An audit of patients seen by genetic counsellors for a history of recurrent miscarriages

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A research report (in the format of a "submissible paper") submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements of the degree of Master of Science in Medicine (Genetic Counselling)

Johannesburg, 2022

Declaration

I, **Zandisiwe Goliath**, declare that this research report (in the format of a "submissible" paper), is my own, unaided work. It is being submitted for the degree of Master of Science in Medicine (Genetic Counselling) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

Z Goliath

25th day of February 2022, in Johannesburg.

Contribution of the candidate to the paper

Declaration: Student's contribution to article and agreement of co-authors

I, Zandisiwe Goliath, student number 806629, declare that this Research Report is my own work and that I contributed significantly towards research findings presented in the paper intended for publication.

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Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article(s) by the student as part of her Research Report.

Article Title: An audit of patients seen by genetic counsellors for a history of recurrent miscarriages

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Dedication

This research report is wholeheartedly dedicated to every woman who has experienced the devastating events of recurrent pregnancy losses. You have shared your stories about the loss and grief you have encountered, and we cannot disregard how difficult that might have been for you.

Abstract

Introduction: Recurrent miscarriage is traditionally defined as three or more early (≤24 weeks' gestation) consecutive pregnancy losses. There are several common causes of recurrent miscarriages, however, 40-60% of cases remain unexplained. Unbalanced translocations resulting from balanced translocations in one of the parents, account for only 2-5% of recurrent miscarriage cases. Therefore, parental karyotyping should not be the first-line investigation for recurrent miscarriages and patients should be thoroughly worked-up prior to being referred for genetic counselling. The recommended investigations for recurrent miscarriage include screening for antiphospholipid antibodies, thyroid screening and the assessment of uterine anatomy.

Aim: The overarching aim of this study was to perform an audit of patients referred for genetic counselling for recurrent miscarriage through the Genetic Clinics of the Division of Human Genetics, National Health Laboratory Service and the University of the Witwatersrand.

Methods: The study was a retrospective file review that used quantitative research methods. A total of 101 files of patients who were referred for genetic counselling for 'recurrent miscarriages' and/or 'poor obstetric history' from the years 2011 to 2021, were assessed. A data collection sheet was designed to collect specific patient details. These details included: maternal age at miscarriage, gravidity and parity, number of miscarriages, trimester in which miscarriages occurred and investigations performed. Data were captured and analysed on Microsoft Excel.

Results: The mean (standard deviation) age of the patients at the time of the consultation was 34.4 ± 5.9 years. The median (interquartile range) number of miscarriages per individual was 4 (3-5) per person. The majority of miscarriages occurred within the first trimester (72.7%, 308/430) of pregnancy. Only 14.0% (14/101) of the patients had all the required investigations performed prior to being referred for genetic counselling. Of the 101 patients that were seen, 43.6% (44/101) of the adult females and 26.7% (27/101) of their male partners with a history of

recurrent miscarriages underwent karyotyping. Out of the patients that underwent karyotyping, only 1.4% (1/71) of the results indicated the presence of a chromosomal abnormality. In 20 of the individuals with a history of recurrent pregnancy losses, their products of conception (POC) were tested and in 50% (10/20) of those cases, an abnormal genetic result was found.

Conclusion: The purpose of this study was to determine what investigations patients with recurrent miscarriages undergo in academic State Hospitals in Johannesburg, South Africa. This study highlighted that very few women were fully worked up prior to their referral for genetic counselling. Furthermore, this study revealed that parental chromosomal abnormalities accounted for a small percentage (44.6%, 1/44) of recurrent miscarriages, therefore, genetic testing should not be a priority the first-line investigation for recurrent miscarriages, and it would be worth prioritising the other recommended investigations over this.

Acknowledgments

This study was initially just an idea in my mind that Ms Elzette Nienaber and Dr Shelley Macaulay helped bring to life. I would like to express my sincere gratitude to my supervisors for your patience and investment in helping me see this project through. You have imparted a great deal of knowledge that I will always value.

To Professor Lombaard, thank you for the input and time you have added to my project. Good luck with your new journey.

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To my friends, I would like to thank you for being readily available to listen to what my research project and career entails. Your support and interest in my goals and desires does not go unnoticed. I hope that you all find your purpose and the same sense of fulfillment that I have.

To my parents and siblings, you have all had to experience the amount of stress and pressure that I have gone through in my chosen career. I know that you are extremely proud of me and I thank you for the love and support that you have shown and given me.

Lastly, to the patients included in this study, you may never get the opportunity to read this research report but I would like to thank you all. You are incredible women and men who have gone through significant challenges, and I wish you nothing but the best of what this world has to offer.

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List of Abbreviations

IQR	Interquartile range
N	Absolute number
SD	Standard deviation
%	Percentage
WHO	World Health Organization
BMI	Body Mass Index
ESHRE	The European Society for Human Reproduction and Embryology
ASRM	The American Society for Reproductive Medicine
APS	Antiphospholipid syndrome
Array-CGH	Microarray-based Comparative Genomic Hybridisation
DNA	Deoxyribonucleic acid
NHLS	National Health Laboratory Service
Wits	The University of the Witwatersrand
QF-PCR	Quantitative Fluorescent Polymerase Chain Reaction
FISH	Fluorescence in Situ Hybridisation
POC	Products of conception
RCOG	The Royal College of Obstetricians and Gynaecologists
AMA	Advanced maternal age

Research report in the format of a submissible paper

This work has been written up as a paper for submission to the Journal of Health Communication. The author guidelines, including word count of abstract, and referencing style have been adhered to and can be found in Appendix A. However, tables and figures have been inserted in text for ease of reference.

An audit of patients seen by genetic counsellors for a history of recurrent miscarriages

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Abstract for paper submission

Running title: An audit of patients seen by genetic counsellors for a history of recurrent miscarriages

Keywords: Chromosome aberrations, genetics, poor obstetric history, recurrent miscarriages, spontaneous abortion

Abstract

Aim: The aim of this study was to perform an audit of patients referred for genetic counselling for recurrent miscarriage through the Genetic Clinics of the Division of Human Genetics, National Health Laboratory Service and the University of the Witwatersrand.

Methods: The study was a retrospective file review that used quantitative research methods. A total of 101 files of patients who were referred for genetic counselling for 'recurrent miscarriages' and/or 'poor obstetric history' from the years 2011 to 2021, were assessed. Data were captured and analysed on Microsoft Excel.

Results: The mean (standard deviation) age of the patients at referral was 34.4 ± 5.9 years. The median (interquartile range) number of miscarriages per individual was 4 (3-5) per person. The majority of miscarriages occurred within the first trimester (72.7%, 308/430). Only 14.0% (14/101) of the patients had all the required investigations performed prior to being referred for genetic counselling. Of the 101 patients that were seen, 43.6% (44/101) of the adult females and 26.7% (27/101) of their male partners with a history of recurrent miscarriages underwent karyotyping. Out of the patients that underwent karyotyping, only 1.4% (1/71) of the results indicated the presence of a chromosomal abnormality.

Conclusion: This study highlights that very few women presenting with recurrent miscarriages are fully worked up prior to their referral to genetic counselling. Furthermore, unbalanced translocations account for a small percentage of recurrent miscarriages and testing of products of conception may be beneficial in giving accurate recurrence risks.

[245 words]

Introduction

Miscarriage is one of the most common complications in pregnancy. Previously, the most widely used definition of miscarriage was one proposed by the World Health Organization (WHO) which was devised in 1977. It defined miscarriage as the "expulsion or extraction from its mother of an embryo or fetus weighing 500 g or less" (1). Currently, miscarriage is defined as the loss of a pregnancy prior to 24 weeks gestation (fetal weight <500 g) when a fetus is not considered viable. The viability is largely influenced by the advances of neonatal care within that population (2).

Recurrent miscarriages are defined as three or more early (<24 weeks gestation) consecutive miscarriages (3). A contrast between primary and secondary recurrent miscarriages has been made; primary recurrent miscarriages are described as having a history of no viable pregnancies beyond 24 weeks, and secondary recurrent miscarriages are defined as having a history of recurrent miscarriages after having one or several pregnancies progressing further than 24 weeks (4). There are several guidelines that propose evidence-base care of recurrent miscarriages. Several similarities and differences have been noted in these guidelines pertaining to the definition of recurrent miscarriages (5). There are four proposed elements in the definition of recurrent miscarriages. The first element considers the type of pregnancy (biochemical, visualised or intrauterine). The second element is the period, in weeks, in which the pregnancy loss is considered a loss. The third element defines recurrence i.e. the number of times a pregnancy loss has occurred and the final element takes into consideration whether the miscarriages are considered to be consecutive or not (3).

Endocrine abnormalities, autoimmune disorders, anatomical uterine anomalies, as well as parental chromosomal abnormalities are considered to be the common causes of recurrent miscarriages. However, approximately 50% of recurrent miscarriages have an unknown aetiology and are largely unexplained (6).

Lifestyle factors such as caffeine, alcohol and smoking are environmental factors associated with spontaneous pregnancy losses but have been difficult to correlate to recurrent miscarriages (7). Obesity, which is defined as body mass index (BMI) ≥ 30 kg/m^2, has been associated with adverse reproductive outcomes. An increased BMI has an effect on oocyte quality and impacts the molecular and endocrinological functioning of the endometrium (8). Obesity is often discussed in the topic of recurrent miscarriages because of its association to

maternal medical conditions such as polycystic ovarian syndrome, Type 2 diabetes mellitus and hormonal imbalances (9).

Amrane and McConnell (2019), reviewed available data on the endocrinopathies associated with recurrent miscarriages. They listed several disorders including thyroid diseases, vitamin D deficiency, luteal phase deficiency, insulin resistance and prolactin disorders (10). In a study performed in Cape Town, South Africa, polycystic ovarian syndrome, impaired glucose tolerance, diabetes mellitus and thyroid dysfunction were found to be the most commonly investigated endocrine disorders associated with recurrent miscarriages in their setting (9). The European Society of Human Reproduction and Embryology (ESHRE) guidelines and the American Society for Reproductive Medicine (ASRM) both recommend thyroid screening as the only endocrine investigation for recurrent miscarriage (3).

Uterine structural abnormalities can be classified as either congenital defects (septate, bicornuate, unicornuate, didelphys and arcuate) or acquired uterine defects (fibroids, polyps and adhesions). Assessment of uterine anatomy is recommended for individuals with a history of recurrent miscarriage (11). Cervical incompetence is the inability of the cervix to maintain the pregnancy until the fetus reaches viability or term. It may be congenital, where it is associated with uterine malformations (acquired) which may be secondary to forced cervical dilatation or physical injuries, or it may be classified as functional where it may be due to molecular or hormonal imbalances (1). An insertion of a cervical cerclage is the recommended management (12).

Several autoimmune conditions have been associated with the potential cause of recurrent miscarriages. Antiphospholipid syndrome (APS) is the most clearly defined autoimmune disorder that affects 15-20% of couples. It is an autoimmune disorder that is characterised by thrombotic episodes and pregnancy complications (13). The evaluation of APS involves routine screening of the following antibodies according to the ASRM and ESHRE guidelines: lupus anticoagulant, anticardiolipin antibody, and anti- β2 glycoprotein I. Other existing tests to investigate autoimmune disorders include antithyroid, antinuclear and antisperm antibodies (4).

Recurrent miscarriages are often assumed to originate exclusively from women, however, it has been reported that men who have partners with a history of recurrent miscarriages might have elevated sperm chromosomal aneuploidy and/or abnormal semen quality (14). However,

the evidence surrounding the effects of male partners on recurrent miscarriage is insufficient. In the case of recurrent miscarriages due to male factors, it is thought that the sperm has deoxyribonucleic acid (DNA) damage which might be exacerbated by lifestyle habits such as smoking, obesity, and excessive exercise. The recommended management for male factors includes assessing lifestyle factors and assessing sperm DNA fragmentation (15).

Parental chromosomal abnormalities account for 2-5% of recurrent miscarriages. Unbalanced translocations, as a result of balanced translocations in parents, are the most common chromosomal aberrations resulting in early pregnancy loss (6). Whilst carriers of balanced translocations are phenotypically normal, their pregnancies are at risk of miscarrying or having a live birth with multiple congenital abnormalities (6). When a chromosomal abnormality is suspected as the cause of recurrent miscarriage, genetic testing should be performed on the products of conception. The recommended genetic testing on products of conception includes karyotyping or microarray-based Comparative Genomic Hybridisation (array-CGH) which is considered to be the ideal test. The array-CGH is a cytogenetic method that is used to detect DNA copy number gains and losses as well as unbalanced chromosomal aberrations. Other genetic testing techniques that can be performed on products of conception include, Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR) which is a molecular cytogenetic technique that is used for aneuploidy detection. Karyotyping is a classic cytogenetic technique that involves the isolation, staining and visual examination of chromosomes and FISH is a molecular cytogenetic method that is used to detect and localise the presence and absence of specific DNA sequences on a chromosome *(22)*.

If an unbalanced translocation is detected in the fetus, parental karyotyping ought to be performed (15).

Pregnancy defines women as being able to reproduce and provides them with the identity of being a mother. Miscarriage has a devastating impact on couples that may leave long-lasting effects (18). The loss of a pregnancy is a traumatic event and in cases of recurring miscarriages, the grief-reaction is magnified (19). The loss of a pregnancy has shown to affect men and women differently. Men have often experienced feelings of frustration, lack of control, rage, guilt and lowered self-esteem whereas women have shown a decline in self-worth, distorted body image and severe marital discord (20). Determining the cause of recurrent miscarriages could provide couples with answers. Finding a cause might allow for intervention that will reduce the recurrence risk, however, this remains a challenging

dilemma for healthcare professionals. Offering psychological support remains imperative for women who experience recurrent miscarriages. Genetic counsellors have the ability to address the psychosocial needs that couples with a history of recurrent miscarriage present with (15).

The aim of this study was to perform an audit of patients seen for recurrent miscarriages through the Genetic Clinics of the Division of Human Genetics, National Health Laboratory Service (NHLS) and the University of the Witwatersrand (Wits).

Methods

Study patients

The Division of Human Genetics, NHLS and Wits offers genetic counselling services at various academic hospitals in Johannesburg, South Africa. This study comprised the review of files from patients that were seen at the Genetic Clinics for recurrent miscarriages and poor obstetric history. Patients who seen for recurrent miscarriages are considered to be high risk for obstetric complications and are managed at tertiary level facilities that offer specialised care for patients referred from regional hospitals, district hospitals and or community health centres (21) .

Sample size

The Division of Human Genetics, NHLS and Wits has an electronic and manual record-keeping system wherein all patients that are seen are recorded. Over a period of almost 11 years, between January 2011- August 2021, a total of 75 patients were recorded under 'recurrent miscarriages', and a total of 26 patients were recorded under 'poor obstetric history'. This allowed for a total of 101 files to be audited for this study. No exclusion or inclusion criteria were used. All the files for patients with a referral for recurrent miscarriage and/or poor obstetric history were included in the study.

Data collection

A data collection sheet was designed to collect specific patient details. These details included: whether they were pregnant at time of referral, maternal age at consultation, gravidity and parity, total number of miscarriages, trimester in which miscarriages occurred, whether the miscarriages were consecutive or not, investigations performed prior to genetic counselling and information surrounding the products of conception. Data were solely extracted from patients' genetic files. No external patient databases were used to collect patient information for the study.

Data analysis

This study made use of basic descriptive statistics that were analysed using Microsoft Excel. The Shapiro Wilk test was used to test the normality of continuous variables. Normally distributed variables were presented as means and standard deviations (SD). Non-normally distributed variables were reported as medians and interquartile ranges (IQR). Categorical variables were represented as frequencies and percentages.

Ethics

Ethics approval was obtained from the Human Research Ethics Committee (HREC) at the University of the Witwatersrand (clearance number: M210264). The identity of the participants were assigned codes in order to maintain confidentiality. The linked data was stored separately from the populated data sheet (Appendix 1 & 2). Appendix B was encrypted with a password that only the principal investigator had knowledge of.

Results

Patient demographics

During the data collection period, files, representing 101 women were reviewed for this study. Table 1 describes the characteristics of the patients. The majority of the patients were of black African ancestry (59.4%, 60/101). The mean (SD) maternal age at the time of referral was 34.5 ± 0.6 years. Around half of the patients were pregnant at time of referral (49.5%, 50/101).

Table 1: Demographics of the patients referred for recurrent miscarriages and/or poor obstetric history

	N (%) or mean ± SD
Characteristics	**n=101**
Ethnicity	
Black	60 (59.4%)
White	7 (6.9%)
Asian	7 (6.9%)
Mixed-race	3 (3.0%)
Unknown	24 (23.8%)
Maternal age at referral (years)	34.5 ± 0.6
<35 years	53 (52.5%)
≥35 years	48 (47.5%)
Pregnant at referral	50 (49.5%)
Reason for referral	
Recurrent miscarriages	75 (74.3%)
Poor obstetric history	26 (25.7%)

Patient's obstetric history

Table 2 provides information regarding the patients' obstetric history. The median (IQR) number of pregnancies that the patients previously had was 5 (4-7). The median number (IQR) number of miscarriages, per person, was 4 (3-5). The majority of the miscarriages occurred within the first trimester (72.7%, 308/43) of pregnancy. A large portion of the miscarriages were consecutive (84.2%, 85/101).

Table 2: Patients' obstetric information

Obstetric information	Median (IQR) or n (%)
Gravidity	5 (4–7)
Parity	0 (0–5)
Total number of miscarriages (n= 430)	4 (3–5)
Two miscarriages	15 (14.9%)
Three miscarriages	31 (30.7%)
Four miscarriages	22 (21.8%)
Five miscarriages	10 (9.9%)
Six miscarriages	9 (8.9%)
Seven miscarriages	3 (3.0%)
Eight miscarriages	6 (5.9%)
Nine miscarriages	4 (4.0%)
Ten miscarriages	1 (1.0%)
Trimester at miscarriage (n= 424) †	
First	308 (72.7%)
Second	98 (22.8%)
Third	18 (4.2%)
Consecutive miscarriages	85 (84.2%)
Family history of miscarriages	11 (11.0%)

†In 6 of the patient files, the trimester in which the miscarriages occurred, was not stated.

Recurrent miscarriage investigations

Table 3 indicates the investigations that were performed on the patients prior to their referral to the Genetic Clinics. Over half of the patients underwent autoimmune testing. Several of the patients had abnormal autoimmune testing results (14.9%, 15/101), however, they were not referred with a confirmed diagnosis of an autoimmune disorder. It is important to note the potential under-reporting of anatomical screening results as patient's results may be reported in files that remain in the gynaecology units. Screening for uterine abnormalities was relatively poor (26.7%, 27/101). Relatively half (51.9% 14/27) of the patients that had uterine screening had abnormal results. The majority (64.3%, 9/14) of the patients that had abnormal uterine screening were noted to have fibroids. Just over half of the patients (56/101, 55.4%) underwent thyroid screening of which a relatively small number of participants (3/56, 5.4%) had an abnormal thyroid test result. Whilst a proportion of patients with abnormal autoimmune and thyroid screening results were noted, no official diagnoses was made by their treating gynaecologist/obstetrician , thus no confirmation of a potential cause was made.

Table 3: Recurrent miscarriage investigations performed on patients prior to referral for genetic counselling

	N (%)
Investigations performed	**n=101**
Autoimmune testing	
Anticardiolipin antibodies	70 (69.3%)
Abnormal anticardiolipin antibody	6 (8.6%)
Anti-β2 glycoprotein-I antibodies	66 (65.3%)
Abnormal anti-β2 glycoprotein-I antibody	1 (1.5%)
Antinuclear antibodies	61 (60.4%)
Abnormal antinuclear antibody	5 (8.2%)
Lupus anticoagulant	56 (55.4%)
Abnormal Lupus anticoagulant	3 (5.7%)
Uterine anatomical screening	**27 (26.7%)**
Abnormal uterine anatomical screening	14 (51.9%)
Fibroids	9 (64.3%)
Cervical incompetence	4 (28.6%)
Bicornuate uterus	1 (7.1%)
Thyroid stimulating hormone (TSH) screening	**56 (55.4%)**
Abnormal TSH result	3 (5.4%)

Recommended recurrent miscarriage investigations

Figure 1 depicts which of the recommended investigations by the RCOG for recurrent miscarriages were performed on the patients in our study. Only 14% of the patients had all the recommended investigations performed prior to their referral for genetic counselling.

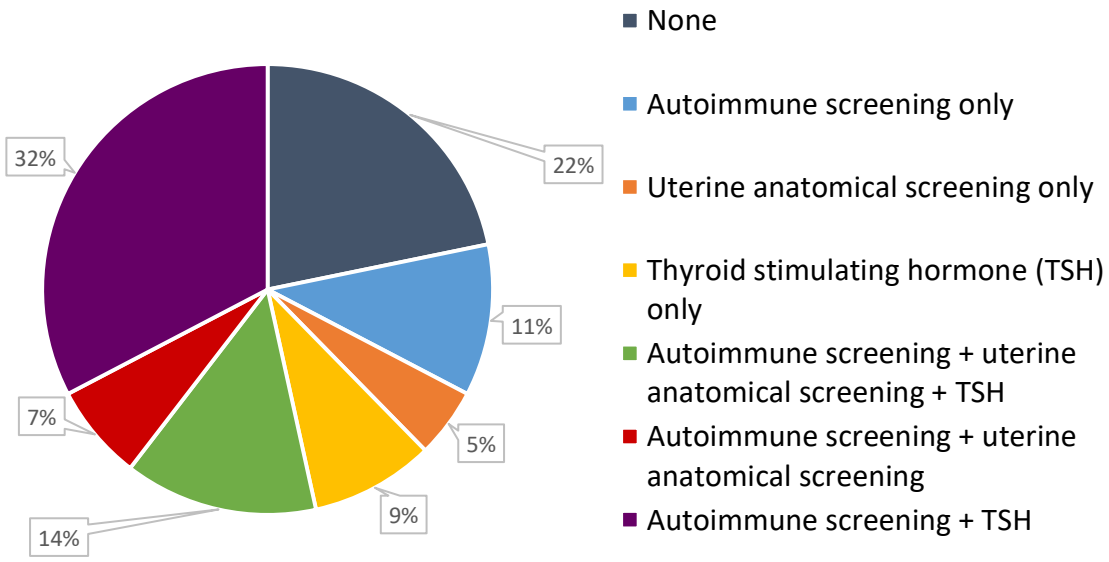

Figure 1: The proportion of recommended investigations performed on patients with recurrent miscarriages prior to referral for genetic counselling

Genetic testing performed on patients

Of the 101 patients that were referred for genetic counselling, only 43.6% (44/101) of the patients underwent karyotyping and only one of the patients had an abnormal karyotype result (47,XX,+17[2]/46,XX [18]). The male partners of patients were also offered karyotyping and only 26.7% of the paternal karyotypes were performed and none yielded an abnormal result. Table 4 indicates the genetic investigations that were offered to patients and those that were performed on the patients and their partners.

Table 4: Karyotype investigations performed on parents

	N (%)
Karyotype analysis	**n=101**
Maternal karyotyping performed	44 (43.6%)
Normal karyotype	40 (90.9%)
Abnormal karyotype§	1 (2.5%)
Unsuccessful karyotype	3 (6.8%)
Paternal karyotyping performed	27 (26.7%)
Normal karyotype	26 (96.3%)
Abnormal karyotype	0 (0.0%)
Unsuccessful karyotype	1 (3.7%)

§Abnormal maternal karyotype: 47,XX,+17[2]/46,XX[18]

Products of conception

A relatively small proportion (20/101, 19.8%) of patients had testing performed on their products of conception (POC). Table 5 indicates the different genetic tests that were used to investigate the POC and the outcomes.

All of the abnormal results on POC testing were aneuploidies. The majority of products of conception that yielded abnormal results (7/10, 70%) were from women of advanced maternal age (≥35 years). The testing of POC might not provide a couple with the cause of their recurrent pregnancy loss. It may, however, give reason as to why that most recent pregnancy resulted in a miscarriage.

Table 2: Products of conception analysis

	N (%)	
Fetus/Products of conception (POC)	**n=20**	**Chromosome abnormalities detected**
Number of foetuses/POC tested	20 (19.8%)	-
Type of genetic test performed		-
QF-PCR	10 (50%)	-
Abnormal results on QF-PCR[§]	4 (40%)	Triploidy Trisomy 16 Trisomy 15 Mosaic Turners
Microarray	4 (20%)	-
Abnormal result on microarray[‡]	4 (100%)	Trisomy 15 Trisomy 7 & 14 Trisomy 14 & 22 Trisomy 15
Karyotype	4 (20%)	-
Abnormal result on karyotype[§§]	1 (25%)	Trisomy 3
Fluorescence In Situ Hybridisation (FISH)	2 (10%)	-
Abnormal result on FISH[††]	1 (50%)	Triploidy
Outcome of genetic test		-
Normal result	9 (45%)	-
Abnormal result	10 (50%)	-
Unsuccessful	1 (5%)	-

Discussion

This is the first study to report on the investigative practices of recurrent miscarriages offered in obstetric and gynaecological units in Johannesburg, South Africa. The topic of recurrent miscarriages is understudied in South Africa. The lack of local guidelines results in the adoption of international guidelines that may not be appropriate to the under resourced clinical settings in South Africa. In this study, referrals for women with recurrent miscarriages were made to our Genetic Clinics. According to RCOG guidelines, couples with three or more consecutive miscarriages, that have been thoroughly worked-up, require a referral for genetic counselling (12).

The impact of maternal age on pregnancy outcome has been well described in the literature. It has been identified as a risk factor for spontaneous miscarriages (23). In our study, the mean age of the women was 34.5 ± 0.6 years and 47.5% (48/101) of the patients were ≥35 years at the time of referral which is considered to be of advanced maternal age (AMA). Advanced maternal age is thought to increase the rate of meiotic errors in the oocyte development leading to nondisjunction which results in embryonic aneuploidy (23). Around 90% of chromosomally abnormal embryos, which are mostly aneuploidies, miscarry because they are

incompatible with life (24). In our study, 1.8% (20/101) POC were tested of which, 50% (10/20) of the POC had an abnormal genetic result and were all aneuploidies. This supports the association of AMA, aneuploidies and miscarriage. In our study, the large majority of the miscarriages (72.7%, 308/424) occurred within the first trimester. This finding is supported in literature where it has been well described that 50% of pregnancies with chromosomal abnormalities have spontaneous miscarriages within the first trimester (25).

Although parental karyotyping is recommended in couples who experience recurrent miscarriages, and who have no other identifiable reason for the miscarriages, only 46.6% (44/101) of the patients in the study underwent karyotyping. Almost half of the patients 47.5% (48/101) were of AMA at referral and 49.5% (50/101) were pregnant at time of referral. It is likely that the patients who did not undergo karyotyping were offered invasive testing for that pregnancy, therefore mitigating the need for maternal karyotyping. Of those 44/101, only 27/101 (26.7%) of their male partners also underwent karyotyping. A study that looked at male partner's views of involvement in maternal health care services that men viewed maternal health issues as a woman's domain. This was mainly determined by their culture which did not promote male involvement in maternal healthcare. Other male partners cited that their employment status affected their ability to participate in their partner's care (26). These findings may support the low uptake of male karyotyping that was noted in our study. In our setting, male partners are not often seen accompanying their female partners to their antenatal visits mainly due to work and in some instances, where males accompany their partners to their antenatal visits, they are often reported to be situated outside the hospital units.

In some cases of recurrent miscarriage, a family history of miscarriages can be observed on the maternal or paternal side. Our findings revealed that 11% (11/101) of the patients had a family history of miscarriages. In our setting, we use the family pedigree to assess if there might be a familial history of recurrent miscarriages which could suggest the presence of a chromosomal abnormality that is being passed on to the offspring. Although family history is not widely accepted as a predictor for recurrent miscarriage, there is increasing evidence that there could be a link between family history of miscarriage and recurrent miscarriage (27). Woolner et al (2020), published the first paper that reviewed the available data on the association of family history of miscarriage and recurrent miscarriage. The authors suggested that more research is needed to describe specific routes of transmission of familial risk from parents, grandparents and other female relatives. The study also cautioned that lifestyle

factors and maternal age, of previous members with a history of miscarriage, need to be taken into consideration when determining a positive family history of miscarriage (27). In light of the fact that 11% of the patients in our study had a family history of miscarriages, genetic counsellors should possibly ask more detailed questions regarding the age of relatives at the time of miscarriage and their lifestyle factors.

In our study, only one patient (2.5%, 1/44) had an abnormal parental karyotype; mosaic trisomy 17 in a female patient. Mosaic trisomy 17 is a rare trisomy that has only previously been reported in 28 cases that were mostly detected prenatally. A full trisomy 17 has never been recorded in live births. There are varying presentations of this condition which have been reported to be related to the level of mosaicism in affected individuals (28). The majority of early recurrent pregnancy losses are caused by numeric chromosomal aberrations (86%), and fewer cases are caused by structural changes (6%) and chromosome mosaicism (8%) (29). Whilst a balanced translocation was not detected in the patients that had karyotypes performed (44/101), there is a possibility that there may have been a proportion of balanced translocation carriers in the 57/101 (56.4%) of participants, that did not undergo parental karyotyping.

The results from this study add to the limited literature on recurrent miscarriages in South Africa. These findings can be used by healthcare professionals in determining what investigations need to be performed prior to genetics referral, taking into account that only 2-5% or recurrent miscarriages are as a result of a balanced translocation. Only 14% of the female patients in this study had all the recommended investigations performed. This could be related to the lack of local guidelines that would assist with providing a framework for the management of recurrent miscarriage in our local setting. Chromosome analysis costs are relatively expensive compared to the other recommended investigations for recurrent miscarriage (30). It may be more cost effective to extensively investigate patients with a history of recurrent miscarriage prior to performing karyotyping which yields low results. Although we know that 50% of recurrent miscarriages are idiopathic, with thorough evaluation of all women with recurrent miscarriages, it may potentially provide the remaining 50% of couples with answers.

One main limitation of this study was that maternal illnesses, weight, and lifestyle factors, were not taken into consideration as risk factors and possible causes of the miscarriages.

Furthermore, this study was not representative of South Africa as a whole and only focused on the patients seen through the WITS/NHLS associated academic hospitals in Johannesburg.

This study highlights the need for thorough investigation of recurrent miscarriages prior to referral for genetic counselling. This speaks to the need of South African based guidelines or protocols for consistency and uniformity for the assessment and management of recurrent miscarriages in all couples with a history of recurrent miscarriage.

Further research could include the development of local guidelines for recurrent miscarriages in South Africa that take into consideration the financial burden that is faced by under-resourced settings. Whilst this study mainly focused on maternal aspects of recurrent miscarriage, it would be interesting to research male factors contributing to recurrent miscarriage and the psychological and cultural implications of male contribution to recurrent miscarriage.

References

1. García-Enguídanos A, Calle ME, Valero J, Luna S, Domínguez-Rojas V. Risk factors in miscarriage: A review. Eur J Obstet Gynecol Reprod Biol. 2002;102(2):111–9.
2. Bhattacharya S, Townend J, Shetty A, Campbell D, Bhattacharya S. Effect of miscarriage on subsequent pregnancies: Author's Reply. BJOG An Int J Obstet Gynaecol. 2009;116(8):1141.
3. Youssef A, Vermeulen N, Lashley EELO, Goddijn M, van der Hoorn MLP. Comparison and appraisal of (inter)national recurrent pregnancy loss guidelines. Reprod Biomed Online [Internet]. 2019;39(3):497–503. Available from: https://doi.org/10.1016/j.rbmo.2019.04.008
4. Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, Middeldorp S, et al. ESHRE guideline: recurrent pregnancy loss. Hum Reprod Open [Internet]. 2018 Apr 1;2018(2). Available from: https://academic.oup.com/hropen/article/doi/10.1093/hropen/hoy004/4963604
5. Hennessy M, Dennehy R, Meaney S, Devane D, O'Donoghue K. A protocol for a systematic review of clinical practice guidelines for recurrent miscarriage. HRB Open Res. 2020;3:12.
6. Bashiri A, Harlev A, Agarwal A. Recurrent Pregnancy Loss. Bashiri A, Harlev A, Agarwal A, editors. Cham: Springer International Publishing; 2016.
7. Toth B, Würfel W, Bohlmann M, Zschocke J, Rudnik-Schöneborn S, Nawroth F, et al. Recurrent Miscarriage: Diagnostic and Therapeutic Procedures. Guideline of the DGGG, OEGGG and SGGG (S2k-Level, AWMF Registry Number 015/050). Geburtshilfe Frauenheilkd. 2018 Apr 27;78(04).
8. Metwally M, Saravelos SH, Ledger WL, Li TC. Body mass index and risk of miscarriage in women with recurrent miscarriage. Fertil Steril [Internet]. 2010;94(1):290–5. Available from: http://dx.doi.org/10.1016/j.fertnstert.2009.03.021
9. Matjila MJ, Hoffman A, van der Spuy ZM. Medical conditions associated with recurrent miscarriage—Is BMI the tip of the iceberg? Eur J Obstet Gynecol Reprod Biol [Internet]. 2017 Jul;214(10):91–6. Available from:

https://linkinghub.elsevier.com/retrieve/pii/S1319562X20302874

10. Amrane S, McConnell R. Endocrine causes of recurrent pregnancy loss. Semin Perinatol. 2019 Mar;43(2).
11. Homer HA. Modern management of recurrent miscarriage. Aust New Zeal J Obstet Gynaecol. 2019 Feb;59(1).
12. RCOG. The Investigation and Treatment of Couples with Recurrent First-trimester and Second-trimester Miscarriage Green-top Guideline No. 17 [Internet]. 2011. Available from: https://www.rcog.org.uk/en/guidelines-research-services/guidelines/gtg17/
13. Limper M, de Leeuw K, Lely AT, Westerink J, Teng YKO, Eikenboom J, et al. Diagnosing and treating antiphospholipid syndrome: A consensus paper. Neth J Med. 2019;77(3):98–108.
14. Khalife D, Ghazeeri G, Kutteh W. Review of current guidelines for recurrent pregnancy loss: new strategies for optimal evaluation of women who may be superfertile. Semin Perinatol [Internet]. 2019;43(2):105–15. Available from: https://doi.org/10.1053/j.semperi.2018.12.008
15. Geyser PJ, Siebert IT. Early Recurrent Pregnancy Loss. Obstet Gynaecol Forum. 2015;25(1):28–31.
16. Stephenson MD, Sierra S. Reproductive outcomes in recurrent pregnancy loss associated with a parental carrier of a structural chromosome rearrangement. 2006;21(4):1076–82.
17. Braekeleer M De, Dao T. Cytogenetic studies in couples experiencing repeated pregnancy losses. 1990;5(5):519–28.
18. van den Akker OB. The psychological and social consequences of miscarriage. Expert Rev Obstet Gynecol [Internet]. 2011 May 10;6(3):295–304. Available from: http://www.tandfonline.com/doi/full/10.1586/eog.11.14
19. Shields R, Hawkes A, Quenby S. Clinical approach to recurrent pregnancy loss. Obstet Gynaecol Reprod Med [Internet]. 2020 Nov [cited 2021 Jan 10];30(11):331–6. Available from: https://www.sciencedirect.com/science/article/abs/pii/S1751721420301640
20. Serrano F, Lima ML. Recurrent miscarriage: Psychological and relational consequences for couples. Psychol Psychother Theory, Res Pract [Internet]. 2006 Dec;79(4):585–94. Available from: http://doi.wiley.com/10.1348/147608306X96992
21. Health N department of. Guidelines for Maternal Care in South Africa: A manual for clinics, community health centres and district hospitals. Pretoria; 2016.

22. Gardner RJM, Amor DJ. Gardner and Sutherland's Chromosome Abnormalities and Genetic Counseling [Internet]. Vol. 1. Oxford University Press; 2018 [cited 2020 Aug 31]. Available from: http://www.oxfordmedicine.com/view/10.1093/med/9780199329007.001.0001/med-9780199329007
23. Marquard K, Westphal LM, Milki AA, Lathi RB. Etiology of recurrent pregnancy loss in women over the age of 35 years. Fertil Steril [Internet]. 2010;94(4):1473–7. Available from: http://dx.doi.org/10.1016/j.fertnstert.2009.06.041
24. El Hachem H, Crepaux V, May-Panloup P, Descamps P, Legendre G, Bouet P-E. Recurrent pregnancy loss: current perspectives. Int J Womens Health [Internet]. 2017 May;Volume 9:331–45. Available from: https://www.dovepress.com/recurrent-pregnancy-loss-current-perspectives-peer-reviewed-article-IJWH
25. Kacprzak M, Chrzanowska M, Skoczylas B, Moczulska H, Borowiec M, Sieroszewski P. Genetic causes of recurrent miscarriages. Rev / Gynecol Ginekol Pol. 2016;87(10):722–6.
26. Nesane K, Maputle SM, Shilubane H. Male partners' views of involvement in maternal healthcare services at Makhado Municipality clinics, Limpopo Province, South Africa. African J Prim Heal Care Fam Med. 2016;8(2):1–5.
27. Woolner AMF, Nagdeve P, Raja EA, Bhattacharya S, Bhattacharya S. Family history and risk of miscarriage: A systematic review and meta-analysis of observational studies. Acta Obstet Gynecol Scand. 2020;99(12):1584–94.
28. Baltensperger A, Haischer G, Rohena L. Rare case of live born with confirmed mosaic trisomy 17 and review of the literature. Clin Case Reports. 2016;4(4):420–4.
29. van den Berg MMJ, van Maarle MC, van Wely M, Goddijn M. Genetics of early miscarriage. Biochim Biophys Acta - Mol Basis Dis. 2012;1822(12):1951–9.
30. Barber J, Cockwell A, Grant E, Williams S, Dunn R, Ogilvie C. Is karyotyping couples experiencing recurrent miscarriage worth the cost? BJOG An Int J Obstet Gynaecol. 2010;117(7):885–8.

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Thank you for your submission! Please do not hesitate to contact us if you have questions.

Appendix B: Participant demographic information

Recurrent miscarriages data collection sheet (linking data) (2011-2021)

Participant number	Year seen	Name	Surname	Date of birth (Age)	Hospital Number	Ethnicity
001.						
002.						
003.						
004.						
005.						
006.						
007.						
008.						
009.						

Appendix C: Data collection sheet

Recurrent miscarriages data collection sheet (2011-2021)

Participant number	Maternal age at miscarriage	Gravidity	Parity	Number of miscarriages	Trimester in which miscarriages occurred	Maternal illnesses	Investigations				Referring hospital	Significant genetic information pedigree
							Antiphospholipid antibodies	Thyroid function tests	Uterine abnormality screening	Genetic testing		
001.												
002.												
003.												
004.												
005.												
006.												
007.												
008.												
009.												
010.												
011.												

Appendix D: Ethics clearance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R49 Ms Z Goliath

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M210264

NAME: (Principal Investigator)	Ms Z Goliath
DEPARTMENT:	School of Pathology Department of Human Genetics Medical School University
PROJECT TITLE:	*An audit of patients seen by genetic counsellors for a history of multiple miscarriages*
DATE CONSIDERED:	2021/02/26
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Ms E Nienaber and Dr S Macaulay
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2021/05/05

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

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 submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **February** and therefore reports and re-certification will be due in the month of **February** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator _____ Date _____

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Postgraduate Affairs)

TO:	Ms Z Goliath School of Pathology Department of Human Genetics Medical School University E-mail: Zandisiwe.Goliath@nhls.ac.za
CC:	Supervisor: Ms E Nienaber and Dr S Macaulay <Elzette.Nienaber@nhls.ac.za> and <HREC-Medical Research Office@wits.ac.za>
FROM:	Mr Iain Burns Human Research Ethics Committee (Medical) Tel: 011 717 1252 E-mail: Iain.Burns@wits.ac.za
DATE:	2021/05/05
REF:	R14/49
PROTOCOL NO:	**M210264** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)
PROJECT TITLE:	*An audit of patients seen by genetic counsellors for a history of multiple miscarriages*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

Appendix E: Turn-it in report

University of the Witwatersrand, Johannesburg

I Zandisiwe Goliath (Student number: 806629) am a student registered for MSc (Med) Genetic Counselling in the year 2021. I hereby declare the following:

I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.

I confirm that the work submitted for assessment for the above course is my own unaided work except where I have explicitly indicated otherwise.

I have followed the required conventions in referencing the thoughts and ideas of others.

I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.

Signature: Z Goliath Date: 25/10/2021

Zandisiwe Goliath Research-compact report.docx

ORIGINALITY REPORT

2%	0%	3%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	"Recurrent Pregnancy Loss", Springer Science and Business Media LLC, 2018 Publication	2%

Exclude quotes	On	Exclude matches	< 2%
Exclude bibliography	On		

Appendix F: Approved protocol

CANDIDATE'S SURNAME: [Please print] **Goliath**		FIRST NAME/S: **Zandisiwe**	STUDENT NUMBER: **806629**
CURRENT QUALIFICATIONS: **Bachelor of Nursing**			
TEL: **011 489 9239**	CELL: **071 895 6973**	E-MAIL: **Zgoliath257@gmail.com**	FAX: **N/A**
DEGREE FOR WHICH PROTOCOL IS BEING SUBMITTED: **MSc (Med) Genetic Counselling**			
PART-TIME OR FULL-TIME: **Full-time**			
FIRST REGISTERED FOR THIS DEGREE:	TERM : **1**		YEAR: **2020**
DEPARTMENT: **Human Genetic**			
TITLE OF PROPOSED RESEARCH: **An audit of patients seen by genetic counsellors for a history of recurrent miscarriages**			
CANDIDATE'S SIGNATURE: Z Goliath			DATE: **20/04/2021**
SUPERVISOR 1 (NAME & SURNAME): **Dr Shelley Macaulay**			**45%** Supervision
SUPERVISOR'S QUALIFICATIONS: **MSc (Med) Genetic Counselling, PhD**			
SUPERVISOR'S DEPARTMENT: **Human Genetics**			
SUPERVISOR'S ADDRESS / TEL / E-MAIL: **Room 15, Jack Metz Building, Cnr Hospital & De Korte Str, Braamfontein/ 011 489 9223/ shelley.macaulay@nhls.ac.za**			
SUPERVISOR 2 (NAME & SURNAME): **Elzette Nienaber**			**45 %** Supervision
SUPERVISOR'S QUALIFICATIONS: **MSc (Med) Molecular and Cellular Biology, MSc (Med) Genetic Counselling**			
SUPERVISOR'S ADDRESS / TEL / E-MAIL: **Room 11, Jack Metz Building, Cnr Hospital & De Korte Str, Braamfontein/ 011 489 9151/ Elzette.Nienaber@nhls.ac.za**			
SUPERVISOR'S (NAME & SURNAME): **Professor Hennie Lombaard**			**10 %** Supervision
SUPERVISOR'S QUALIFICATIONS: **MBChB, MMed (OetG), FCOG (SA)**			
SUPERVISOR'S ADDRESS/ TEL/ E-MAIL: hennie.lombaard83@gmail.com			
SYNOPSIS OF RESEARCH:(Brief summary of proposed research project; between 200-300 words only; with sub-headings: an introduction and justification for study, aim/s, proposed methodology and expected outcome/s) [Use reverse side of this page if more space is required] PTO (see separate page)			
WITS ETHICS NOT REQUIRED: Yes No WITS ETHICS PENDING: (Yes) No WITS ETHICS APPROVED: Yes No (circle appropriate symbol)* ***Please note the final human ethics clearance certificate or animal ethics certificate must be available prior to starting research**			IF Y SUPPLY ETHICS CLEARANCE CERTIFICATE AS ATTACHMENT AND INCLUDE ETHICS NUMBER HERE:
As supervisor/s, I/we confirm that I have read the protocol which has been submitted for assessment.			
SIGNATURE OF SUPERVISOR/S:	SMacaulay	Nienaber	
SIGNATURE PG OFFICE STAFF ……	REGISTERED YES….. NO…..		STAMP

An audit of patients seen by genetic counsellors for a history of recurrent miscarriages

Zandisiwe Goliath

806629

MSc (Med) Genetic Counselling

Supervisor 1:

Dr. Shelley Macaulay

MSc (Med) Genetic counselling, PhD

Supervisor 2:

Ms. Elzette Nienaber

MSc (Med) Molecular and Cellular Biology, MSc (Med) Genetic Counselling

1. Introduction

Miscarriage is a frequent pregnancy complication that occurs in about 15-20% of all pregnancies. It is a spontaneous termination of a foetus prior to viability which is <24 weeks' gestation (Serrano and Lima, 2006). Recurrent miscarriages is traditionally defined as three or more early (<12 weeks gestation) consecutive pregnancy losses and is said to affect approximately 1% of women (Shields *et al.*, 2020). Several entities have established common aetiologies that contribute to miscarriage which will be further discussed.

2. Aetiology of recurrent miscarriages

There are several factors that are commonly recognised as causes of recurrent miscarriages. Figure 1 depicts the proportions in which the causes of recurrent miscarriages are distributed.

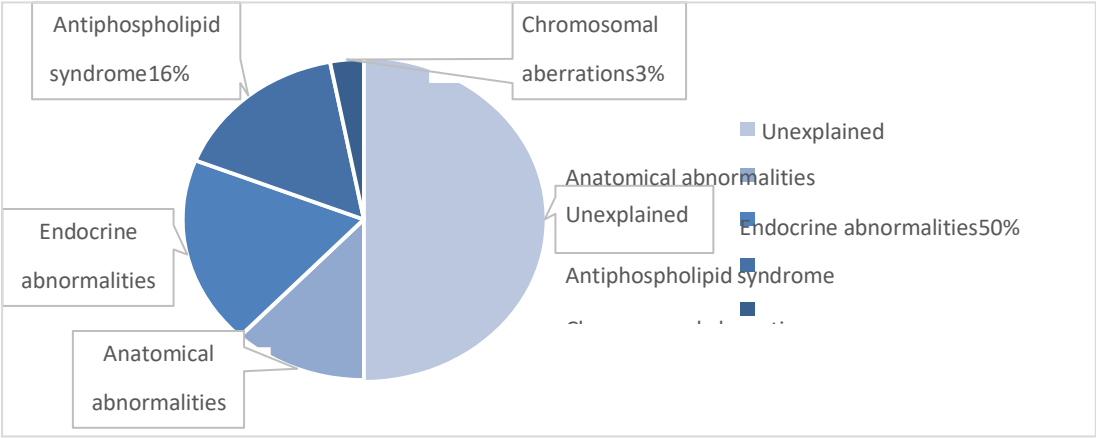

Figure 1: Aetiology of recurrent miscarriage (Bashiri et al., 2016)

2.1. Lifestyle and behavioural factors

Previous studies have associated higher stress levels, coffee consumption and nicotine consumption with recurrent miscarriages. These factors have been difficult to prove because some studies have been small and others did not show a linear association to recurrent miscarriages (Toth et al., 2018).

2.2. Hormonal and metabolic factors

Thyroid disorders have been associated with several adverse pregnancy outcomes as well as recurrent

miscarriages. There is controversial evidence that links thyroid disorders to recurrent pregnancy loss. However, the impact of thyroid disorders have been associated with adverse pregnancy outcomes such as premature delivery, gestational hypertension, low birth weight, congenital anomalies and foetal death (Amrane and McConnell, 2019).

Other studies have shown an association between increased thyroid antibodies and recurrent miscarriages in patients with normal thyroid function (Bashiri et al., 2016).

Maternal endocrine disorders such as diabetes have been associated with miscarriage. Women that present with elevated HbA1c levels (≥6.5% or 48mmol/mol) during the first trimester are at increased risk of miscarriage and foetal anomalies (RCOG, 2011).

Recent investigations indicate an association between vitamin D deficiency and recurrent miscarriages (Homer, 2019). Recurrent miscarriage guidelines were published prior to these recent investigations, therefore, recommendations on vitamin D testing for recurrent miscarriages have not yet been included (Khalife et al., 2019).

In order for successful implantation of an embryo to occur, progesterone is required to prompt changes to the endometrial lining. Luteal phase deficiency refers to deficient progesterone production that results in the inadequate development of the endometrium, resulting in poor implantation of the embryo and pregnancy loss. Deficient levels of progesterone may lead to pregnancy loss (Khalife et al., 2019). The relationship between the luteal phase deficiency and recurrent miscarriages is difficult to make due to controversy regarding clinical relevance (Bashiri et al., 2016).

Polycystic ovarian syndrome is a multifactorial endocrinopathy that affects females. It is characterised by: (1) oligo- or anovulation, (2) excess of androgen as well as androgenic features (Bashiri et al., 2016). It has been associated with a risk of miscarriage but the mechanism has not been clearly defined in

literature. The risk for miscarriage in women with polycystic ovarian syndrome has been previously linked with insulin resistance (RCOG, 2011).

2.3. Antiphospholipid syndrome

Antiphospholipid syndrome (APS) is an autoimmune disorder that is characterised by recurrent venous or arterial thrombosis, recurrent pregnancy loss as well as adverse pregnancy outcomes (Bashiri et al., 2016). It is considered to be one of the most important treatable causes of recurrent miscarriages. The syndrome may exhibit laboratory abnormalities in antiphospholipid antibodies which include Lupus anticoagulant (LA), Anticardiolipin antibodies (aCL) and anti-β_2 glycoprotein-I antibodies. APS is associated with adverse pregnancy outcomes which include (1) three or more consecutive pregnancy losses prior to ten weeks gestation, (2) unexplained loss of morphologically normal foetus at or before ten weeks of gestation or (3) a history of preterm births delivered prior to 34 weeks gestation due to placental-related conditions (RCOG, 2011). The antiphospholipid antibodies cause harm in pregnancy through various mechanisms which include an inflammatory response and interference in the coagulation cascade (Khalife et al., 2019).

Antinuclear antibodies are autoantibodies that target the nucleus of the cell. The antibody titer levels have been reported to be increased in women with a history of recurrent miscarriages. (Toth et al., 2018).

2.4. Anatomical factors

Uterine abnormalities are found in approximately 19% of recurrent miscarriage cases (El Hachem et al., 2017). They can be classified as congenital or acquired uterine abnormalities which will be discussed below.

Uterine malformations

There have been several types of uterine abnormalities observed in women with a history of recurrent miscarriages. These malformations have been described as either congenital or acquired uterine defects

(Khalife et al., 2019). Congenital defects include uterine septate, bicornuate, unicornuate, didelphys and arcuate uteri (figure 2). Uterine malformations have been noted to be higher in women with second trimester miscarriages than those who suffer first trimester miscarriages. (RCOG, 2011). Acquired uterine abnormalities include fibroids, polyps and adhesions. While it is known that acquired uterine abnormalities increase the risk of recurrent miscarriage, it is still unclear how it occurs. Possible mechanisms include disruption of blood flow to the uterus, inflammation of the uterus as well as reduced capacity of the uterus (Homer, 2019)

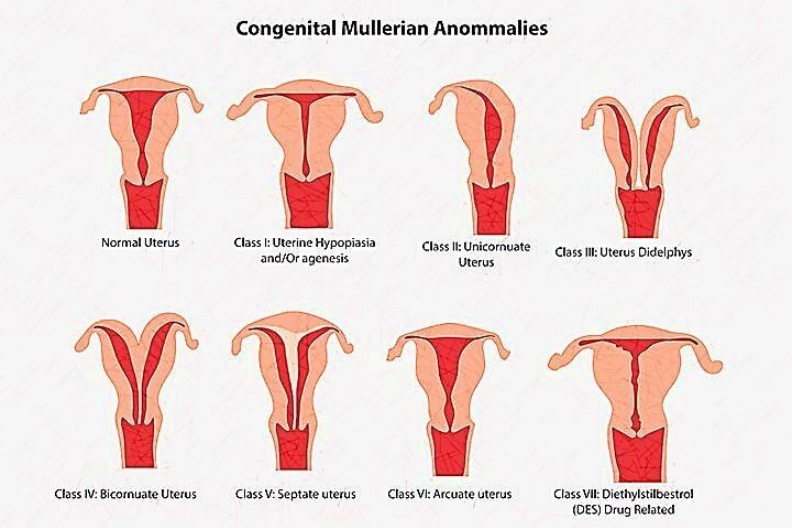

Figure 2: Congenital uterine abnormalities (Mahmood Daood, 2019)

Cervical incompetence

Cervical incompetence has been associated with second trimester pregnancy loss but the incidence has not been clearly defined in literature. There is no test that can identify women with cervical weakness in a non-pregnant state. It is usually identified in women who have a history of second trimester miscarriages and is often followed by premature rupture of membranes and painless dilatation of the cervix (RCOG, 2011). Currently in the clinical setting in South Africa, cervical length is monitored and measured for women who have a history of mid-trimester fetal losses. Cervical length is assessed between the 11-13 weeks and between 18-22 weeks scans. The decision to insert the cervical cerclage ismade at the 11-13

week scan, however, if the funneling of the cervix presents late and is missed in the first trimester screening, a rescue cerclage is inserted between 18-22 weeks (Wits, 2013). A cervical length below 25mm would indicate an insertion of a cerclage to prevent preterm labour (Shields et al., 2020).

2.5. Inherited thrombophilias

Thrombophilia is a condition that is associated with the increased risk of developing venous thromboembolism which can be acquired or inherited. Inherited thrombophilias include Factor V Leiden mutation, Prothrombin mutation, Protein C, Protein S and antithrombin III deficiency, all of which have been reported to have an undetermined role in recurrent miscarriage (Toth et al., 2018).

2.6. Genetic factors of recurrent miscarriages

Lastly, recurrent miscarriages could be attributed to genetic factors which include parental chromosomal rearrangements as well as foetal chromosomal abnormalities.

Parental chromosomal rearrangements

In about 2-5% of couples who present with recurrent miscarriages, one of the partners carries a balanced structural chromosomal rearrangement. Carriers of balanced translocations are phenotypically normal but their pregnancies are at risk of miscarrying or they are at risk of having a live birth with multiple congenital malformations due to an unbalanced chromosomal rearrangement. The outcome of the pregnancy is often influenced by the size of the rearrangement, the position and the region of the rearranged genetic content(Kacprzak et al., 2016).

Embryonic/fetal chromosomal abnormalities

Embryonic aneuploidies are the most common cause of spontaneous miscarriages. Due to the natural selection mechanism, 90% of chromosomally abnormal embryos are miscarried. The most common embryonic or fetal chromosomal abnormalities are numeric abnormalities such as trisomies, polyploidies and monosomy X. Trisomy 15, 16 and 22 are reported to be the most common non-viable trisomies (El

Hachem et al., 2017). The reason as to why these specific trisomies occur more commonly is because women of advanced maternal age (≥35 years) are at increased risk of chromosomal defects which result in the increased risk of fetal trisomy. As women age, the number of oocytes and the quality of these oocytes decrease which make them prone to error which could result in a nondisjunction event (Toth et al., 2018).

Monogenic Disease

Some X-linked dominant disorders have been reported to be lethal in males and increase the risk of miscarriage such as Incontentia pigmenti. Autosomal recessive conditions such as haemoglobinopathies and some skeletal dysplasias result in severe malformations that increase the risk of intrauterine death (Toth et al., 2018).

2.7 Studies on the causes of recurrent miscarriages in South Africa

In South Africa, the topic of recurrent miscarriages remains an understudied topic. A local study in Cape Town looked at commonly investigated medical disorders associated with recurrent miscarriages (Matjila et al., 2017). The results of the study revealed that polycystic ovarian syndrome accounted for 22% of the causes, impaired glucose intolerance was responsible for 11% of the cases, and diabetes mellitus was responsible for 3%. Fifteen percent of the cases remained unexplained. Genetic contributions for recurrent miscarriage were not taken into consideration for this study. This study is not a reflection of the medical disorders that are associated with recurrent miscarriage in the whole of South Africa, but it offers insight on the topic of recurrent miscarriage in units outside of the Division of Human Genetics, NHLS & Wits.

3. Management of recurrent miscarriages

The Royal College of Obstetricians and Gynaecologists, has recommended that the investigation of recurrent miscarriage includes a detailed family and medical history, endocrine investigations, anatomic

evaluation and antiphospholipid antibody testing (RCOG, 2011). The proposed management of recurrent miscarriage is discussed below (Table 1).

Table 1: Summary of recommended management for recurrent miscarriages (RCOG, 2011)

Possible cause	Investigation	Treatment
Lifestyle and behavioural factors	Detailed medical history	
Thyroid diseases	TSH screening only if patient is known to have disease and/or if there are clinical manifestations	Hypothyroidism: supplementation of levothyroxine Hyperthyroidism: carbimazole Euthyroid women with thyroid antibodies: levothyroxine
Diabetes mellitus	In literature an HbA1c test is a screening test recommended only if patient is known to have diabetes and or if there are clinical manifestations. In the South African clinical space, random blood glucose testing is done if there is a strong suspicion of diabetes. If random test yields results of ≥ 7.8 mmol/L, 75g oral glucose tolerance test is done (Wits, 2013).	Metformin Diabetic diet
Vitamin D	Vitamin D level testing is advised but not recommended.	Vitamin D supplementation
Luteal phase deficiency	There is no standardised diagnostic method available	If progesterone is supplemented, it should bedone from ovulation to 7-9weeks gestation.
Antiphospholipid syndrome	Lupus anticoagulant, anticardiolipin antibodies and anti-β2 glycoprotein-I antibodies testing should be done. European guidelines suggest antibody testing should be done 6 weeks after pregnancy loss and confirmed 12 weeks later (Bender Atik et al., 2018).	Unfractionated heparin andlow dose aspirin
Anatomical factors	All women with RM should have assessment of uterine anatomy. Hysteroscopy: all patients Cervical sonographic surveillance for cervical incompetence	Cervical cerclage for cervical incompetence
Inherited thrombophilias	Only recommended if there is a personal or strong family history of thrombosis	Unfractionated heparin or low molecular weight heparin

Chromosomal rearrangements	Karyotype of products of conception should be done from 3rd miscarriage. If testing of products of conception reveals unbalanced structural chromosomal abnormality in fetus, karyotypingin both partners should be performed	Genetic counselling

4. Genetic counselling for patients with recurrent miscarriages

The definition of genetic counselling, as developed by the Task Force of The National Society of Genetic Counsellors (NSGC) "is the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease" (Resta *et al.,* 2006). The roles and responsibilities of genetic counsellors involves taking a family history and drawing a family pedigree, determining the risk of inheritance of a condition, discussing the natural history of genetic conditions, providing patients and other health care professionals with education on genetics, discussing available options, assessing the psychosocial impact of a diagnosis and providing support to patients and their families (Skirton *et al.,* 2015).

The role of genetic counsellors in the management of recurrent miscarriages is to evaluate medical and family histories to determine the likelihood of a genetic contribution to recurrent miscarriages in couples, to facilitate the process of genetic testing, to determine the likelihood of future successful pregnancies and to offer psychosocial support to couples and families.

4.1 Psychological impact of multiple miscarriage

The loss of a pregnancy has shown to affect men and women differently. Men have often experienced feelings of frustration, lack of control, rage, guilt and lowered self-esteem whereas women have shown a decline in self-worth, distorted body image and severe marital discord (Serrano and Lima, 2006). Women with a history of recurrent miscarriage display depressive symptoms and are at an increased risk of adverse psychological effects which include pregnancy-related anxiety, depression andfear (Tavoli et al., 2018).

The loss of a pregnancy is a traumatic event for women and couples. Evidence suggests that the recurrent nature of recurrent miscarriages can magnify the grief reaction (Shields et al., 2020). Determining the cause of recurrent miscarriages, in order to reduce the recurrence risk, remains one of the most challenging dilemmas for health care professionals. However, offering psychological support remains imperative for women who experience recurrent miscarriages. Genetic counsellors have the ability to address the psychosocial needs that couples with a history of recurrent miscarriage present with.

5. Motivation for research

As mentioned above, the topic of recurrent miscarriage is understudied in the South African context and because South Africa currently uses international guidelines on how to manage recurrent miscarriages, the approach differs in each local organisational setting. The lack of a national standardised protocol results in patients being referred to the genetics department without being fully investigated. Chromosomal abnormalities only account for 2-5% recurrent miscarriages. This indicates that genetic investigations should not be the first-line investigation. It has been noted, in practice, that not all the investigations thatmay assist in identifying the cause of recurrent miscarriages have been done prior to patients being referred for genetic investigations.

The psychological impact that miscarriage has on couples cannot be ignored. In some cases, receiving a diagnosis can offer couples a sense of relief because they are able to avoid the recurrence of miscarriage. It is unlikely that every couple will be provided with answers because the cause of miscarriages remain unexplained in a large percentage of cases. With the appropriate management, couples can at least be reassured that the necessary investigations have been done. This study will provide valuable insight on the number of patients that have been seen with recurrent miscarriage by the Genetics Department over a period of 10 years, the management implemented prior to referral and whilst seen by the Genetics department and the outcomeof the genetic testing that has been done.

6. Study aims and objectives

The aim of this study is to perform an audit of patients seen for recurrent miscarriages through the Genetic Clinics of the Division of Human Genetics, NHLS & Wits.

The objectives are:

1. To assess the demographic factors of the participants who are referred for recurrent miscarriages
2. To evaluate the reason behind the referral of the patient
3. To identify what investigations had been done prior to referral
4. To calculate how many patients and their partners underwent karyotyping
5. To evaluate the results of genetic testing performed

7. Methodology

7.1 Research design

This study will be a retrospective file review using quantitative research methods. This will involve retrieving files from the Division of Human Genetics filing room at the National Health Laboratory Service (NHLS) in Johannesburg. Permission to access these files has been granted by the Head of the Division, Professor Amanda Krause.

7.2 Participants

The study will comprise of files for patients that were seen at the Genetics Clinic for recurrent miscarriages. All patients that were referred with a diagnosis of recurrent miscarriages or poor obstetric history will be included. The terms recurrent miscarriages and poor obstetric history are used interchangeably in the clinical setting. We have therefore included patients that have been referred for both to ensure that all possible participants are included into the study. The Division of Human Genetics, NHLS and Wits offers genetic counselling services at various state hospitals (Charlotte Maxeke Johannesburg Academic Hospital, Rahima Moosa Mother and Child Hospital and Chris Hani Baragwanath Academic Hospital) around Johannesburg every week or every two weeks. Patients that

are seen for recurrent miscarriages are considered to be high risk and are seen at tertiary level facilities that offer specialised care for patients who are referred from regional hospitals, district hospitals and or community health centers.

7.3 Sample size

The Division of Human Genetics, NHLS and Wits has an electronic and manual system in the department wherein patients that are seen for recurrent miscarriages and poor obstetric history are recorded. Over a period of 10 years, between 2011-2020, a total of 102 patients for recurrent miscarriage have been recorded and a total of 27 patients for poor obstetric history have been recorded. This allows for the potential of 129 files to be audited for this study.

7.4 Data collection

A data collection sheet has been designed to collect specific patient details (Appendix 1). These details include: maternal age at miscarriage, gravidity and parity, number of miscarriages, trimester in which miscarriages occurred, maternal illnesses, investigations performed, management, referring hospital and any unusual or significant genetic information from pedigree.

7.5 Data analysis

This study will make use of basic descriptive statistics that will be analysed using Excel. The Shapiro Wilk test will be used to test normality of continuous variables. Normally distributed variables will be presented as means and standard deviations. Non-normally distributed variables will be presented as medians and interquartile ranges. Categorical variables will be represented as frequencies and percentages

8. Ethics

This research protocol will be submitted to Human Research Ethics Committee (HREC) at the University

of the Witwatersrand on the 5th of February 2021 for ethical clearance. The identity of the participants will be protected in order to maintain confidentiality. This will be done by using a coding system that will protect patient names. The linked data will be stored separately from the populated data sheet (Appendix 1 & 2). All data will be stored in a password protected computer.

9. Timing:

This study will commence as soon as ethical approval and clearance is obtained.

	Jan 2021	Feb 2021	Mar 2021	Apr 2021	May 2021	June 2021	Jul 2021	Aug 2021
Internal protocol assessment								
Submission of protocol to Faculty of Health Sciences								
Submission of protocol to ethics								
School of Pathology assessment of protocols								
Data collection								
Data analysis								
Write up and submission								

10. Funding

This research does not require any funding.

11. Potential limitations

- The sample size will be affected by the availability of files.
- The information in the files may not be comprehensive e.g. all the investigations done outside of the Division of Human Genetics may not be included in patient files.

- This study is a representation of patients that have been seen in Johannesburg, therefore, it does not represent other genetic units in South Africa.

References

1. Amrane, S., McConnell, R., 2019. Endocrine causes of recurrent pregnancy loss. Semin. Perinatol. 43.https://doi.org/10.1053/j.semperi.2018.12.004
2. Bashiri, A., Harlev, A., Agarwal, A., 2016. Recurrent Pregnancy Loss. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-27452-2
3. Bender Atik, R., Christiansen, O.B., Elson, J., Kolte, A.M., Lewis, S., Middeldorp, S., Nelen, W., Peramo, B., Quenby, S., Vermeulen, N., Goddijn, M., 2018. ESHRE guideline: recurrent pregnancy loss. Hum. Reprod. Open 2018. https://doi.org/10.1093/hropen/hoy004
4. El Hachem, H., Crepaux, V., May-Panloup, P., Descamps, P., Legendre, G., Bouet, P.-E., 2017. Recurrent pregnancy loss: current perspectives. Int. J. Womens. Health Volume 9, 331–345.https://doi.org/10.2147/IJWH.S100817
5. Homer, H.A., 2019. Modern management of recurrent miscarriage. Aust. New Zeal. J. Obstet. Gynaecol. 59. https://doi.org/10.1111/ajo.12920
6. Kacprzak, M., Chrzanowska, M., Skoczylas, B., Moczulska, H., Borowiec, M., Sieroszewski, P., 2016.
7. Genetic causes of recurrent miscarriages. Rev. / Gynecol. Ginekol. Pol. 87, 722–726. https://doi.org/10.5603/GP.2016.0075
8. Khalife, D., Ghazeeri, G., Kutteh, W., 2019. Review of current guidelines for recurrent pregnancy loss: new strategies for optimal evaluation of women who may be superfertile. Semin. Perinatol. 43. https://doi.org/10.1053/j.semperi.2018.12.008
9. Mahmood Daood, M., 2019. FEMALE INFERTILITY: ULTRASOUND AND HYSTEROSALPOINGOGRAPHY. https://doi.org/10.24941/ijcr.34061.01.2019
10. Matjila, M.J., Hoffman, A., van der Spuy, Z.M., 2017. Medical conditions associated with recurrent miscarriage—Is BMI the tip of the iceberg? Eur. J. Obstet. Gynecol. Reprod. Biol.214,

91–96, .https://doi.org/10.1016/j.ejogrb.2017.05.003

11. RCOG, 2011. The Investigation and Treatment of Couples with Recurrent First-trimester and Second-trimester Miscarriage Green-top Guideline No. 17.
12. Resta, R., Biesecker, B.B., Bennett, R.L., Blum, S., Estabrooks Hahn, S., Strecker, M.N., Williams, J.L., 2006. A New Definition of Genetic Counseling: National Society of Genetic Counselors'Task Force Report. J. Genet. Couns. 15, 77–83. https://doi.org/10.1007/s10897-005-9014-3
13. Serrano, F., Lima, M.L., 2006. Recurrent miscarriage: Psychological and relational consequences for couples. Psychol. Psychother. Theory, Res. Pract. 79, 585–594. https://doi.org/10.1348/147608306X96992
14. Shields, R., Hawkes, A., Quenby, S., 2020. Clinical approach to recurrent pregnancy loss. Obstet.
15. Gynaecol. Reprod. Med. 30, 331–336. https://doi.org/10.1016/j.ogrm.2020.09.005 Skirton, H., Cordier, C., Ingvoldstad, C., Taris, N., Benjamin, C., 2015. The role of the genetic
16. counsellor: a systematic review of research evidence. Eur. J. Hum. Genet. 23, 452–458. https://doi.org/10.1038/ejhg.2014.116
17. Tavoli, Z., Mohammadi, M., Tavoli, A., Moini, A., Effatpanah, M., Khedmat, L., Montazeri, A., 2018.
18. Quality of life and psychological distress in women with recurrent miscarriage: a comparativestudy. Health Qual. Life Outcomes 16, 150. https://doi.org/10.1186/s12955-018-0982-z
19. Toth, B., Würfel, W., Bohlmann, M., Zschocke, J., Rudnik-Schöneborn, S., Nawroth, F., Schleußner, E., Rogenhofer, N., Wischmann, T., von Wolff, M., Hancke, K., von Otte, S., Kuon, R., Feil, K., Tempfer, C., 2018. Recurrent Miscarriage: Diagnostic and Therapeutic Procedures. Guideline of the DGGG, OEGGG and SGGG (S2k-Level, AWMF Registry Number 015/050). Geburtshilfe Frauenheilkd. 78. https://doi.org/10.1055/a-0586-4568
20. Wits, O., 2013. Guidelines for obstetric care at referral hospitals in Johannesburg.

Appendices

Appendix 1

Student name: Zandisiwe Goliath

Student number: 806629

Study title: An audit of patients seen by genetic counsellors for a history of recurrent miscarriages

Study reference number: **M210264**

Recurrent miscarriages data collection sheet (2011-2020)

Participant number	Maternal age at miscarriage	Gravidity	Parity	Number of miscarriages	Trimester in which miscarriages occurred	Maternal illnesses	Investigations				Management	Referring hospital	Significant genetic information pedigree
							Antiphospholipid antibodies	Thyroid function tests	Uterine abnormality screening	Genetic testing			
1.													
2.													
3.													
4.													
5.													
6.													
7.													
8.													

Appendix 2:

Student name: Zandisiwe Goliath

Student number: 806629

Study title: An audit of patients seen by genetic counsellors for a history of recurrent miscarriages

Study reference number: **M210264**

Recurrent miscarriages data collection sheet (linking data) (2011-2020)

Participant number	Year seen	Name	Surname	Date of birth	Hospital number
1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					
9.					