less likely to have a high risk screening result or diagnosis of a fetal anomaly, but they were more likely to decline TOP when offered.

There were 18 aneuploidies diagnosed (Trisomy 21: 9, Trisomy 18: 7, Trisomy 13: 2). Of those with a diagnosis of trisomy 21, 5 had a termination of pregnancy and 4 continued with the pregnancy (one being already 32weeks pregnant and termination of pregnancy was not offered to her). Six of the 7 pregnancies with trisomy 18 had a termination and one continued with the pregnancy. This baby demised shortly after birth. Both women who received the diagnosis of trisomy 13 decided to continue with the pregnancy. The other genetic diagnoses included Robert's syndrome (termination at 20 weeks gestation) and Beemer Langer type lethal short rib polydactyly syndrome (declined invasive testing and termination of pregnancy, unfortunately we do not have any delivery notes on this baby).

Comparison between women opting for termination of pregnancy and those who declined.

Table 4: Comparison between women opting for termination of pregnancy (TOP) and those who declined, expressed in number (%), mean ± standard deviation or median (range).

	TOP accepted	**TOP declined**	**p-value**
Number (% of TOP offered)	25 (65.8%)	13 (34.2%)	
Urban **Rural**	17 (68%) 8 (32%)	7 (53.8%) 6 (46.2%)	0.3
Age	40.6 ± 2.3	39.9 ± 1.9	0.3
≥ 40 years	11 (44%)	5 (38.5%)	0.07
Gravidity	3 (1-8)	4 (2-7)	0.3
Parity	2 (0-6)	2 (1-5)	0.5
Previous obstetric history			
Neonatal death	0	0	-
Term loss	1	1	0.6
T3 loss	1	1	0.6
T2 loss	2	2	0.5
T1 loss	4	6	0.04
Ectopic	0	0	-
T1 TOP	0	0	-
T2 TOP	0	0	-
First trimester assessment			
Number (%)	9/25 (36%)	4/13 (30.8%)	0.7
Gestational Age (days)	90.3 ± 5.1 [92 (81-96)]	89 ± 5.5 [89 (83-95)]	0.7
1: Background Risk	76.3 (9-150)	86.5 (43-142)	0.3
1: Adjusted Risk	3 (2-2854)	129.5 (9-429)	0.4
Risk Adjustment	0.2 (0.02-20.1)	1.6 (0.1-5.6)	0.3
Risk calculated Low Risk (< 1:165) High Risk (≥ 1:165) High Risk with Anomaly	9 (36%) 4 (44.4%) 1 (11.1%) 4 (44.4%)	4 (30.8%) 2 (50%) 1 (25%) 1 (25%)	0.4
Second trimester assessment			
Number (%)	20 (80%)	13 (100%)	

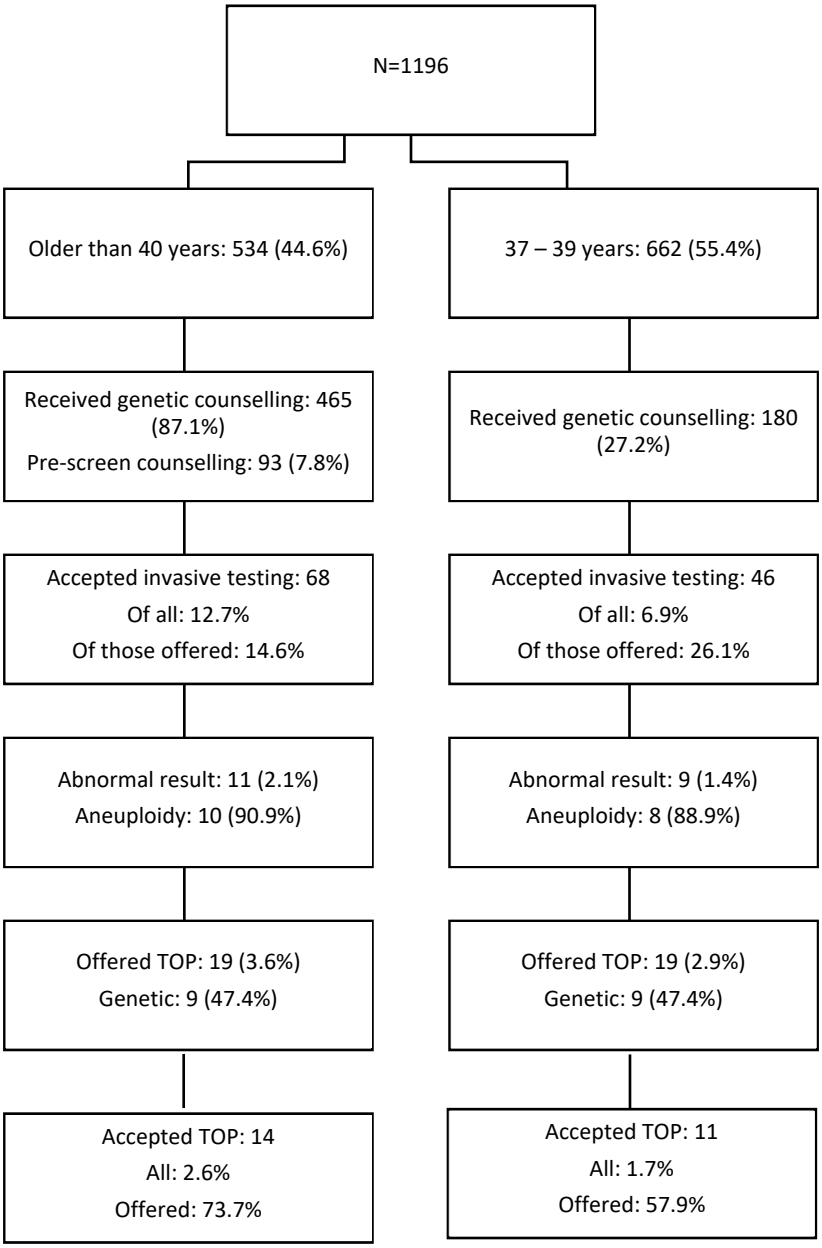
	TOP accepted	TOP declined	p-value
Gestational age (days)	150.6 ± 20.7 [144.5 (114-219)]	149.1 ± 14.2 [144 (135-187)]	0.8
1: Background Risk	103 (28-2854)	96 (9-929)	0.2
1: Adjusted Risk	25.5 (2-1299)	8 (2-946)	0.4
Risk Adjustment	7.6 (0.02-9.06)	7.6 (0.04-8.58)	0.5
Risk calculated	17 (85.0%)	11 (84.6%)	0.5
Low Risk with Anomaly	5	1	0.4
High Risk, no Anomaly	2	1	
High Risk with Anomaly	10	9	
Any trimester			
Ever High Risk result	23 (92%)	12 (92.3%)	0.7
Ever Fetal Anomaly	19 (76%)	10 (76.9%)	0.9
Genetic counseling			
Indication	24/25 (96%)	13 (100%)	0.7
≥ 40 Low Risk	3 (12.5%)	1 (7.7%)	
≥ 40 High Risk	1 (4.2%)	1 (7.7%)	
≥ 40 with Anomaly	10 (41.7%)	8 (61.5%)	
< 40 High Risk	2 (8.3%)	0	
< 40 with Anomaly	8 (33.3%)	3 (23.1%)	
Pre-screen	5 (20%)	0	0.4
Who received counseling	24 (96%)	13 (100%)	0.5
Woman	17 (70.8%)	8 (61.5%)	
Couple	4 (16.7%)	5 (38.5%)	
Woman with other	3 (12.5%)		
Invasive testing			
Invasive test offered	24 (96%)	13 (100%)	-
Invasive test accepted	15/24 (62.5%)	7/13 (53.8%)	0.6
Genetic disorder diagnosed	12/25 (48%)	7/13 (53.8%)	0.3
Prenatal aneuploidy*	11/25 (44%)	6/13 (46.2%)	0.9
Termination of pregnancy			
TOP without invasive testing	10 (40.0%)	0	-
Indication TOP offered			0.7
Genetic	12/25 (48.0%)	6/13 (46.2%)	

	TOP accepted	TOP declined	p-value
Central nervous system	5 (20.0%)	2 (15.4%)	
Cardiac	0	2 (15.4%)	
Multiple anomalies	6 (24.0%)	1 (7.7%)	
Renal	1 (4.0%)	1 (7.7%)	
Skeletal	0	1 (7.7%)	
Maternal disease	1 (4.0%)	0	

*TOP: Termination of pregnancy; T 1-3: trimester 1-4; *: One with trisomy 21 not offered TOP as gestation was 32 weeks.*

TOP was offered to 38 patients and 25 (65.8%) accepted (10 without prior invasive genetic testing), with no significant differences in gravidity, parity, place of residence or who was counselled. There were marginally more women over 40 accepting TOP when offered (p 0.07) and more women with a previous early miscarriage declining (p 0.04). The decision to accept a TOP was not influenced by gestational age at presentation, background risk or risk adjustment in both first and second trimester assessments, or the indication to offer TOP.

Diagram 1: Cumulative overview of findings



Discussion:

Low uptake of invasive testing – decline over time

Women older than 37 years make up a large proportion of the case load seen at the Fetal Medicine unit at Tygerberg hospital (N=1195 patients, 24%; 1492 visits). Most underwent genetic screening (N=1092, 91.4%) and more than half of them received standardised genetic counselling (N=645, 54%) and were offered invasive testing (54%). Of all women who were offered invasive testing only 17.8% accepted (114/640) and another 10 opted to terminate the pregnancy without invasive testing. In total, only 124 women (19.4%) acted on the results discussed during the genetic counselling session. The remainder continued the pregnancy without confirmatory testing, irrespective of the risk or ultrasound findings.

The current uptake rate of amniocentesis of 17.8% was lower than recorded in previous studies. In a retrospective analysis done by Urban et al in 2008 - 2009 the uptake rate in AMA women of Cape Town was 31% (13). In 2006-7 L Geerts reported an uptake rate of 52.3% in AMA women (12), and Viljoen et al reported it to be 74% in 1992-1994 (33). There is a clear decrease in the uptake of invasive testing over the years in Cape Town. The cause of this decline is multifactorial. An interaction of availability of services and culture specific values is at play. Previously screening was done by maternal age alone, at that stage the uptake was high. Another reasons for this could have been health care services was mostly utilized by the higher socioeconomic groups, who are more likely to agree to invasive testing. Later on ultrasound assessments was introduced and 50% of women of advanced maternal age may decline invasive testing based on reduced risk after ultrasound assessment (12). Fetal abnormalities and high risk ultrasound screening increased as indications for invasive testing. One would expect the introduction of ultrasound based risk assessment to have increased the uptake of invasive testing with our current protocol. We see the exact opposite in this audit, suggesting that the uptake of invasive testing goes beyond absolute risk assessment.

A resource-poor country like South Africa cannot afford not including a routine ultrasound service in their obstetric service delivery(12). Besides the clear advantage this hold for genetic screening, the World Health Organization recommends, in their antenatal care recommendations of 2016, that all pregnant women should have one ultrasound scan before 24 weeks gestation. This will aid in estimating gestational age, detecting fetal anomalies (including chromosomal abnormalities) and multiple pregnancies, and improve a women's pregnancy experience.

Factors influencing the uptake of invasive testing

Prenatal screening and diagnosis is a complex interaction between risk assessment, patient perceived risk and decision making constructs. A systemic review by Gadino et al classified factors influencing women's decision about invasive testing as external and psychosocial factors. External factors being opportunity for screening, screening results and the use of genetic counselling. Psychosocial factors included ethnicity, socio-demographic status and attendance of partners during counselling (34).

Policy

The first external factor influencing uptake of invasive testing is the policy governing the availability of prenatal screening and testing. Policies will include guidelines on both counselling, screening and testing. It will address the timing of counselling, for instance pre- or post-screen counselling, as well as method and timing of screening. Testing guidelines will determine whether or not invasive testing is offered to all or only those with a high risk after screening. For example in China there was a policy change in 2006. Prior to 2006 all women was offered invasive testing or screening. After 2006 only women with a high risk screening result or a fetal anomaly was offered invasive testing. Before the policy changed 40.5% of women older than 35 years opted for invasive testing and after only 10.6% (35).

At Tygerberg hospital we now offer age- and ultrasound based screening to all women 37 years and older at conception. Before 2008 we use to offer invasive testing to all women older than 37 at conception, and found that the yield was extremely low if invasive testing was done after a normal scan. This study and population-based findings has shown a much higher screening sensitivity and efficiency by ultrasound than by maternal age alone (12). Another contributing factor is the focus of the counselling. Before 2006, much emphasis was placed on the risk associated with advanced maternal age. After the introduction of ultrasound based screening most of the counselling is focused on individualized risk after screening. In the current study, 358 of the 662 women who were younger than 40 (54.1%) had normal ultrasound findings and a low risk result, and therefore were reassured and not offered further testing. The change in screening policy dramatically decreased the amount of tests being done in women aged 37-39 and the cost associated with karyotyping. This reduction in cost may be more than the cost to introduce a routine obstetric ultrasound service.

Another cost reduction strategy would be to introduce pre-screen counselling for patients at their referral site. This will especially be beneficial for rural patients, as this will avoid unnecessary referrals. Only 18% of patients older than 40 years received pre-screening genetic counselling, but in this group only 45% (42/93) accepted further screening or testing. Pre-screen counselling enables the patient to

make informed, preference-based screening decisions and is fundamental in prenatal screening. Pre-screen counselling is beneficial to the patient and the obstetric service budget.

Screening result

Higher risk – higher uptake?

The second external factor is the screening result. Overall, 318 (30.5%) women in this study were ever considered to be at high risk for aneuploidy after their ultrasound assessment and the uptake of invasive testing was significantly influenced by a higher adjusted risk and a smaller risk adjustment in both first and second trimester assessments, and the disclosure of a high adjusted risk (above the cut-off). Vergani et al showed that a normal ultrasound is three times more likely to change a women's initial interest in amniocentesis than an abnormal ultrasound finding in a patient who is not interested in invasive testing from the start (20% versus 7%, $p < 0.001$, OR = 3.2, 95% CI 1.8; 5.8)(36). Kuppermann et al (25) found that women who attached high value to the screening result were more likely to undergo invasive testing but this was not always true as 6.6% of women who obtained a low risk result still opted for invasive testing and 29.5% of women who had a high risk result declined testing.

Prior opinion more important

Our findings were more in line with those of Marini et al (38), who found that 52% of women with high risk screening results still declined invasive testing. We found that 210 of 296 women (70.9%) with a high risk screening result still declined testing, while 28 of 434 women with a low risk result (who were offered testing based on their age) opted for an invasive procedure (6.5%).

Furthermore Vergani et al (36) found that the greatest predictor of uptake of invasive testing is the patients prior opinion regarding testing. They found that the majority of patients stayed with their initial decision (83% proceeded with testing who was in favour of testing and 96% who initially declined, still declined). Most women (74.3%) in our study said they would accept a child with Down syndrome and as they would not terminate the pregnancy, they also declined invasive testing. Other reasons for declining testing were the risk of miscarriage and perceiving the adjusted risk as acceptable.

Patient characteristics

The psychosocial factors like ethnicity and socio-demographic status were not fully assessed in this study but we found that gravidity, parity and place of residence (rural or urban) did not have a significant influence on the uptake of invasive testing. We found that older women (more likely of rural residence) were more likely to be offered invasive testing, but less likely to accept it. Women with a history of first trimester losses were also more likely to decline. In keeping with other studies, the

decision was not influenced by the presence of a partner or family member during the counselling session. Other reasons why there was such a low uptake could be that most of the higher socioeconomic status patients attend antenatal care in the private sector and these are the patients who are more likely to accept prenatal testing.(39) And the majority of South Africans are largely still traditional/religious and survival orientated (40), which was evident in the fact that 74% said they would not consider termination of pregnancy. On the other hand patients are much more aware of their "patient's rights" now and this could also influence their decision regarding prenatal testing.

The effects of genetic counselling (group sessions and individual counselling) on decisional conflict, anxiety and perceived risk were not assessed in this study. Kaiser et al suggests psychoeducational interventions for specific vulnerable population groups. These targeted population groups may include women who have a high level of uncertainty, decisional conflict or anxiety and women with an inflated or diminished perception of risk. This may enable one to deliver specialized targeted counselling or support services.(41)

Timing of assessment

Most procedures were done (91.2%) and most genetic disorders and fetal anomalies detected after a second trimester assessment, considering only 455 (38%) of women were assessed in the first trimester and 933 (78%) in the second trimester. The uptake of invasive testing (when it was offered) after the first trimester assessment was only 20.4% (11/54) but 296 women were reassessed in the second trimester (65%, more for women who had a previous pregnancy loss). The uptake after second trimester screening was 17.9% (104/580, p = 0.6) overall. It was quite low for those who had been reassessed (9.1%) but this group increased the total uptake for those with a T1 assessment by 145% (26 versus 11). First-trimester ultrasound combined with maternal age has a high sensitivity and low false-positive rate to detect trisomy 21 and resulted in a significantly greater risk adjustment than in the second trimester, with a low-risk result in 91.9% (136/148) of cases (81.8% (372/455) when also including those that were reassessed later).

Patients who had only a T2 assessment had the highest adjusted risk and only 66.4% were at low risk after screening (447/673). While a second trimester sonogram is indicated in all pregnancies to detect fetal abnormalities, this will also increase the detection rate of trisomy 21 (42) as it increases the number of women with access to screening in communities where late booking is common.

If patients decline prenatal genetic screening, one should still offer a second trimester fetal anomaly scan as this led to an additional 43 anomalies being detected in the current study (an extra 10 after a T1 assessment). This is in keeping with other local studies that show a community based ultra-sound

service has many additional benefits.(43) When no structural fetal anomaly is found, the value of recalculating the risk in the second trimester seems limited as it only changed the decision in 9 women in whom the risk category changed (2 tested because the risk became high and 7 did not test because the risk became low).

Fetal anomaly

The uptake of invasive testing in patients where a fetal anomaly was seen on ultrasound assessment was much higher (52.8%) when compared AMA alone (17.8%). An audit done in the Cape Town Metropole West area found the uptake of amniocentesis after an abnormal scan to be considerably higher (66%) than only a high risk screen result (54%) and maternal age alone (31%).(14) The routine ultrasound scan in the public sector has been undervalued over the years, due to the questionable contribution to the change in perinatal outcome.(44) However, Talip et al has shown an increase in the relative role of fetal anomalies to perinatal losses over the decades, from 7.9% in 1986 to 11.4% in 2007.(45) According to the savings babies report (2014-2016) congenital abnormalities is the fourth most common cause of neonatal death in the Western Cape. Additional advantages of an routine ultrasound service is: more accurate dating and its sequelae and early diagnosis of multiple pregnancies.(44) Women and their partners are more likely to attend an ultrasound assessment than genetic screening based on advanced maternal age alone.(14)

Yield of genetic testing.

We found 20 genetic diagnoses (1.67% of all patients) including 18 abnormal karyotypes from 114 tests resulting in a yield of 15.8%, which is high compared to screening by maternal age alone (4.7%), by serum screening (7.5%)(46) or NT screening (3%) (47) and is (partially) due to us using multiple markers and structural anomalies as the screening protocol. The yield is higher than in the previous study in this area that found a yield of 2.9% in all women over 37 undergoing testing (2.3% for the common trisomies), with a yield of 9.3% (1:11) for aneuploidy in those who had screened HR after US assessment and no aneuploidies found in 88 karyotype results if ultrasound findings had been normal.(12) The higher yield may be reflection of our more recent policy to restrict the offer of invasive testing in women between 37 and 39 years of age to those who screen HR after US assessment, either based on the presence of soft markers or a fetal anomaly. A retrospective study from a single centre in United states found an diagnostic yield of 9.6% for chromosomal abnormalities. They also found the combination of ultrasound and maternal serum testing was most effective in detecting chromosomal abnormalities (sensitivity of 92%).(46) Our yield is higher because we used a high risk population based exclusively on advanced maternal age and high risk ultrasound assessment while the other study included many other inclusion criteria for example maternal serum screening and anxiety.

Uptake of TOP

Termination of pregnancy was offered to 3.2% of patients and 65% accepted (25/38) and this decision was not influenced by maternal characteristics except previous early miscarriage, or by adjusted risk. This correlates with data from a study done in Johannesburg where the uptake was 63%.(48) Stewart et al (2008) fond the uptake of termination of pregnancy to be predominantly influenced by the type of fetal anomaly detected.(49) The most common indication for termination of pregnancy in our study was genetic, followed by CNS abnormalities and multiple fetal abnormalities. TOP was accepted by 61.1% (11/18) of patients with a diagnosis of aneuploidy, 5 of which was trisomy 21.

In Denmark if Down syndrome is diagnosed the termination rate is more than 95%. They found in a qualitative interview study that all couples already made the decision regarding termination before the diagnosis of Down syndrome. Therefore, awareness of a couple's a priori decision will assist patient centered communication during prenatal screening and testing. It also illustrates the importance of pre-screen counselling. All patients should receive adequate information regarding prenatal genetic screening and testing. This information can be given in a group session but should consider the patients' culture and educational level. An individual session should follow the group session to answer any question and enable the patient to make informed, preference-based screening decisions.

Limitations:

The study population only included patients who were referred to Tygerberg Hospital and not all AMA women in the catchment area. There may be a large proportion of AMA patients who did not present for antenatal care or who were not referred to Tygerberg hospital in time for screening. As formal pre-screen counselling was not offered at the time, the chance of selection bias based on patient choice is considered to be small and, if present, would probably have resulted in a cohort more interested in prenatal screening than the background population, not less. The quality, timing and method of counselling was not assessed but all counseling was provided by medical geneticists or qualified genetic counsellors. No information on prior intent or prior knowledge of the patients was available during the study period and only the patients who received pre-screen counseling provide some indication of common views, at least in women over 40.

There was no post-natal follow up of the infants to determine the sensitivity and false positive rate of our screening protocol but this was assessed in 2006-2007 in a similar cohort of women over 37 and found to be highly effective. Retrospective data analysis prevented us from collecting all the necessary data for all the patients.

Conclusion:

The uptake of invasive testing is low and showed a clear decline over time. One would expect uptake to increase after the introduction of ultrasound based screening, but despite this change in policy we continue to see a decline. Pre-screen counselling is identified as an important first step to avoid unnecessary referral and imposing additional strain on the tertiary services. The aim is to offer pre-screen counselling at all referral centres in the future. This process has started by a standardised informative video on T21, screening and testing, followed by individualised post-video counselling done by a trained midwife at district and secondary level hospitals. Only women who express interest in screening and testing, or those who are undecided, are referred.

The uptake of invasive testing is affected by the adjusted risk. First trimester screening offers the best risk reduction and highest percentage for classification as low risk, therefore emphasis should be placed to start screening with a first trimester assessment as it reduces the need for formal genetic counseling in women aged 37-39 and reduces the number of women opting for invasive testing.
The use of an effective screening algorithm in the second trimester (FMF) is also paramount to stratify risk, although it is less effective in risk reduction. In this study there was no significant difference in diagnostic yield whether or not screening started in the first or second trimester. For women who decline prenatal genetic screening or who screen low risk at the first trimester screen, a second trimester fetal anomaly scan is still important, with the main aim to detect structural anomalies. Second trimester scanning for all women is important, especially in South Africa due to late presentation and referral which precludes women from utilising the more optimal aneuploidy screening in the first trimester.

Even though a routine, well integrated, ultrasound service has many advantages to providing antenatal care, one should always remember culture specific concerns. In other African settings the diagnostic power and therapeutic abilities of ultrasound have been misunderstood and many patients expected too much once an abnormality was detected.(50) This can specifically be a problem in South Afrika because there is very limited, if any, access to intrauterine procedures, which are available in the developed world. South Africa, and specifically the population who uses the public health sector still includes strongly tradition/religious based decision makers. Therefore, most women will not consider termination of pregnancy for Trisomy 21. Trisomy 21 is quite well accepted in our society and the government offers disability grants to support families. For this reason, the introduction of more expensive screening modalities (maternal serum or NIPT) is not a priority within the public health care

system. To the contrary, ultrasound based screening has many other advantages besides identifying possible Trisomy 21. Ultrasound based screening will therefore remain the mainstay of prenatal screening in this country, it is one small step for genetic screening and one giant leap for antenatal care.

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