AN EVALUATION OF PREIMPLANTATION GENETIC DIAGNOSIS OUTCOMES IN JOHANNESBURG, SOUTH AFRICA

Bianca Carzis

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 for the degree of Master of Science in Medicine (Genetic Counselling)

Johannesburg, 2017

Declaration

I, **Bianca Carzis**, 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 at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(signature of candidate)

16th day of November in 2017, in Johannesburg

Contribution of the candidate to the paper

Declaration: Student's contribution to article(s) and agreement of co-author(s)

I, Bianca Carzis, student number 1260293, declare that this Research Report is my own work and that I contributed significantly towards research findings presented in the paper intended for publication below.

Signature of Student

Date 16/11/2017

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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 Evaluation of Preimplantation Genetic Diagnosis Outcomes in Johannesburg, South Africa

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This research report is dedicated to my parents, Foti and Marina Carzis,

and to Devon Rossouw.

Presentations arising from this research project

1. SASHG Biennial Congress 2017, 13th-16th August, Durban, KwaZulu-Natal (oral presentation)
2. World Congress on Genetic Counselling 2017, 4th-6th October, Cambridge, United Kingdom (poster presentation)
3. University of the Witwatersrand Cross-Faculty Postgraduate Symposium, 25 October, School of Public Health, Wits (poster presentation)
4. Division of Human Genetics Departmental Seminar, 8 November, National Health Laboratory Service (oral presentation)

Abstract

Preimplantation genetic diagnosis (PGD) involves testing embryos that were created using artificial reproductive technology for a familial genetic condition before being transferred to a woman's uterus. The aim of this study was to determine the number and nature of PGD cases managed through the Division of Human Genetics at the University of the Witwatersrand and National Health Laboratory Service, and assess the outcomes at various stages of the PGD process, including a cost estimation of PGD. The files of 22 PGD couples were included in a retrospective case review. In total, there were 42 IVF/ICSI cycles included in the analysis. The clinical pregnancy rate per embryo transfer was 29.3%. Overall, 10/22 (45.5%) couples had a successful cycle resulting in a liveborn baby. On average, one cycle of PGD costs USD 9,525 (ZAR 125,578). This study shows that our PGD success rates are comparable to those achieved globally.

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Thank you to my parents, Foti and Marina Carzis, and Devon Rossouw, for the continuous support, love and guidance. To my new friends, Elzette and Melissa, it's been a privilege sharing this journey with the two of you.

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List of Abbreviations

aCGH	Array comparative genomic hybridisation
β-hCG	Beta-human chorionic gonadotropin
ESHRE	European Society of Human reproduction and Embryology
HLA	Human leukocyte antigen
ICSI	Intracytoplasmic sperm injection
IQR	Interquartile range
IVF	*In vitro* fertilisation
NGS	Next-generation sequencing
NHLS	National Health Laboratory Service
No.	Number
PGD	Preimplantation genetic diagnosis
PGS	Preimplantation genetic screening
RE	Reproductive event
SA	South Africa
SD	Standard deviation
SNP	Single nucleotide polymorphism
UK	United Kingdom
USA	United States of America
USD	United States Dollar
Wits	University of the Witwatersrand
ZAR	South African Rand

Research report in the format of a "submissible" paper

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ix. **What's already known about this topic?**

- Preimplantation genetic diagnosis (PGD) success rates are limited by the success rates of *in vitro* fertilisation.
- The costs of PGD are high, making PGD largely inaccessible to couples in developing countries who do not have access to government-funded PGD initiatives.

What does this study add?

- This is the first study to report on PGD outcome measures and success rates in South Africa.
- This study demonstrates a cost estimation of the PGD process in South Africa.

x. **Abstract:**

Objective: To determine the number and nature of preimplantation genetic diagnosis (PGD) cases managed through a South African academic and diagnostic Human Genetics unit and assess the outcomes at various stages of the PGD process, including a cost assessment.

Method: A retrospective file review was performed. Couples' genetic counselling, IVF/ICSI and PGD-specific records were analysed.

Results: Among the 22 PGD couples included in the study, 42 IVF/ICSI cycles were completed and included in the analysis. Most of the conditions for which PGD was requested were autosomal recessive. Of the biopsied embryos, 71/228 (31.1%) were suitable for transfer and 41/71 (57.7%) embryos were transferred. Of these, 14/41 (34.0%) embryos successfully implanted and 11/14 (78.6%) resulted in a liveborn baby. The clinical pregnancy rate per embryo transfer was 29.3%. Overall, 10/22 (45.5%) couples had a successful cycle resulting in a liveborn baby. On average, one cycle of PGD costs USD 9,525 (ZAR 125,578).

Conclusion: This study showed that the PGD success rates in this South African sample are comparable to those achieved globally as reported in the literature. South Africa has the infrastructure and the expertise to provide PGD as a service. Future research should aim to evaluate couples' experiences of PGD.

Introduction

Preimplantation genetic diagnosis (PGD) is a process that involves testing embryos that were created using *in vitro* fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) for a familial genetic condition before being transferred to a woman's uterus for implantation. The first successful use of PGD in humans was reported in 1990[1] and by 2004 the first 1,000 PGD births were reported[2]. In South Africa (SA), PGD is still a relatively new service, with 2006 marking the first PGD case managed through the Division of Human Genetics at the National Health Laboratory Service (NHLS) and the University of the Witwatersrand (Wits) Johannesburg, SA.

The PGD process has evolved over the years including changes in the time of embryo biopsy and techniques employed. The stage at which the biopsy is performed influences the outcome of the IVF process and the accuracy of genetic testing[3]. Currently, PGD laboratories are using genome-wide karyomapping[4] as the assay of choice. This technique involves linkage analysis using genome-wide single nucleotide polymorphism (SNP) genotyping and alleviates the need to optimise mutation-specific tests. Achievement of a successful pregnancy is limited by the success rate of embryo biopsy (30% for cleavage stage biopsy and 51% for blastocyst stage biopsy[3]) and therefore cannot be guaranteed. Patients who are undergoing PGD may opt to have preimplantation genetic screening (PGS) performed simultaneously, to screen for aneuploidies and increase the pregnancy success rate[5]. Preimplantation genetic diagnosis and PGS offer parents an opportunity to have a child unaffected with a molecularly confirmed familial genetic condition and reduce the chance of having a termination of an affected pregnancy.

In SA, the Division of Human Genetics (NHLS/Wits) has collaborated with a specialised reproductive genetics laboratory in the United Kingdom (UK) and United States of America (USA), and more recently a local laboratory and fertility centre in SA, to provide PGD as a service to couples. The current PGD process in Johannesburg involves attending an initial genetic counselling consultation which also includes completion of a PGD eligibility assessment, enrolment and referrals. If the specific familial mutation is unknown a diagnostic workup, in a reputable genetics laboratory, is initiated to identify the familial mutation. Once the mutation has been identified, the reproductive genetics laboratory optimises the PGD test. Previously, this took eight to twelve weeks, but newer technology has allowed for a decreased turnaround time. The couple also attends a fertility centre for a baseline fertility assessment and initiation of IVF/ICSI. The embryos that are produced are biopsied, and embryos that are

unaffected with the familial mutation are transferred. The process is not always linear. Certain parts of the process may occur simultaneously and may not always follow the same order.

Unlike the situation in Italy, France and the UK, for example, where there are strict regulatory policies that guide the use of PGD[6], SA's legislative framework lacks such regulation[7]. South Africa is governed by three acts which deal with various reproductive issues: the Human Tissues Act No. 65 of 1983, the National Health Act No. 61 of 2003 and the Children's Act No. 38 of 2010 (https://www.gov.za/documents/acts). There is no direct reference to PGD in these acts, however, the content is broad enough to permit the use of PGD for genetic conditions, as well as for human leukocyte antigen (HLA) matching for donor siblings[7]. The National Health Act No. 61 of 2003 does, however, state that sex selection for family balancing is not permitted. Furthermore, unlike the government-funded PGD initiatives in these European countries, there are no PGD government funding initiatives available for South African couples. In France, for example, the government funds up to four IVF cycles[6]. In contrast, approximately 17.4% of the South African population is covered by private medical insurance,[8] which may not cover the cost of PGD, and so couples are obliged to pay for the procedure themselves. The remaining 82.6% of the population is dependent on the government funded healthcare system which does not offer PGD as a service. The high cost of PGD coupled with the lack of medical insurance coverage is a limiting factor, making this procedure inaccessible to most couples and only accessible to individuals of a higher socioeconomic status.

During PGD-related genetic counselling, some queries that are usually raised by South African couples centre around the potential high costs involved, the length and complexity of the entire PGD process, as well as the relatively low IVF success rates. To make an informed decision about whether to undergo PGD, couples need to understand all the limitations involved and the possible outcomes that they may experience. Current PGD-related genetic counselling in the Division of Human Genetics (NHLS/Wits) can only provide generalisations of PGD outcomes and expenses based on the published experiences of other non-South African countries. Though several PGD cases have been seen in the Division since 2006, no audit of this service has ever taken place. Therefore, the aim of this study was to conduct an audit of the PGD service at the Division of Human Genetics (NHLS/Wits) to provide accurate, local data to South African couples. The specific objectives were to determine the number and nature of PGD cases that have been managed through the Division of Human Genetics (NHLS/Wits), the demographics of couples choosing to undergo PGD, and to

evaluate the outcomes at various stages of the PGD process. Furthermore, we conducted a basic cost assessment of the PGD process.

Methods

Study sample

This study was a retrospective file review. The target population consisted of 33 couples who consulted the Division of Human Genetics (NHLS/Wits) between January 2006 and December 2016 for PGD-related genetic counselling (seen by a registered medical geneticist, genetic counsellor or genetic nurse). These couples were at risk of having a child with a monogenic condition, and initiated the PGD process. Only couples seen at a single genetic counselling centre and single fertility clinic were included, allowing for ease of accessibility and consistency of processes. Couples who had PGS without PGD, or who had PGD for a chromosomal abnormality, were excluded. After all exclusions, a total of 22 couples' files were available for data extraction and analysis. The sample selection process is summarised in Figure 1.

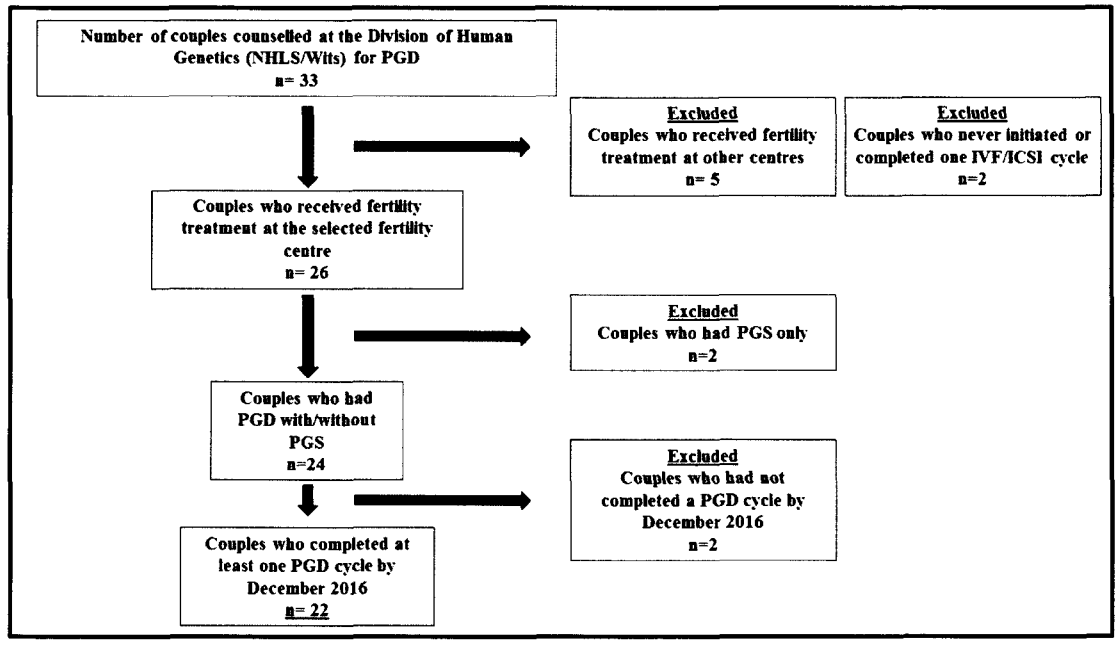

Figure 1: Study sample composition.

Study design

This was a descriptive study that involved an audit of couples' genetic counselling, IVF/ICSI and PGD records. Documented information included the types of genetic conditions, family and obstetric history, the number of IVF/ICSI cycles initiated, the number of embryos created, biopsied and transferred, the time elapsed until a successful pregnancy was achieved or the process was terminated, and the number of successful pregnancies achieved. Each IVF cycle was analysed separately at various stages of the process. A cost assessment of the PGD process was conducted using invoices contained within IVF/ICSI and PGD records. The cost assessment included the costs of genetic counselling consultations, all IVF/ICSI-related costs, courier costs, as well as the cost of PGD and PGS. The cost of the molecular genetic workup needed to identify the familial genetic mutation was not included in the cost assessment. Couples signed consent at the time of PGD initiation allowing for the anonymous use of their data. Ethics clearance was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (Ethics Clearance Certificate no. M170257).

Statistical analysis

The data analysis and statistical software package STATA version 12 (StataCorp. 2011. College Station, TX: StataCorp LP.) was used to analyse the data in this study. All data was checked for normality using the Shapiro-Wilk test. Continuous variables that were normally distributed were reported as means ± standard deviation. Continuous variables that were not normally distributed were reported as median (interquartile range). Categorical variables were described as frequencies and percentages.

Results

Participant demographics

Of the 22 couples' included in this study sample, 19/22 (86.4%) were Caucasian of European ancestry and 3/22 (13.6%) were Indian. There were no Black or Mixed Ancestry couples in this study sample. Furthermore, 7/22 (31.8%) of the couples were of Ashkenazi Jewish ancestry. The mean ages of the female and male partners at their initial PGD genetic counselling consultation were 31 ± 4.08 and 34.5 ± 3.73 years respectively. Of the female partners, 6/22 (27.3%) were considered to be of advanced maternal age (> 35 years of age). There was no mention of medical insurance coverage at the time of PGD enrolment in the files of two couples.

Genetic conditions for which PGD was requested

Preimplantation genetic diagnosis was requested for a range of monogenic conditions as depicted in Figure 2.

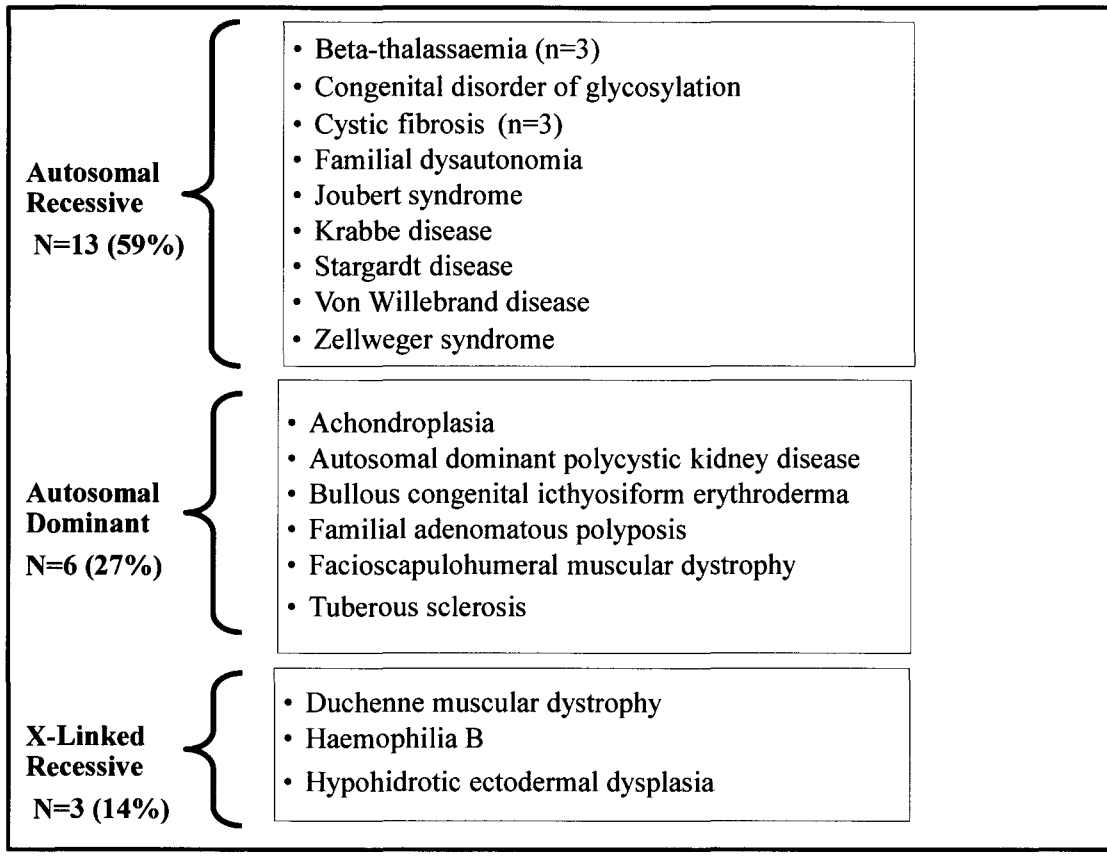

Figure 2: Genetic conditions for which PGD was requested

Family and reproductive histories of PGD couples

The family and reproductive histories captured are limited to what was documented at the couples' initial genetic counselling consultation. There was a single consanguineous couple (third-cousin union) in the study sample who requested PGD for an autosomal recessive condition. Regarding the couples' family histories, there was a median of 1 (1-2) individual affected with the monogenic condition in each family. Reproductive histories represented in Table 1 refer exclusively to pregnancies that occurred prior to PGD enrolment and do not consider any pregnancies (natural or assisted) that occurred during or after PGD. Of the 41 pregnancies, prior to PGD enrolment, 9/41 (17.1%) had prenatal testing. All nine of these prenatal tests were positive for the various monogenic conditions. In total, 8/9 (88.9%) affected pregnancies were terminated. Furthermore, 13/22 (59.1%) couples had at least one affected liveborn child each.

Table 1: Family and reproductive histories of PGD couples prior to PGD enrolment (N=22)

Reproductive event (RE)	**No. of couples with ZERO RE**	**No. of couples with ONE RE**	**No. of couples with TWO RE**	**No. of couples with THREE RE**	**No. of couples with ≥ FOUR RE**
Gravidity	3	8	7	1	3
Parity	7	9	4	1	1
Miscarriages	18	3	0	0	1
Terminated pregnancies	16	4	2	0	0
Affected liveborn children	9	10	3	0	0

No. number; RE reproductive event

PGD indications

Couples' reasons for seeking PGD are reported in Table 2. Preimplantation genetic diagnosis indications were inferred from family and reproductive histories, as well as notes recorded in patient files. Couples had more than one indication for undergoing PGD.

Table 2: Couples' reasons for undergoing PGD (N=22)

Indication†	Number of PGD couples
To avoid having an affected child	22
Previous affected child	13
HLA-matching for donor sibling	2
Previous failed sex-selection for X-linked condition	1
Poor fertility history	3
To avoid termination of pregnancy	5
Previous termination of pregnancy	6

HLA Human leukocyte antigen; PGD preimplantation genetic diagnosis

†Couples had more than one indication for undergoing PGD.

PGD cycle outcomes

Preimplantation genetic diagnosis outcomes for this study sample were analysed per cycle rather than per couple. Some PGD cycles had to be excluded due to missing information in patient records or an inability to locate patient records, therefore limiting the number of PGD cycles reported. A total of 45 PGD cycles were initiated among the 22 couples in the timeframe of the study. Out of 45 cycles, three cycles resulted in failed oocyte harvesting and were therefore discontinued. Outcomes for the remaining 42 cycles were analysed at different stages of the PGD process as indicated in Table 3 and Table 4. A median of 2 (1-2) cycles per couple were completed. Of the transferred embryos, 14/41 (34.0%) successfully implanted (indicated by positive beta-human chorionic gonadotropin (β-hGG) tests) and 11/14 (78.6%) of the implanted embryos resulted in a liveborn baby. The clinical pregnancy rate per embryo transfer was 29.3%. Overall, 10/22 (45.5%) couples in this study sample had a successful cycle resulting in a liveborn baby.

Table 3: Pooled data of cycle outcomes at various stages of the PGD process for 22 couples over a period of 10 years from 2006-2016

Stage of the PGD process	Outcome	n	%
Total number of cycles	Failed cycles (failed oocyte harvesting)	3/45	6.7
	Completed IVF/ICSI cycles (from oocyte harvesting to embryo)	42/45	93.3
IVF/ICSI[†]	Oocytes retrieved	532	-
	Oocytes fertilised/inseminated	343/532	64.5
Embryo biopsy	Embryos biopsied	220/343	64.1
	Embryos re-biopsied	8/220	3.6
	Day 3 biopsies	88/228	38.6
	Day 5/6 biopsies	132/228	57.9
	Unknown embryo stage	8/228	3.5
Testing of biopsied embryos	Embryos tested (PGD)	222/228[‡]	97.4
	Embryos tested (PGS)	137/228	60.1
	Embryos tested (HLA-typing)	66/228	28.9
Biopsy results	Unaffected embryos (PGS and PGD) suitable for transfer	71/228	31.1
	Total number of affected embryos	117/228[§]	51.3
	Affected embryos with abnormal karyotype only (PGS)	42/137	30.7
	Affected embryos with monogenic condition only (PGD)	67/222	30.2
	Affected embryos with abnormal karyotype and monogenic condition (PGS and PGD)	8/228	3.5
	Inconclusive results (PGD/PGS/Both)	47/228[¶]	20.6
Embryo transfer	Embryos transferred	41/71	57.7
Final pregnancy outcome	Embryos implanted (positive β-hCG)	14/41	34.1
	Prenatal genetic tests recorded	1/14	7.1
	Miscarriages	2/14	14.3
	Clinical pregnancies	12/14	85.7
	Liveborn babies	11/14	78.6
	Postnatal genetic tests recorded	2/11	18.2

β-hCG Beta-human chorionic gonadotropin; HLA Human leukocyte antigen; ICSI intracytoplasmic sperm injection; IVF *in vitro* fertilisation; PGD preimplantation genetic diagnosis; PGS preimplantation genetic screening

†Number of oocytes retrieved and fertilised were taken to be the same as the number of embryos biopsied for four cycles where this information was missing from records.

‡Some embryos were tested for chromosome abnormalities prior to PGD. Six embryos were chromosomally abnormal (detected by PGS) and therefore did not have PGD.

§There were 66 embryos that were also tested for HLA-matching. Of these, 8/66 (12.1%) were HLA matches but were unsuitable for transfer.

¶Eight of these were re-biopsied, of which seven were reclassified and are therefore also represented in the affected/unaffected category.

Table 4: Average outcomes per cycle at various stages of the PGD process for the 42 cycles completed by the 22 couples in this study sample

Number of events per cycle	Mean ± SD /Median (IQR)
Oocytes retrieved	12 (11-14)
Oocytes fertilised/inseminated	8.2± 4.59
Embryos biopsied	5.2 ± 2.72
Unaffected embryos (PGS and PGD) suitable for transfer	1 (1-2)

IQR interquartile range; PGD preimplantation genetic diagnosis; PGS preimplantation genetic screening; SD standard deviation

Time to complete the PGD process

On average, couples took 21 months from their initial PGD genetic counselling consultation to complete the PGD process, i.e. achieve a biochemical pregnancy or discontinue the process, during the study timeframe. Furthermore, it took an average of 17.5 months from the initial fertility consultation until completion of the PGD process, and an average of 10 months from the initiation of IVF/ICSI (hormone stimulation) until completion of the PGD process. The time it takes couples to complete the process is, however, variable. For example, one couple took 40 months to complete two PGD cycles, which resulted in a single successful pregnancy. Contrastingly, another couple took 35 months in which seven cycles were completed, but there were no successful pregnancies.

Cost assessment of one PGD cycle

A cost assessment of the PGD process was conducted to determine the average cost per PGD cycle. The analysis included costs involved from the initiation of PGD up until the completion of a PGD cycle, as shown in Table 5. The point of cycle completion can vary; some couples may not go further than embryo biopsy, whereas other cycles may include embryo transfer, and this will in turn influence the cost of each cycle. Furthermore, the cost of fertility assessments and medication will vary depending on the fertility of the couple and whether IVF/ICSI was performed during a natural cycle or medically stimulated cycle. Seven cycles with insufficient financial records were omitted from the cost evaluation. The cost assessment was divided into three analyses: the first considered the cost of couples' first cycles only. On average, the total cost was USD 9,734 (ZAR 128,336 as per exchange rate on 25/08/2017) per cycle. The second analysis considered all the cycles undertaken. On average, the total cost per cycle was USD 9,525 (ZAR 125,578 as per exchange rate on 25/08/2017). The final analysis only included couples' most expensive cycles. On average, the total cost per cycle was USD 10,283 (ZAR 135,572 as per exchange rate on 25/08/2017). Three different analyses were

done because of the variability of the costs involved during each cycle. The costs reflected here do not include the cost of the molecular genetic diagnostic workup to identify the familial mutation, which is required prior to the initiation of PGD. These costs were excluded because of their variability. The cost for the molecular testing will be influenced by the type of genetic test performed, the laboratory used (local or international) as well as the number of people in the family who need to be tested to identify the familial mutation.

Table 5: Cost assessment of the PGD process per cycle given in USD, excluding molecular genetic testing costs

Stage of PGD process	Costs involved	Average cost of couple's first cycle (n=20)	Range	Average cost of all cycles (n=35)	Range	Average cost of couple's most expensive cycles (n=20) †	Range
Pre-PGD enrolment	Genetic Counselling	61	57-64	35	0-64	36	0-64
IVF/ICSI	Fertility Assessments‡	326	115-648	189	0-648	47	0-648
	Medication	729	137-1,514	741	28-1,514	803	38-1,514
	IVF/ICSI§	3,523	1,507-6,925	3,782	573-7,878	4,501	1,582-7,878
	Total	4,578	1,898-8,995	4,712	1,525-9,189	5,351	2,748-9,189
Testing	PGD	3,190	3,022-4,575	3,025	1,033-4,575	3,094	2,115-3,668
	PGS	1,562	1,294-2,209	1,474	992-2,209	1,511	992-2,209
	Courier fees	314	57-561	250	0-561	248	0-561
	Other fees¶	29	0-379	29	0-379	43	0-379
	Total	5095	4373-7724	4778	2025-7724	4896	3107-6817
Grand Total of PGD cycle††		**9,734**	6,398-14,554	**9,525**	4,052-14,554	**10,283**	6,577-14,554

ICSI intracytoplasmic sperm injection; IVF *in vitro* fertilisation; PGD preimplantation genetic diagnosis; PGS preimplantation genetic screening, USD United States Dollar

†Some couples only had one cycle, which was included.

‡Includes, but may not be limited to, fertility screening, blood tests, follicle counts, semen analysis, hysteroscopy, doctor's consultation

§Includes ovarian stimulation, oocyte retrieval procedure, fertilisation by IVF/ICSI, embryo culturing, embryo biopsy, embryo vitrification, embryo transfer, embryo storage, fertility specialist and embryologist fees, facility fees, laboratory fees.

¶Other fees include antenatal consultation fees, ultrasound fees and other non-routine medical procedures that may have been necessary.

††Costs are given in USD for easy comparison to other published figures.

Discussion

PGD outcomes

This is the first study to report on measures of PGD outcomes in SA. In this study, we report a clinical pregnancy success rate of 29.3% per embryo transfer. This finding is in keeping with the clinical pregnancy success rate of 29.0% per embryo transfer reported in 2012 by the European Society of Human Reproduction and Embryology (ESHRE) PGD consortium[9]. In 2010, the ESHRE PGD consortium reported that out of 8111 PGD-only cycles, 61% of oocytes retrieved were fertilised, of which 74.2% were biopsied and 33% of these embryos were suitable for transfer. Of the embryos transferred, 18.0% implanted. The clinical pregnancy success rate per embryo transfer was reported as 26%[10]. We report a similar rate of embryos that were suitable for transfer in our study sample (31.1%) despite a significantly smaller sample size. However, we report a higher embryo implantation success rate (34.1%). The difference in these results is likely to be attributed to the inclusion of PGS in a high number of embryos in our study sample (n=137), as well as potential differences in the IVF process. Overall, our findings are comparable to previously reported global PGD outcome measures and will be integral in providing evidence-based genetic counselling in the local context.

Preimplantation genetic diagnosis is still a relatively new service that is offered in SA. In recent years, changes to the timing of embryo biopsy, new testing strategies and methodologies, robust embryo vitrification technologies, and the introduction of PGS, appear to have improved the IVF/ICSI pregnancy success rates. The use of next generation sequencing (NGS) and array comparative genomic hybridisation (aCGH) for PGS, and karyomapping for PGD, have led to an increase in the accuracy and efficiency of testing, with less false-negative and false-positive results reported[4,5]. Biopsy on day five and day six embryos is also associated with increased success rates[3,5]. With the introduction of new, robust vitrification technology in 2015, there has been an improvement in the pregnancy success rates in patients who have a frozen thawed transfer cycle where centres vitrify rather than slow-freeze the embryos[11,12]. One would expect to see higher pregnancy success rates in a PGD couple cohort since many will have normal fertility. Most of the PGD couples included in this study sample had PGD pre- 2015. With a general increase in IVF/ICSI success rates we expect to see a concomitant rise in the PGD pregnancy success rates in the coming years.

Time to complete the PGD process

The time it took couples included in this study to complete the PGD process was variable. We hypothesise that the timing will be influenced by many factors including whether a family-specific mutation has been identified, whether couples' have completed/had a baseline fertility workup, and if financial resources are readily available. Other factors such as the age of the couple, emotional wellbeing, and life-stage may also influence how long couples take to complete the process. This study only considered the time to complete the PGD process and did not investigate the factors contributing to the timing, as this fell out of the scope of the current study. Follow-up research will aim to identify the factors that contribute to the timing of the PGD process.

The cost of PGD

The cost of PGD coupled with the lack of medical insurance coverage is a limiting factor, making this procedure inaccessible to most couples in SA. A previous study found that couples in the USA felt that using PGD to avoid having affected children was worth the financial burden, however, couples also stressed that the cost of PGD was a major barrier to its use[13]. In 2013, the cost range of one IVF cycle in the USA was USD 9,226–12,513 while PGD cost an additional USD 2,500–6,000 per cycle[13]. A fertility centre in Chicago estimated the cost of one PGD cycle to amount to USD 20,895[14]. We estimate the cost of one PGD cycle (including PGS) to be approximately USD 9,525. As the median number of cycles per couple in this sample was 2 (1-2), the average cost of PGD in SA can be estimated as USD 19,050 (ZAR 251,156 as per exchange rate on 25/08/2017). The costs reflected here are likely to underestimate the total cost slightly, however, as the evaluation did not consider discounts that may have been offered, and medical insurance that may have covered some of the costs for which couples were then not invoiced. For example, some medical insurance companies may provide some financial cover for consultation fees, medication and other aspects of IVF. Furthermore, the cost of molecular genetic testing needed to identify familial mutations for the PGD workup was not included in this evaluation.

The cost of PGD limits access for many couples in SA. However, the Malka Ella Fertility Fund is a charity which assists South African Orthodox Jewish couples. This organisation will fund up to two IVF/ICSI cycles per couple per year and will also fund PGD and PGS[15]. There was a noteworthy representation of Ashkenazi Jewish couples in this study sample (31.8%), which could be explained by their access to private funding for PGD. No other such services exist for South African couples who are not Ashkenazi Jewish.

Benefits of PGD

Whilst the costs of PGD are high, the financial burden of caring for a child with a genetic condition is significant. As reviewed in Anderson et al. (2007), children with disabilities utilise healthcare services at a significantly higher rate compared to children without disabilities, and therefore incur higher healthcare expenditures[16]. The cost of caring for a child with disabilities varied between USD 108 to USD 8,742 per year, with costs exceeding 5%-12% of families' incomes[16]. Low-income families are particularly vulnerable to the financial burden of caring for a child with disabilities or severe medical problems. Furthermore, caring for a child with a genetic condition who may have physical and intellectual disability, or may need continuous medical intervention and care, can carry a significant emotional burden. The psychological effects of terminating an affected pregnancy are just as significant[17]. South Africa is a resource limited country. The unemployment rate was reported as 27.7% in the third quarter of 2017[18], leaving many citizens dependent on government-funded healthcare, which at present does not provide any funding towards PGD. South Africa's current legislative framework lacks appropriate regulation regarding the use and accessibility of PGD which needs to be addressed.

Access to equal healthcare is a basic human right. The ESHRE argue that the decision to have children should not be dependent on income as this is considered part of basic healthcare, and PGD provides an equal opportunity to have an unaffected, yet genetically related, child[19]. PGD further allows couples to avoid having to terminate an affected pregnancy. In this sample, 11/22 (50%) couples requested PGD to avoid terminating a pregnancy, or because they had a previous termination of pregnancy and were not prepared to undergo another termination of pregnancy. This highlights the significant negative impact that a termination of pregnancy can have on a couple, and the significance of avoiding a termination of pregnancy as an indication for PGD.

Conclusion

This retrospective file review of 22 PGD couples shows that the success rates achieved in our South African centre are comparable to those achieved globally. South Africa has the infrastructure and the expertise to provide PGD as a service; the limiting factor is the lack of government-funded or medical insurance-funded PGD initiatives, despite the financial burden children affected with genetic conditions place on healthcare resources. We suggest that the potential benefits that can be garnered through the use of PGD as a prevention mechanism for monogenic disorders outweigh the cost implications, and that the South African government support the idea of increasing public access to PGD. We advocate for the essential inclusion of genetic counselling services as a standard part of the PGD process. It is a cost-effective service relative to the costs involved in the PGD process. Couples need to be counselled about the PGD process, the limitations and the potential outcomes. Couples also need to be aware of other reproductive options available to them, for example prenatal testing, so that they can make informed decisions. Genetic counsellors are equipped with the necessary skills to convey this information in a non-directive manner and can assist with the adaptation process. The findings from this study will enable genetic counsellors to offer couples interested in PGD evidence-based and locally accurate information regarding PGD outcomes, success rates and costs involved. Although this study was limited in its sample size, it reflects the uptake and success of PGD services in Johannesburg, SA. Additional limitations of this study include omitted and incomplete information in records which impact on the accuracy of the data presented. Meticulous record keeping in healthcare is of utmost importance to ensure the quality of future research in the field. Future research should focus on collecting larger data sets over a longer period to assess the success rates of PGD overtime accurately. The authors of this paper are currently conducting a qualitative study assessing couples' experiences of the PGD process. These results, together with the information from this study, are important in guiding PGD-related genetic counselling practices and assisting with the implementation of appropriate PGD-related training of genetic counsellors in SA.

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Thank you to Ms Shelley Macaulay, Dr Jaysen Knezovich, Ms Lynn Frylinck, Ms Calista Hardwick and Ms Angela Turner for your contributions to this study. Thank you to the National Health Laboratory Service for hosting me. We are truly grateful.

Appendix A

Approved research protocol

CANDIDATE'S SURNAME:	Carzis	FIRST NAME/S:	Bianca	STUDENT NUMBER:	1260293
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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: 2016			
DEPARTMENT: Human Genetics					
TITLE OF PROPOSED RESEARCH: An Evaluation of Preimplantation Genetic Diagnosis Outcomes in Johannesburg, South Africa					
CANDIDATE'S SIGNATURE: 		DATE: 27-01-2017			
SUPERVISOR #1'S NAME: Ms. Tasha Wainstein					
% OF SUPERVISION TO BE PROVIDED BY SUPERVISOR #1: 50%					
SUPERVISOR #1'S QUALIFICATIONS: MSc (Med) Genetic Counselling					
SUPERVISOR #1'S DEPARTMENT: Human Genetics					
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SUPERVISOR #2'S NAME: Prof. Amanda Krause					
% OF SUPERVISION TO BE PROVIDED BY SUPERVISOR #2: 40%					
SUPERVISOR #2'S QUALIFICATIONS: MBBCh, PhD					
SUPERVISOR #2'S DEPARTMENT: Human Genetics					
SUPERVISOR #2'S ADDRESS / TEL / **E-MAIL**: Room 104, First Floor, Watkins Pitchford Building, , Cnr Hospital & De Korte Str, Braamfontein/Tel: 011-489-9219/ Email: amanda.krause@nhls.ac.za					
SUPERVISOR #3'S NAME: Dr. Lawrence Gobetz					
% OF SUPERVISION TO BE PROVIDED BY SUPERVISOR #3: 10%					
SUPERVISOR #3'S QUALIFICATIONS: MBChB (Pret), F.C.O.G (SA) with Reproduction Medicine					
SUPERVISOR #3'S DEPARTMENT: N/A					
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SYNOPSIS OF RESEARCH:

Preimplantation genetic diagnosis (PGD) is a process that involves testing embryos that were created using artificial reproductive techniques (ART) for genetic conditions before being transferred to a woman's uterus for implantation. For couples who are at risk of having a child affected with a genetic condition, PGD provides an alternative option to prenatal testing and has the potential to avoid an unwanted termination of pregnancy. In Johannesburg, South Africa, the Division of Human Genetics at the National Health Laboratory Service (NHLS) and the University of the Witwatersrand (Wits) in collaboration with Genesis Genetics and Vitalab Fertility Clinic has offered PGD services to private patients for ten years. To date, no audit of this service has ever taken place. The aim of this study is to determine the number and nature of PGD cases that have been seen through the Division of Human Genetics, NHLS, Johannesburg, and assess the outcomes at various stages of the PGD process. This study is a retrospective case review involving an audit of patients' genetic counselling, fertility and PGD records. Information regarding family and obstetric history, the number of IVF cycles underwent, the number of embryos tested and transferred, pregnancy outcomes, as well as the cost and time to complete PGD will be analysed quantitatively. The results of this study will enable genetic counsellors and other healthcare professionals involved in the PGD process to provide patients with more accurate information regarding PGD outcomes and costs in South Africa based on the information obtained from patients in this study population. Furthermore, patients will be able to compare these outcomes to other centers. This will allow patients to make informed decisions when considering PGD as a reproductive option.

ETHICS PENDING: (Ψ) ETHICS APPROVED: (circle appropriate symbol)	IF Y SUPPLY ETHICS CLEARANCE No:

SIGNATURE OF SUPERVISOR/S:			

An Evaluation of Preimplantation Genetic Diagnosis Outcomes in Johannesburg, South Africa:

Bianca Carzis

1260293

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PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

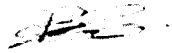
SENATE PLAGIARISM POLICY: APPENDIX ONE

I **Bianca Carzis** (Student number: **1260293**) am a student

registered for the degree of **MSc (Med) Genetic Counselling** in the academic year 2017.

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 degree 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: 

Date: 26/01/2017

1. Background literature analysis and critique

1.1 Introduction

Preimplantation genetic diagnosis (PGD) is a process that involves testing embryos that were created using artificial reproductive techniques (ART) for genetic conditions before being transferred to a woman's uterus for implantation. PGD is indicated in couples who are at high-risk of having a child with a genetic condition, either because they are carriers of a monogenic disease mutation or of a chromosomal structural rearrangement. A second indication for PGD is human leucocyte antigen (HLA) typing for the purposes of HLA matching for donor siblings (Ben-Nagi et al., 2016).

Preimplantation genetic screening (PGS) involves a similar process, but is indicated in individuals who do not carry a known genetic condition, but whose embryos are screened after going through *in vitro* fertilisation (IVF) for large chromosomal aneuploidies to increase their chances of a successful pregnancy (Dahdouh et al., 2015). Patients who are undergoing PGD may opt to have PGS performed simultaneously. Traditional prenatal testing options, including chorionic villus sampling and amniocentesis, are invasive procedures which are associated with a miscarriage risk and the decision of whether or not to terminate an affected pregnancy. PGD and PGS offer parents an opportunity to have a child unaffected with the family genetic condition and avoid an unwanted termination of pregnancy (Ben-Nagi et al., 2016). PGD rather than PGS will form the main focus of this study.

The first successful use of PGD in humans was reported in 1990 (Handyside et al., 1990) and by 2004 the first 1,000 PGD births were reached (Verlinsky et al., 2004). In South Africa, PGD is still a relatively new service that is being offered. While PGD has been available since the 1990's in developed countries in the United States and Europe, the first PGD case seen through the Division of Human Genetics at the National Health Laboratory Service (NHLS) and the University of the Witwatersrand (Wits) Johannesburg, South Africa was in 2006 (A. Krause,

pers. comm., 5 January 2017). Though a number of cases have been seen in the Division since then, no audit of this service has ever taken place.

1.2 **The basic PGD workflow**

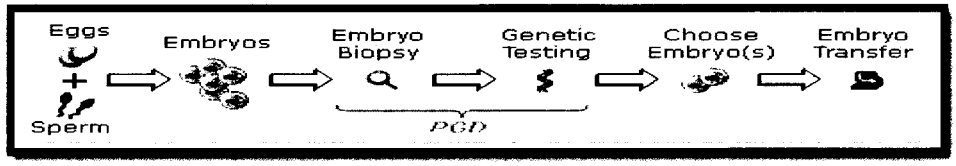


Figure 1: Diagram illustrating the basic process for PGD. After a complete genetic workup and optimisation of a family-specific genetic test is completed, embryos are produced by artificial reproductive techniques and are then tested for genetic conditions before being selectively transferred for implantation if unaffected.
(Retrieved from: http://www.ingender.com/gender-selection/PGD/)

The process of PGD is summarised in Figure 1. Embryos are created using IVF. Intracytoplasmic sperm injection (ICSI), which involves injecting a single sperm directly into the egg, is the technique used when PGD for monogenic conditions is being performed. ICSI is used to maximise fertilisation and prevent maternal and paternal contamination, allowing for improved diagnostic accuracy (Ben-Nagi et al., 2016). A biopsy is then preformed either on a polar body, one cell (blastomere) from a cleavage stage embryo, or on trophectodermal cells from a blastocyst stage embryo. The stage at which the biopsy is performed is associated with various advantages and disadvantages and can influence the outcome of the IVF process and the accuracy of genetic testing (Scott et al., 2013) Embryo biopsy is then followed by genetic diagnostic testing. Different genetic testing techniques are employed, depending on the genetic condition being tested. Previously, the mutation analysis assay was tailored specifically for each family. Currently, PGD labs are using single-nucleotide polymorphism (SNP) arrays as an alternative. This allows for more comprehensive and more efficient screening of chromosomes which improves IVF and pregnancy outcomes (Dahdouh et al., 2015). Fresh or frozen embryos found not to have the genetic defect of interest are then transferred to the uterus for implantation. Embryos that are unaffected but were not transferred are cryopreserved for use in future cycles. Embryos that are affected with the genetic defect of interest are discarded.

1.3 **Limitations of PGD**

Couples who decide to undergo PGD will have to go through IVF, even if they are fertile. Achievement of a successful pregnancy is limited by the success rate of IVF and genetic testing, and therefore a successful pregnancy cannot be guaranteed. The success rates of IVF are influenced by multiple factors. Advancing maternal age is associated with a decreased number of follicles, reduced oocyte quality, poor ovarian reserve and reduced response to ovarian stimulation (Ben-Nagi et al., 2016). Therefore, the success rate for IVF in older women may be lower than for younger women. The type of biopsy performed also influences the success rate of PGD. Cleavage stage biopsy is associated with a 30% success rate, and a blastocyst stage biopsy is associated with a 51% success rate (Scott et al., 2013). The type of genetic condition involved can also influence the success of PGD. The pregnancy rate is higher for monogenic disorders compared to chromosomal rearrangements. This is as a result of a higher proportion of unaffected embryos being available for transfer for monogenic compared to chromosomal conditions (Harper et al, 2012).

There are technical limitations associated with genetic testing and these may also affect the outcome of the PGD process. Polymerase chain reaction (PCR) is used to test a single or small number of cells from an embryo for a monogenetic condition. Limitations of using PCR-based techniques on a single cell include DNA contamination and allelic dropout (Ben-Nagi et al., 2016), which both affect the diagnostic accuracy. Previously, fluorescence *in situ* hybridisation (FISH) was used to diagnose chromosomal abnormalities, which was associated with a misdiagnosis rate of 0.1% per embryo transfer (Harper et al., 2012). Currently, SNP arrays and comparative genomic hybridisation arrays are routinely used which has alleviated some, but not all, of the technological limitations previously experienced.

The cost of PGD coupled with the lack of medical insurance coverage is also a limiting factor, making this procedure inaccessible to most couples. Drazba et al. (2013) found that couples in

the United States felt that using PGD to avoid having affected children was worth the financial burden, however, couples also stressed that the cost of PGD was a major barrier to its use. In 2013 the average cost of one IVF cycle in the United States was US$9,226–12,513 (~R129,936-R176,229) while PGD cost an additional US$2,500–6,000 per cycle (~R35,209-R84,502) (Drazba et al., 2013). In South Africa, the costs for PGD are high but need to be evaluated. There are many factors which will influence the overall cost of the PGD process including patient fertility, the type and amount of hormone medication required, IVF cost, embryo biopsy cost, genetic counselling consultation cost, number of IVF cycles and the type of genetic test used.

1.4 **Religious, cultural and ethical concerns**

PGD has been contested by certain religious and cultural groups, largely on the basis of the status of the embryo. PGD has been the topic of many ethical debates and its applications have been seen as controversial. PGD is permissible in Judaism and in Islam as well as some Christian denominations but not by the Roman Catholic Church (Fasouliotus & Schenker, 1998; Alsulaiman et al., 2010). From an ethical standpoint, the application of PGD or PGS for adult-onset conditions, HLA-typing for the creation of 'saviour siblings', discarding affected embryos and sex selection for family balancing have been met with much controversy. In South Africa, sex selection for family balancing is not permitted by law (National Health Act No. 61 of 2003).

1.5 **PGD for South African patients**

Genesis Genetics, a commercial reproductive genetic testing laboratory and one of the pioneers of PGD services, has been involved in offering PGD and performed many of the first successful cases of PGD. Genesis Genetics currently have twelve laboratories located across the globe. In South Africa, the Division of Human Genetics (NHLS/Wits) collaborates with Genesis Genetics in the United Kingdom and United States, and more recently South Africa

(http://www.genesisgenetics.co.za/), as well as Vitalab fertility clinic in South Africa (http://www.vitalab.com/index.html) to provide PGD as a service to South African patients (A. Krause , pers. comm., 5 January 2017). As a result of the cost implications involved with PGD, this service is only accessible to private patients of a higher socio-economic status in South Africa. PGD is not offered at state hospitals. Figure 2 illustrates the current PGD process that South African patients in Johannesburg undergo. The process is not static and can change, with certain stages of the process occurring simultaneously.

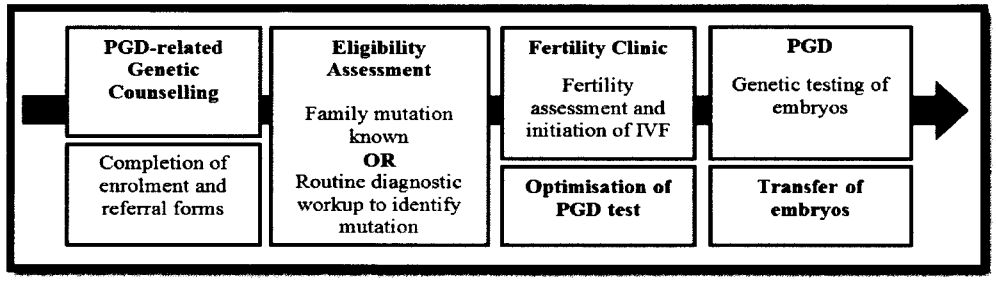


Figure 2: Standard operating procedure for PGD patients in Johannesburg. The Division of Human Genetics (NHLS/Wits) collaborates with Genesis Genetics and Vitalab to provide PGD to its patients.

When patients are seen for PGD-related counselling, the main concerns that are usually raised centre around the high costs involved, the complexity and length of the entire PGD process, as well as the relatively low IVF success rates (A. Krause, pers. comm., 5 January 2017). In order to make an informed decision about whether or not to undergo PGD, patients need to have an understanding of all the limitations involved and the possible outcomes that they may experience.

Therefore, the aim of this study is to determine the number and nature of PGD cases that have been seen through the Division of Human Genetics (NHLS/Wits), and assess the outcomes at various stages of the PGD process. The results of this study will enable genetic counsellors and other healthcare professionals involved in the PGD process to provide patients with more accurate information regarding PGD outcomes and costs in South Africa based on the information obtained from patients in this study population. Furthermore, outcomes to other

PGD centers will be compared. This will allow patients to make informed decisions when considering PGD as a reproductive option.

2. Aim and Objectives

2.1 Aim

To determine the number and nature of PGD cases seen through the Division of Human Genetics (NHLS/Wits) and assess the outcomes at various stages of the PGD process by conducting a retrospective case review.

2.2 Objectives

- To determine patient demographics, the associated genetic condition and relevant family history including affected family members, poor obstetric history and previous prenatal testing and pregnancy terminations and to identify patients' reasons for seeking PGD.
- To evaluate the outcome of the PGD process by quantifying information such as the number of IVF cycles undertaken, the number of embryos produced, tested and transferred, and the number of successful pregnancies achieved.
- To establish the total cost of the PGD process and the time that it takes to complete the PGD process until a successful pregnancy is achieved.

3. Methods

3.1 Study Participants

The study sample will consist of patients who have been seen for PGD-related genetic counselling (either by a registered medical geneticist, genetic counsellor or genetic nurse, through the Division of Human Genetics [NHLS/Wits]) who then initiated the PGD process. An information document and informed consent form will be Emailed to eligible participants (Appendix one). The genetic counselling, IVF and PGD records of all of the patients who have been seen through the Division for PGD-related genetic counselling will be reviewed and

included in the study (upon receipt of informed consent). The expected sample size is 25-30 participants. This number is based on the estimated number of patients who have been seen by the Division of Human Genetics (NHLS/Wits) who meet the inclusion criteria. The Division of Human Genetics (NHLS/Wits) works closely and predominantly with Vitalab. Therefore, only patients who have been managed through the Division of Human Genetics (NHLS/Wits) and who have had their IVF treatment at Vitalab will be included in this study, allowing for ease of accessibility and consistency of processes. Appendix two contains permission letters from the relevant authorities at the Division of Human Genetics (NHLS/Wits) and Vitalab allowing for the access to patient records.

3.2 **Inclusion criteria**

1. Patients who initiated the PGD process after an initial PGD-related genetic counselling consultation, through the Division of Human Genetics (NHLS/Wits), for any monogenic genetic condition.
2. Patients who were seen between January 2006 and December 2016.
3. Patients who are older than 18 years of age.
4. Patients who attended Vitalab fertility clinic for their IVF treatment.

3.3 **Exclusion Criteria:**

1. Patients who underwent PGS only.
2. Patients who underwent PGD for a chromosomal abnormality.

3.4 **Study Design**

This study is a retrospective case review and will therefore involve an audit of patients' genetic counselling, IVF and PGD records. The genetic counselling records include family pedigrees, relevant obstetric and medical history, and notes regarding their genetic counselling consultations. This information is routinely collected when patients are seen for genetic counselling in the Division of Human Genetics (NHLS/Wits). The Vitalab records include

fertility and PGD information pertaining to patients' IVF cycles and the genetic status of tested embryos. Ethics clearance from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand will be sought to use the information from the patients' files. The data collected from the files will be anonymised to protect the individuals' confidentiality. The data will be coded and will not be attached to any personal identifiers.

Information regarding the number of cases that have been seen for PGD-related genetic counselling and the related genetic conditions, relevant family and obstetric history, the number of IVF cycles underwent, the number of embryos generated, tested and transferred, the time elapsed until a successful pregnancy was achieved and the number of successful pregnancies will be retrieved from the patients' files and will be documented using Research Electronic Data Capture (REDCap) Software. A detailed data collection sheet can be found in Appendix three.

3.5 **Data Analysis**

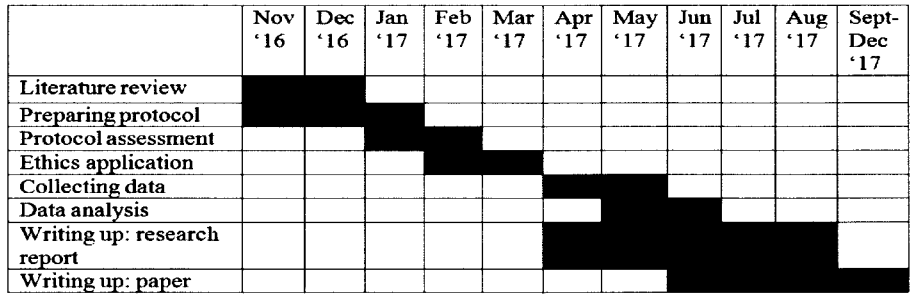
The data collected during this study will be analysed quantitatively. Descriptive statistics will be used to examine the distribution of data. Frequencies of observations will be reported, as well as the mean, median, mode and standard deviation of the data collected. Excel 2016 will be used to analyse the data. IVF outcomes at various stages of the processes will be evaluated. Each IVF cycle will be analysed separately and averages will be reported for each couple. Average outcomes for the entire study sample will also be reported. A cost analysis of the PGD process will be conducted using invoices contained within patient records. Input from Vitalab will be obtained where necessary. A breakdown of the components of the cost analysis is included in Appendix three.

4. **Ethics**

Ethics clearance pertaining to the use of data recorded within patients' genetic counselling, IVF and PGD records has been sought. Patients will be contacted via Email to inform them of the

study and obtain their consent to access the information contained within their genetic counselling, IVF and PGD records. The choice to Email the information document and informed consent form to participants is seen as appropriate due to ease of access for the participants. Furthermore, the participants are widely dispersed across the country so face-to-face informed consent may be difficult. The data will be anonymised and coded and only the researcher and the supervisors will have access to the identifying information. Submission to the Human Research Ethics Committee (Medical) of the University of the Witwatersrand was made on 7 February 2017. At a meeting of the committee held on 24.02.2017, the proposal was granted ethical clearance (Reference number: M170257).

5. Timing



6. Funding

6.1 Predicted Budget

Resource	**Cost**	**Source of Funding**
Researcher's travel expenses (three trips between NHLS and Vitalab = 90km) at 82.4c/km	R74,16	Self-funded
Printing and binding of research report (~80 pages) (X2) at 39c per page	R82.40	Self-funded
REDCap Software and Excel 2016	R0.00	Freely available
Total:	**R156.56**	

7. Possible problems and study limitations

This study will contain a small sample size, as it is limited to the amount of patients who have been seen through the Division of Human Genetics (NHLS/Wits) for PGD-related genetic counselling. This may limit the statistical power of the study. Although the sample is small, a

large volume of data will be collected from each patient relating to each IVF cycle. Therefore, the number of IVF cycles will outnumber the amount of patients included, generating a larger data set. Some of the patient records may also be incomplete and certain information omitted.

8. References

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Ben-Nagi, J., Serhal, P., SenGupta, S., et al. (2016). Preimplantation genetic diagnosis: an overview and recent advances. *The Obstetrician & Gynaecologist*, 18(2), 99-106.

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Fasouliotis, S. J., & Schenker, J. G. (1998). Preimplantation genetic diagnosis principles and ethics. *Human Reproduction*, 13(8), 2238-2245.

Handyside, A. H., Kontogianni, E. H., Hardy, K., et al. (1990). Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature*, 344(6268), 768-770.

Harper, J. C., Wilton, L., Traeger-Synodinos, J., et al. (2012). The ESHRE PGD Consortium: 10 years of data collection. *Human reproduction update*, 18(3), 234-247.

Scott, R. T., Upham, K. M., Forman, E. J., et al. (2013). Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. *Fertility and sterility*, *100*(3), 624-630.

Verlinsky, Y., Cohen, J., Munne, S., et al. (2004). Over a decade of experience with preimplantation genetic diagnosis: a multicenter report. *Fertility and sterility*, *82*(2), 292-294.

Appendix one

INFORMATION DOCUMENT

Study title: An Evaluation of Preimplantation Genetic Diagnosis Outcomes in Johannesburg, South Africa.

Dear Sir/Madam

Introduction:
My name is Bianca Carzis and I am an MSc (Med) Genetic Counselling Masters student in the Division of Human Genetics, National Health Laboratory Services (NHLS) and University of the Witwatersrand (Wits). I, along with my supervisors, am conducting research to determine the number and nature of PGD cases seen through the Division of Human Genetics, (NHLS/Wits), and assess the outcomes at various stages of the PGD process. Research is just the process used to learn the answer to a question. In this study we would like to review couples' Genetic Counselling, IVF and PGD records to gain insight into the family history, indications for PGD, number of IVF cycles undertaken, the number of embryos produced and tested, pregnancy outcomes, as well as the cost and time to complete the PGD process. Reviewing these records is not routine care and will be done for purposes of this research study. The results of this study will enable genetic counsellors and other healthcare professionals involved in the PGD process to provide patients with more accurate information regarding PGD outcomes and costs in South Africa based on the information obtained from patients in this study population. This will allow patients to make informed decisions when considering PGD as a reproductive option.

Invitation to participate: We are requesting your permission to review your genetic counselling, IVF and PGD records and include the data obtained from these records in our study. All information will remain anonymous and no personal identifiers will be attached to your data.

What is involved in the study: This study is a retrospective case review and will therefore involve an audit of patients' genetic counselling, IVF and PGD records. The genetic counselling records include family pedigrees, relevant obstetric and medical history, and notes regarding patients' genetic counselling consultations. The data collected during this study will be analysed and will be reported as totals, averages and percentages.

Risks: There are no risks to participants
Benefits: There will be no direct benefits to participants

Participation is voluntary; refusal to participate will involve no penalty or loss of benefits to which the participant is otherwise entitled

Confidentiality: Efforts will be made to keep personal information confidential. Absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law (This is extremely unlikely).

Organizations that may inspect and/or copy our research records for quality assurance and data analysis include groups such as the Research Ethics Committee and the Medicines Control Council (This is extremely unlikely).
If results are published, results may lead to individual / cohort identification.

Contact details of researcher/s: Please feel free to contact us if you require further information

Researcher: Miss Bianca Carzis | 011 489 9223 | bianca.carzis@nhls.ac.za
Supervisor 1: Miss Tasha Wainstein | 011 489 9151 | tasha.wainstein@nhls.ac.za
Supervisor 2: Professor Amanda Krause | 011 489 9219 | amanda.krause@nhls.ac.za
Supervisor 3: Dr Lawrence Gobetz | 0861 882 522 | lawrenceg@vitalab.com

Contact details of REC administrator and chair – for reporting of complaints / problems.

Ms. Zanele Ndlovu | 011 717 1252/2700/1234/2656 | zanele.ndlovu@wits.ac.za
Mr. Rhulani Mkansi | 011 717 1252/2700/1234/2656 | rhulani.mkansi@wits.ac.za

Informed Consent

With regards to the research study entitled **"An Evaluation of Preimplantation Genetic Diagnosis Outcomes in Johannesburg, South Africa"** I (Name), _____ consent to my genetic counselling, PGD and IVF records being reviewed and analysed by the researcher and the supervisors.

- I consent to the use of data contained within these records for the purposes of the above mentioned research project.
- I acknowledge that I have read and understood the information document.
- I understand that findings from this study will not be reported back to me and that results may be published within accredited journals.
- I understand that my information will remain anonymous unless required by law.

Participant Signature_____ Date_____

Witness Signature_____ Date_____

Record ID

Appendix Two: A

NATIONAL HEALTH LABORATORY SERVICE

School of Pathology, University of the Witwatersrand

DIVISION OF HUMAN GENETICS

WITS UNIVERSITY

Hospital Street, Johannesburg, 2001 | PO Box 1038, Johannesburg, 2000
[T] +27 11 489 9211 | [F] +27 11 489 9226 | [E] human.genetics@nhls.ac.za

Date: 28/01/2017

To whom it may concern

Re: Permission to access PGD-related patient records at the NHLS, Braamfontein

I, **Professor Amanda Krause,** hereby grant the researcher, **Ms. Bianca Carzis**, as well as the Supervisors, **Ms. Tasha Wainstein and Dr Lawrence Gobetz**, permission to access PGD-related patient records at the Division of Human Genetics, National Health Laboratory Service (NHLS) and University of the Witwatersrand (Wits), Braamfontein. Records of patients seen between January 2006 and December 2016 will be made available for data extraction and analysis. This is in connection with the project entitled, "An evaluation of preimplantation genetic diagnosis outcomes in Johannesburg, South Africa".

I permit the use of information obtained from these records for the purposes of the research being conducted. I have seen the MSc (Med) research protocol for the proposed research study as well as a data sheet of the information to be collected from the files.

The appropriate ethical considerations have been incorporated into the proposal to ensure the confidentiality and anonymity of the patients' information.

The patient files are the property of the Division of Human Genetics (NHLS/Wits) and will not be removed from the site for any reason.

AMKrause

Professor Amanda Krause, Medical Geneticist and Head of Division of Human Genetics

MBBCh (Wits), PhD

School of Pathology, National Health Laboratory Service, Faculty of Health Sciences, University of the Witwatersrand.

Tel: 011 489 9219

Email: amanda.krause@nhls.ac.za

The Inner Circle, 159 Rivonia Road, Morningside, 2196 | P.O. Box 652837, Benmore, 2010
Tel: (011) 911 4700 or 0861 882522 | Fax: 0865100951
www.vitalab.com | info@vitalab.com

Appendix Two: Date: 30/01/2017

To whom it may concern

Re: Permission to access Vitalab IVF-related patient records

I, **Dr Lawrence Gobetz,** hereby grant the researcher, **Ms. Bianca Carzis**, as well as the supervisors, **Ms. Tasha Wainstein** and **Professor Amanda Krause**, permission to access IVF-related patient records of Division of Human Genetics, (NHLS/Wits) patients who underwent fertility treatment at Vitalab as part of the PGD process. Records of patients seen between January 2006 and December 2016 will be made available for data extraction and analysis. This is in connection with the research proposal entitled, "An evaluation of preimplantation genetic diagnosis outcomes in Johannesburg, South Africa" of which I am also a supervisor.
I permit the use of information obtained from these records for the purposes of the research being conducted. I have seen the MSc (Med) research protocol for the proposed research study as well as a data sheet of the information to be collected from the files.
I note that the appropriate ethical considerations have been incorporated into the proposal to ensure the confidentiality and anonymity of the patients' information.
The patient files are the property of Vitalab and will not be removed from the site for any reason.
I understand that I will be consulted in the interpretation and publication of these results.

Dr Lawrence Gobetz
MBChB (Pret), F.C.O.G (SA) with Reproduction Medicine, Reproduction Medicine Specialist, Vitalab Centre for Assisted Conception.
Tel: 0861 882 522/ 011 911 4744
Email: lawrenceg@vitalab.com

Patient Demographics

Record ID	1
Population Group	◯ White ◯ Black ◯ Indian ◯ Mixed Ancestry ◯ Asian ◯ Other
Nationality	_____
Female partner's age at initial PGD consultation	_____
Male partner's age at initial PGD consultation	_____
Female partner's Occupation	_____
Male Partner's Occupation	_____
Medical Aid	◯ Yes ◯ No

Genetics

Genetic Diagnosis _____

Female Partner's Genetic Status
- ◯ Affected
- ◯ Carrier
- ◯ Unaffected
- ◯ Unknown

Male Partner's Genetic Status
- ◯ Affected
- ◯ Carrier
- ◯ Unaffected
- ◯ Unknown

Family History

Consanguineous couple
- ◯ Yes
- ◯ No

Affected couple?
- ◯ No
- ◯ Yes, both affected
- ◯ Yes, one partner affected

How many affected children does the couple have?
- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6

Total number of affected relatives
- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6
- ◯ 7
- ◯ 8
- ◯ 9
- ◯ 10
- ◯ 10+

Fertility history
- ◯ Poor
- ◯ Normal
- ◯ Unknown

Gravidity
- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5

Parity
- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5

How many miscarriages have the couple had?
- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5

Number of miscarriages in family

- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4
- ◯ 5
- ◯ 6

Other relevant family fertility or obstetric history

Number of previous prenatal tests performed

- ◯ 0
- ◯ 1
- ◯ 3
- ◯ 4
- ◯ 5

Type of prenatal test (* question to be repeated for each previous prenatal test)

- ◯ NIPT
- ◯ CVS
- ◯ Amnio

Previous TOP

- ◯ 0
- ◯ 1
- ◯ 2
- ◯ 3
- ◯ 4

Indications for PGD

Reasons for undergoing PGD

- ☐ Avoid having affected child with a monogenic disorder
- ☐ PGS
- ☐ HLA
- ☐ Sex selection
- ☐ Poor fertility history
- ☐ Avoid TOP
- ☐ Previous TOP
- ☐ *Other

*Other _____

IVF (Harvesting)

Number of failed cycles

- ○ 0
- ○ 1
- ○ 2
- ○ 3
- ○ 4
- ○ 5
- ○ 5+

Number of completed cycles

- ○ 0
- ○ 1
- ○ 2
- ○ 3
- ○ 4
- ○ 5
- ○ 5+

Cycle 1 (These questions will be repeated for each IVF cycle completed)

Number of eggs retrieved _____

Number of embryos produced _____

Number of embryos tested _____

Stage of embryo when tested (*repeated for each embryo tested)
- ◯ 3-day cleavage stage embryo
- ◯ 5-day Blastocyst

Number of affected embryos _____

Number of unaffected embryos _____

Number of inconclusive results _____

Number of unaffected carriers (if recessive condition) _____

Number of embryos transferred _____

Day at which embryo is transferred (*repeated for each transferred embryo) _____

Number of embryos implanted _____

Prenatal test performed
- ◯ No
- ◯ Yes, CVS
- ◯ Yes, Amnio

Outcome of prenatal test
- ◯ Affected
- ◯ Unaffected
- ◯ Inconclusive
- ◯ Resulted in a miscarriage
- ◯ N/A

Pregnancy Outcome
- ◯ Pregnancy not achieved
- ◯ Pregnanacy achieved (positive hCG test)
- ◯ Miscarriage (*gestation to be indicated)
- ◯ Stillborn
- ◯ Premature live born
- ◯ Full-term live born

Postnatal genetic test performed
- ◯ Yes:affected
- ◯ Yes:unaffected
- ◯ Yes:inconclusive
- ◯ No

Financial Cost

Genetic Counselling consultations	_____
Fertility assessment	_____
Genetic workup	_____
Hormonal Medication	_____
IVF	_____
PGS	_____
PGD	_____
Prenatal testing	_____
Postnatal Testing	_____
Courier fee	_____
Other	_____
Total Cost	_____

Time of process

From genetic counselling consultation to termination of process/pregnancy _____

From initiation of IVF to termination of process/pregnancy _____

Appendix four

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

STATEMENT OF PRINCIPLES FOR POSTGRADUATE SUPERVISION

IN A CONTEXT OF ACADEMIC FREEDOM AND WITHIN A FRAMEWORK OF INDIVIDUAL AUTONOMY AND THE PURSUIT OF KNOWLEDGE THIS STATEMENT IS WRITTEN IN THE BELIEF THAT THERE IS A RECIPROCAL RELATIONSHIP AND MUTUAL ACCOUNTABILITY BETWEEN SUPERVISOR AND STUDENT.

THE SUPERVISOR AND THE STUDENT:

1. Will establish agreed roles and clear processes to be maintained by both parties. In the case of join supervision everybody's role needs to be clarified.
2. Will meet regularly and as frequent as is reasonable to ensure steady progress towards the completion of the proposal, research report, or dissertation or thesis. This time varies but the normal minimum requirement for face-to-face contact spread across each year of registration is: 10 contact hours for an Honours project, 15 contact hours for a Masters by a research report and 24 contact hours for a Masters by dissertation and a PhD.
3. Will keep appointments, be punctual and respond timeously to messages.
4. Will keep one another informed of any planned vacations or absences as well as changes in his/her personal circumstances that might impact on the work schedule. Unplanned absences or delays should be discussed as soon as possible and arrangements should be made, to catch up lost time.
5. Will ensure that research on animal or human subjects is concluded according to the procedures and the requirements of the relevant University Ethics committee.
6. Will together complete progress reports on the research project, as requested by each Faculty Graduate Studies Committee.

THE SUPERVISOR

1. Undertakes to provide guidance for the student's research project in relation to the design and scope of the project, the relevant literature and information sources, research methods of data analysis.
2. Has a responsibility to be accessible to the students.
3. Will be prepared for the meeting with student. This includes being up to date on the latest work in his/her area of expertise.
4. Will expect written work as jointly agreed, and will return that work with constructive criticism within a timeframe (a suggestion of 2-4 weeks) jointly agreed at the outset of the research.
5. Will provide advice that can help the student to improve his/her writing. This may include referrals for language training and academic writing. The supervisor will provide guidance on technical aspects of writing such as referencing as well as on the discipline specific requirements. Detailed correction of drafts and instruction in aspects of language and style are not the responsibility of the supervisor.

6. Will support the student in the production of a research report, dissertation or thesis. Provision should be allowed for adequate, mutually respectful, discussion around recommendations made.
7. Will assist with the construction of a written time schedule, which outlines the expected completion dates of successive stages of the work.
8. Will ensure the student has the opportunity to present work at postgraduate/staff seminars/national/international conferences as appropriate.
9. Will assist with the publication of research articles appropriate.
10. Will discuss the ownership of research conducted by the student in accordance with the University guidelines and rules on intellectual property, co-authorship and copyright.
11. Will ensure that the research is conducted in accordance with the University's policy on plagiarism.
12. Will ensure that the student is made aware in writing of the inadequacy of progress and/or of any work where the standard is below par. Acceptability will be according to criteria previously supplied to the student.
13. Has a duty to refuse to allow the submission of sub-standard work for examination, regardless of the circumstances. If the student chooses to submit without the consent of the supervisor, then this should be clearly recorded and the appropriate procedures followed.

THE STUDENT

1. Undertakes to work independently under the guidance of the supervisor. This includes reading widely to ensure that the literature pertinent to his/her chosen topic has been identified and consulted.
2. Is obliged to make appointments to see the supervisor and will arrange meeting times well in advance.
3. Will think carefully about how to get maximum benefit from these contact sessions by planning what s/he wants in these sessions.
4. Should submit written work for discussion with the supervisor well in advance of a scheduled meeting. The kind and frequency of written work should be agreed with the supervisor at the outset of the research.
5. Written work that is submitted should be relatively free from basic spelling mistakes, incorrect punctuation and grammatical errors. Responsibility for the accuracy of language, the overall structure and coherence of the final research report, dissertation or thesis rests with the student.
6. Undertakes to heed the advice given by the supervisor and to engage in discussion around suggestions made. Ultimately the student has to take responsibility for the quality and presentation of the work.
7. Should strive, within reasonable bounds, to maintain a focus on his/her research area and to work within the agreed time schedule.
8. Will prepare material for presentations at seminars and conferences.
9. Undertakes to submit papers for publication.
10. Agrees to honour agreements about ownership of the research and in accordance with the University's guidelines and rules in relation to co-authorship, copyright and intellectual property.
11. Will ensure that the work contains no instances of plagiarism and that all citations are properly referenced and that the list of references is accurate, complete and consistent.
12. Agrees to work in accordance with the criteria of acceptability as supplied by the supervisor.
13. Undertakes not to place the supervisor under undue pressure to submit work for examination until the supervisor is satisfied that it has reached an acceptable level of quality. We confirm that we have read and understood this statement and agree to be guided by its principles for as long as we continue to work together.

Please note: The University and Faculty endorse the **Singapore Statement** on research integrity. The principles of the Singapore Statement include honesty, accountability, professionalism and stewardship. Our responsibilities as researchers (i.e. both as students and supervisors) are as pledged in the Singapore Declaration: the assurance of appropriate "data integrity, data sharing, record keeping, authorship, publication, peer review, conflict of interest, reporting misconduct and irresponsible research, communicating with the public, complying with regulations, education, and social responsibilities (World Conference on Research Integrity, 2010)". These principles and responsibilities are important in training our postgraduate students and in promoting global research integrity. Ref: Resnik, DB and Shamoo AE (2011). The Singapore Statement on Research Integrity. Account Res. 18:71-75.

Name of student: Bianca Carzis — Student's signature:

Name of Supervisor 1: Miss Tasha Wainstein — Supervisor's signature:

Name of Supervisor 2: Professor Amanda Krause — Supervisor's signature:

Name of Supervisor 3: Dr Lawrence Gobetz — Supervisor's signature:

The broad area of study is: An evaluation of Preimplantation Genetic Diagnosis Outcomes in Johannesburg, South Africa

Provisional submission date is: 31 August 2017 — Degree: MSc (Med) Genetic Counselling

School: Pathology — Faculty: Health Sciences

Date: 30-01-2017

Specific agreement pertaining to: ownership and joint publication, funding, may be attached and signed.

GRIEVANCE PROCEDURES: It should be acknowledged that during the course of the research that both students and supervisors can feel aggrieved. In this event, these should be dealt with as swiftly as possible by the parties involved and, if necessary, the Postgraduate Coordinators and Committees. There is, in addition, a University Grievance Policy to help guide deliberations.

Appendix B

Ethics clearance certificate

R14/49 Miss Bianca Carzis

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170257

NAME: (Principal Investigator)	Miss Bianca Carzis
DEPARTMENT:	Human Genetics - Cninical and COunselling Section National Health Laboratory Service
PROJECT TITLE:	An Evaluation of Preimplantation Genetic Diagnosis Outcomes in Johannesburg, South Africa
DATE CONSIDERED:	24/02/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Ms T. Wainstein, Prof A. Krause and Dr L. Gobetz
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	27/02/2017

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in February and will therefore be due in the month of February each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 13.03.2017

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix C

Turn-it-in report

1260293:MSc_Manuscript_Main_text_incl_tables_and_Figs_2..

ORIGINALITY REPORT

7%	5%	5%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of Nottingham Student Paper	1%
2	www.health.gov.au Internet Source	1%
3	Caryn Todd. "Genetic Counseling for Fetal Abnormalities in a South African Community", Journal of Genetic Counseling, 02/05/2010 Publication	1%
4	Submitted to Laureate Higher Education Group Student Paper	<1%
5	www.medimond.com Internet Source	<1%
6	wiredspace.wits.ac.za Internet Source	<1%
7	Submitted to University of Edinburgh Student Paper	<1%
8	www.samj.org.za Internet Source	<1%

9	Iwarsson, E.. "Preimplantation genetic diagnosis: twenty years of practice", Seminars in Fetal and Neonatal Medicine, 201104 Publication	<1%
10	Screening the Single Euploid Embryo, 2015. Publication	<1%
11	Submitted to University of Keele Student Paper	<1%
12	onlinelibrary.wiley.com Internet Source	<1%
13	www.eshre.eu Internet Source	<1%
14	www.ncbi.nlm.nih.gov Internet Source	<1%
15	L. Xanthopoulou. "When is old too old for preimplantation genetic diagnosis for reciprocal translocations?", Prenatal Diagnosis, 10/2011 Publication	<1%
16	lrd.yahooapis.com Internet Source	<1%
17	toolsforschool.net Internet Source	<1%
18	www.scielo.br Internet Source	<1%

19	bmcfampract.biomedcentral.com Internet Source	<1%
20	medpolicy.ibx.com Internet Source	<1%
21	King, E. A., D. McCoy, S. Desai, T. Nyirenda, and K. Bicking. "Vancomycin-resistant enterococcal bacteraemia and daptomycin: are higher doses necessary?", Journal of Antimicrobial Chemotherapy, 2011. Publication	<1%
22	kar.kent.ac.uk Internet Source	<1%
23	www.mdpi.com Internet Source	<1%
24	Baine, Fiona K, Chris Kay, Maria E Ketelaar, Jennifer A Collins, Alicia Semaka, Crystal N Doty, Amanda Krause, L Jacquie Greenberg, and Michael R Hayden. "Huntington disease in the South African population occurs on diverse and ethnically distinct genetic haplotypes", European Journal of Human Genetics, 2013. Publication	<1%
25	Eshetu Bekle Worku, Arlene M Davis, Brenda Morrow. "A critical review of health research ethical guidelines regarding caregiver consent for HIV research involving minors in South	<1%

Africa: Ethical and legal issues", South African Journal of Bioethics and Law, 2016
Publication

26	J. C. Harper. "The ESHRE PGD Consortium: 10 years of data collection", Human Reproduction Update, 02/16/2012 Publication	<1%

Exclude quotes	On	Exclude matches	< 3 words
Exclude bibliography	On		

Appendix D

Prenatal Diagnosis author guidelines

Prenatal Diagnosis

- **1. SUBMISSION**
- Thank you for your interest in *Prenatal Diagnosis*. Note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium.
- **Once you have prepared your submission in accordance with the Guidelines, manuscripts should be submitted online at** https://mc.manuscriptcentral.com/pd
- The submission system will prompt you to use an ORCID iD (a unique author identifier) to help distinguish your work from that of other researchers. Click here to find out more.
- Click here for more details on how to use ScholarOne
- For help with submissions, please contact: prenataldiagnosis.wiley@gmail.com
- We look forward to your submission.
- **2. AIMS AND SCOPE**
- *Prenatal Diagnosis* welcomes submissions in all aspects of prenatal screening and diagnosis with a particular focus on areas in which molecular biology and genetics interface with prenatal care and therapy, encompassing:
- • prenatal cytogenetics, including microarrays
  • prenatal molecular genetics, including whole exome and whole genome sequencing
  • prenatal diagnosis of single gene disorders, including metabolic disorders
  • fetal transcriptome, proteome and metabolome studies
  • fetal cells and cell-free nucleic acids in maternal blood and other fluids
  • preimplantation genetic diagnosis and screening
  • fetal and placental development and pathology
  • fetal therapy
  • prenatal and preconceptional genetic screening
  • development and evaluation of laboratory services for prenatal testing
  • psychosocial, legal, ethical and economic aspects of prenatal testing
  • preconceptual, preimplantation and prenatal genetic counseling

- The overriding criteria for publication are scientific merit, originality, and interest to a multidisciplinary audience.

- **3. MANUSCRIPT CATEGORIES AND REQUIREMENTS**

- *Prenatal Diagnosis* publishes a number of different article types including:

- **• Original Articles**

  Original articles should contain reports of new research findings or conceptual analyses that make a significant contribution to knowledge. They must include a structured abstract (maximum 200 words), and should not exceed 3,500 words of body text. Original articles must also include bulleted statements (maximum 70 words) in answer to the following questions: what's already known about this topic?; what does this study add?

  • Review Articles

  Review Articles will typically be solicited by the Review Editor. Authors who wish to submit an unsolicited review should first contact the Review Editor email: prenataldiagnosis.wiley@gmail.comto determine its suitability for publication in the Journal. All reviews will be peer-reviewed. Review Articles must include an unstructured abstract (maximum 200 words), and should not exceed 3,500 words of body text, and are limited to 150 references. Review articles must also include bulleted statements (maximum 70 words) in answer to the following questions: what's already known about this topic?; what does this study add?

  • Research Letters

  Case reports and clinical observations are rarely published and will only be considered if they are of exceptional educational interest, value or novelty. If accepted, they will be published as Research Letters. All Research Letters are peer-reviewed. Research Letters should not exceed 1,500 words, and are limited to 1 table, 1 figure, and 10 references. No abstract or key words are required, and text should be formatted in one continuous section. Research Letters must include bulleted statements (maximum 70 words) in answer to the following questions: what's already known about this topic?; what does this study add?

  • Correspondence

  Correspondence (letters to the Editor) may be in response to issues arising from recently published articles, or short, free-standing pieces expressing an opinion. Letters are not subject to external peer-review.

- **4. PREPARING YOUR SUBMISSION**

- **Parts of the Manuscript**

  Manuscripts must be submitted as a file and should be written in English. The manuscript should be submitted in separate files: title page; main text file; figures; tables.

- Covering Letters are encouraged; if you do include a covering letter please consider suggesting an Associate Editor to handle your submission.

- **Title Page:**

- The single title page should be presented in the following order:

  i. Title

  ii. A short running title of less than 70 characters

  iii. Manuscript word, table and figure count

  iv. The full names of the authors

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  vi. Name, address, telephone number and email address of corresponding author

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