



Evaluating the ratio of mature oocytes to immature oocytes in oocyte cohorts aspirated from HIV seropositive and HIV seronegative females post-controlled ovarian hyperstimulation.

Submitted in fulfilment of the requirements for the Master of Health Science in Clinical Technology degree in the Faculty of Health Sciences at the Durban University of Technology.

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AUGUST 2024

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ABSTRACT

Introduction

A woman's reproductive potential can be threatened by disrupting normal oocyte development, often associated with advancing maternal age, chronic viral infections, medical conditions/interventions, pharmaceutical and recreational drugs, and an individual's Body Mass Index (BMI). Often, these factors affect the outcomes of assisted reproductive techniques (ART); however, they are not always addressed well, especially in developing countries such as South Africa.

Research Aim and Objectives:

This study aimed to provide a comparative analysis of the mature and immature oocyte yields in oocyte cohorts obtained from HIV-infected females against HIV-uninfected women of reproductive age, post-controlled ovarian hyperstimulation (COH). Additionally, the study examined the potential influence of factors associated with HIV infection, infertility, and ART, such as age, BMI, viral load, CD4+ lymphocyte counts, antiretroviral medication intake, ovarian stimulation protocols, and ovulation trigger injections on oocyte maturation outcomes.

Methodology:

This study evaluated n=80 women aged between 18-39 years attending ART between 2021 and 2023. GnRH-Antagonist protocol (Cetrotide® antagonist) for COH was used on all patients, and the exogenous gonadotropins for follicular stimulation were initiated on day 2/3 of the menstrual cycle. Ovitrelle® at 250mcg or Lucrin® at 40IU or 80IU were the ovulation triggers used. The oocytes were aspirated 36 hours after administration of the ovulation trigger. Oocyte denudation and assessment were done 2 hours after the TVOA, after which insemination using the ICSI technique was conducted 2 hours after incubation.

Only the mature (MII) oocytes were inseminated at Day 0/Day 1, the appearance of 2-pronuclei (PN) and 2-Polar bodies (PB) at 16-18hours signified successful fertilisation, and single embryo culture in one-step media (under oil) was carried out for the next 3-5 days in a benchtop incubator.

Results:

The means for MII oocytes (p-value= 0,264), MI oocytes (p-value= 0,352), and GV oocytes (p-value= 0,258) between the HIV-infected population and HIV uninfected controls showed no statistical differences (all p-values> 0.05).

Furthermore, when the total population was matched by age, BMI, ovarian stimulation protocol, ovulation trigger injection, and serum AMH, outcomes (follicle number, oocyte retrieval rates, and MII oocyte number) were comparable (all p-values> 0.05). Age was, however, positively associated with the relationship between AMH, follicle number, and MII oocyte number in the entire population.

Conclusion

In this study, the quantities of mature (MII) oocytes in oocyte cohorts from the HIV positive females against the HIV negative females were similar. Furthermore, there were no significant differences in ART outcomes between the two groups after matching patients by age, BMI, ovarian stimulation protocol, and ovulation trigger.

Keywords: Infertility, ART, HIV, Age, BMI, Antiretroviral therapy, Oocyte maturation.

Author Declaration of Originality

I, Nombuso Felicia Ndlela, hereby assert that,

- This dissertation is my work and has never previously been published or submitted in fulfilment of any degree or examination by me or any other person of this university or another institute of higher learning.
- I declare that this submission does not contain, in part nor in full, any other person's data, pictures, and materials unlawfully, and complete acknowledgement was given in the sourced materials.

This research study was carried out in a private fertility clinic, FemiCare Fertility Clinic, under the Department of Biomedical and Clinical Technology with supervision from Dr D.R. Prakaschandra (PhD, HoD & Senior Lecturer-DUT) and co-supervision from Mr E. Gabriel (BSc, BSc (Hons), MSc, Part-time lecturer-DUT).

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Ethical Clearance Certification

The study design for this dissertation was approved by the Durban University of Technology (DUT) Institutional Research Ethics Committee (IREC).

Ethical Clearance Number: IREC 138/22

N.F.

Student initials

Signature

2024-08-22

Date

Dedication

I dedicate this work to:

Firstly, my Lord and Saviour, Christ Jesus, without whom I would have never chosen this amazing career path. Thank you for being my heart and soul.

My mother, Nompumelelo Ndlela, and sisters Nonkululeko Ndlela and Sabelisiwe Ndlela- Thank you for the constant push, encouragement, and never-ending support. Thank you for always believing in me and constantly praying for and with me.

My friends and confidants, Carolina Seoka, Nkateko Baloyi, and Mlondi Shandu- Thank you for the motivation to do this qualification. Thank you for waiting with me while I worked and never complained about your time spent. Thank you for caring beyond measure.

Lastly, this work is dedicated to my heart in human form, my son Hlumelo- Thank you for giving me a reason to push even on my bad days. Thank you for the pick-me-up hugs, smiles, and laughs. Life will always be hard, but we will always be tougher.

Acknowledgement

I would like to extend my gratitude to the following people who greatly assisted with the completion of this dissertation,

- My supervisor, Dr. D.R. Prakashchandra, for always directing, supporting, and encouraging me, and mostly for the invaluable knowledge that helped shape the completion of this project.
- My co-supervisor, Mr. E. Gabriel, for always pointing out and assisting me with plugging the holes in my research to ultimately produce results that are sound and solid.
- Dr. K.T. Maoto for answering all my questions and editing my work from time to time to help me reach my goal with the highest standards.
- Prof. G. Mathews for doing and redoing so much statistical analysis of my data so many times. Thank you for your hard work, patience, and impeccable response time.
- Mr. O. Sapo for the continued guidance and support during the writing of the dissertation.

I would also like to acknowledge and thank Dr. J. Ndhlovu and FemiCare Fertility Management for their willingness to grant me access and provide all the support I required to complete my research. Finally, I would like to immensely appreciate all the patients who, regardless of the burdens they were experiencing throughout the entire ART process, still took their time to participate in this study, knowing this research study would not increase their ART outcomes at the time. Without them, this research would have never come to fruition. I am grateful always.

TABLE OF CONTENTS

ABSTRACT	II
AUTHOR DECLARATION OF ORIGINALITY	IV
ETHICAL CLEARANCE CERTIFICATION	V
DEDICATION	VI
ACKNOWLEDGEMENTS	VII
TABLE OF CONTENTS	VIII
LIST OF FIGURES	XII
LIST OF TABLES	XIII
LIST OF APPENDICES	XVI
LIST OF ABBREVIATIONS	XVII

CHAPTER 1	2
INTRODUCTION	2
1.1 Structure of the thesis	4

CHAPTER 2	6
LITERATURE REVIEW	6

2.1 Background	6
2.1.1 Infertility	6
2.2 Human Immunodeficiency Virus (HIV) and Female Fertility	8
2.2.1 Background	8
2.2.2 Current Literature on HIV Infection and Female Fertility	11
2.3 Antiretroviral Therapy and Female Fertility	15
2.4 Controlled Ovarian Hyperstimulation and Ovulation Trigger Injections	18
2.5 Age and Female Fertility	21
2.6 Body Mass Index (BMI) and Female Fertility	22
2.7 Follicular Milieu and the Oocyte	24
2.8 Conclusion	25

CHAPTER 3	27
MATERIALS AND METHODOLOGY	27
3.1 Background	27
3.2 Study Setting and Study Design.....	27
3.3 Recruitment Process	28
3.4 Study Population	28
3.5 Data Collection Tool	30
3.6 Research Process	30
3.6.1. Clinical Process	30
3.6.1.1 Controlled Ovarian Hyperstimulation	30
3.6.1.2 Ovarian Stimulation Protocol	31
3.6.1.3 <u>Exogenous Gonadotropins for Follicular Growth</u>	31
3.6.1.4 Ovulation Triggering	33
3.6.1.5 Ovarian Stimulation Monitoring	34
3.6.2 Laboratory Process	34
3.6.2.1 Transvaginal Oocyte Aspiration (TVOA)	35
3.6.2.2 Laboratory Analysis	37
3.6.2.3 Denudation and Insemination	39
3.6.2.3.1 Oocyte Denudation	39
3.6.2.3.2 Oocyte Maturation Assessment	39
3.6.2.3.3 Insemination	42
3.6.2.4 Fertilisation and Embryo Development	43
3.7 Statistical Analysis	44
3.7.1 Software and Statistical Representation	44
3.7.2 Ethical Considerations	45
3.7.3 Confidentiality and the POPI Act	46
3.7.4 Storage of Records	46

3.8 Conclusion	46
CHAPTER 4	48
RESULTS	48
4.1 Introduction, Aim and Objective	48
4.2 Study Population and Sample Size	48
4.3 Descriptive Statistics	51
4.4 Inferential Statistics	59
4.4.1 Independent-Samples Wilcoxon Mann-Whitney U Test	59
4.4.2 Independent-Samples Median Test	62
4.4.3 Independent-Samples T-test (Two-sample t-test)	63
4.4.4 Pearson Correlation Coefficient Test	70
4.4.5 Fisher-Freeman-Halton Exact Test	73
4.5 Conclusion	75
4.6 Supplementary Statistics	76
4.6.1 Outcomes When the Diagnosis is the Same- Independent-Samples Wilcoxon Mann-Whitney U Test	76
4.6.2 Outcomes in Rescue <i>in vitro</i> matured (R-IVM) Oocytes-Independent-Samples Wilcoxon Mann-Whitney U Test	78
4.6.3 Fertilisation and Embryo Development	79
4.7 Conclusion	83
CHAPTER 5	84
DISCUSSION	84
5.1 Oocyte Maturation Rates in HIV Infected and HIV Uninfected Women Attending ART	84
5.2 Relationship Between HIV Infection (Viral Load) and Female Fertility	88

5.3 Relationship Between CD4 ⁺ Lymphocyte Counts and Female Fertility	89
5.4 Relationship Between Antiretroviral Therapy and Female Fertility	90
5.5 Relationship Between Ovarian Stimulation, Ovulation Trigger Injection and Female Fertility	92
5.6 Relationship Between Age and Female Fertility	94
5.7 Relationship Between BMI and Female Fertility	97
5.8 Relationship Between Serum AMH, Follicle Number and MII Oocytes	98
5.9 Supplementary Statistics	99
5.9.1 Outcomes in Rescue in vitro Matured (R-IVM) Oocytes	99
5.9.2 Outcomes when the Diagnosis is the Same	100
5.9.3 Fertilisation and Embryo Development	101
5.10 Study Limitations	103
5.11 Study strengths and weaknesses	104
 CHAPTER 6	 105
CONCLUSION	105
RECOMMENDATIONS	106
 CHAPTER 7	 107
REFERENCES	107
 APPENDICES	 128

LIST OF FIGURES

Figure 1: Key features in the fight against HIV in South Africa.

Figure 2: Prevalence of HIV in adults aged 15-49 years in 2017 in sub-Saharan countries.

Figure 3: HIV prevalence in South Africa among males and females in different age groups.

Figure 4: Factors which affect circulating anti-Müllerian hormone levels

Figure 5: Flow chart of ART recommendations in the era of HIV.

Figure 6: The process of IVF/ICSI: From ovarian hyperstimulation-Ovulation Trigger-Transvaginal oocyte aspiration, to Luteal phase support-Embryo transfer.

Figure 7: Effects of changes in the gut microbiota, inflammation, and oxidative stress on the ovarian oestrous cyclicity and oocyte quality.

Figure 8: Image depicting Controlled Ovarian Hyperstimulation using the GnRH-antagonist protocol.

Figure 9: Sonographic image portraying multi-follicular growth in the ovaries, often observed during ovarian stimulation monitoring.

Figure 10: Image describing the laboratory process that follows ovarian stimulation, from oocyte aspiration-oocyte insemination-embryo culture-embryo transfer.

Figure 11: Photomicrograph depicting an enclosed cumulus-oocyte-complex in follicular fluid in a screening dish during the oocyte aspiration procedure.

Figure 12. a: Criteria used to assess oocyte quality as presented in a study by Lemseffer *et al.* (2022:1).

Figure 12. b: Oocyte Photomicrograph depicting Lemseffer *et al.* (2022:1) oocyte quality assessment characteristics.

Figure 13: Photomicrograph of Oocytes at different stages of maturity.

Figure 14: Sperm selection technique for IVF/ICSI.

Figure 15: Fertilised oocyte (Zygote) photomicrograph showing 2 Pronuclei (2PN) and 2 Polar bodies (2PB) depicting normal fertilisation.

Figure 16: Photomicrograph of embryo developmental stages from the 2-cell stage to the blastocyst stage.

Figure 17: Scatter plot diagram of oocyte retrieval rates and maturation rates in the HIV-infected and HIV-uninfected groups. The diagram represents the quantity and heterogeneity of oocyte cohorts from each patient group.

Figure 18: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the follicular growth rates observed between the HIV-infected and HIV-uninfected females.

Figure 19: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the total number of oocytes aspirated between HIV-infected and HIV-uninfected females.

Figure 20: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the total number of MII oocytes aspirated between HIV-infected and HIV-uninfected females.

Figure 21: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the number of MI oocytes aspirated between HIV-infected and HIV-uninfected females.

Figure 22: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the number of GV oocytes aspirated between HIV-infected and HIV-uninfected females.

Figure 23: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the follicular growth rates observed between HIV-infected and HIV-uninfected females with the same infertility diagnosis.

Figure 24: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution of the total number of oocytes aspirated between HIV-infected and HIV-uninfected females with the same infertility diagnosis.

Figure 25: Independent-Samples Wilcoxon Mann-Whitney U Test illustrating the frequency distribution patterns of the number of MII oocytes aspirated between HIV-infected and HIV-uninfected females with the same infertility diagnosis.

Figure 26: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the number of rescue-IVM oocytes that were initially immature (MI oocytes) on the day of oocyte retrieval, but matured following extended culture, between HIV-infected and HIV-uninfected women.

Figure 27: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the number of rescue-IVM oocytes that were initially immature (GV oocytes) on the day of oocyte retrieval, but matured following extended culture, between HIV-infected and HIV-uninfected women.

LIST OF TABLES

Table 1: List of African countries and the number of registered IVF units within each country (Ombelet and Onofre 2019:68).

Table 2: Study outcome measures.

Table 3: Example of patient injection timetable (FemiCare Fertility Clinic laboratory protocol, 2019).

Table 4: Study population baseline characteristics and reproductive outcomes table.

Table 5: Frequency distribution of MII oocytes among the HIV-positive and HIV-negative populations oocyte cohorts.

Table 6: Representation of the average number of mature-MII and immature (MI+GV) oocytes in oocyte cohorts aspirated from HIV-positive women and HIV-negative women.

Table 7: Distribution of patients across different types and doses of exogenous gonadotropins used for ovarian stimulation.

Table 8: Distribution of patients across different types and doses of ovulation trigger injections.

Table 9: Follicular growth (number and size) measurements observed during ovulation monitoring, before the transvaginal oocyte aspiration procedure.

Table 10: Mean endometrial thickness measurements observed in the women living with HIV and HIV uninfected females.

Table 11: Frequency distribution of patients across different age categories.

Table 12: Frequency distribution of patients across different BMI categories.

Table 13: Representation of average population age and body mass index (BMI) by HIV serostatus.

Table 14: Independent-Samples Median analysis depicting the comparison in means of mature MII oocytes between the HIV-infected females and HIV-uninfected females.

Table 15: Independent-Samples Median analysis depicting the comparison in means of immature-MI oocytes between the HIV-infected females and HIV-uninfected females.

Table 16: Independent-Samples Median analysis depicting the comparison in means of immature GV oocytes between the HIV-infected females and HIV-uninfected females.

Table 17: Outcomes of follicle number, oocyte number and MII oocyte number based on different exogenous gonadotropins used for ovarian induction.

Table 18: Outcomes of follicle growth, oocyte number and MII oocyte number based on the different ovulation triggers in the HIV-infected and HIV-uninfected females.

Table 19: Two-sample t-test analysis of the serum AMH levels of the HIV-infected test group over the HIV-uninfected control group.

Table 20: T-test analysis of the comparison in serum AMH levels between the HIV-infected and HIV-uninfected females matched by age.

Table 21: T-test analysis comparing the outcomes- follicle number, total oocyte number and MII oocyte number in the HIV-infected females and HIV-uninfected control women, matched by age.

Table 22: T-test analysis comparing the outcomes- follicle number, total oocyte number and MII oocyte number observed between the HIV seropositive women and the HIV uninfected control group, matched by BMI category.

Table 23: Pearson correlation coefficient analysis of the relationship between AMH, follicle number and MII oocyte number in the HIV-infected females and HIV-uninfected women by age.

Table 24.: A comparative analysis of the total oocyte number and MII oocyte numbers in oocyte cohorts obtained among the HIV-uninfected versus the HIV infected females.

Table 25: Fertilisation rates observed between HIV-infected and HIV uninfected women.

Table 26: Distribution analysis showing the quality and the number of embryos transferred on the day of embryo transfer (Day 3/Day 5) in the population of women living with HIV.

Table 27: Distribution analysis showing the quality and the number of embryos transferred on the day of embryo transfer (Day 3/Day 5) in the HIV-negative women.

Table 28: SART grading 'Good', 'Fair', and 'Poor' embryo frequency distributions between the HIV-infected and HIV-uninfected women.

Table 29: Number of patients who froze surplus embryos and had freeze-all cycles where all embryos were frozen. Categorised according to HIV serostatus and day of cryopreservation.

LIST OF APPENDICES

Appendix A: Permission request letter.

Appendix B: Confirmation letters from the patient's clinician and the research study's facility management granting access to proceed with the research.

Appendix C: Ethical Clearance Letter.

Appendix D: Letter of information.

Appendix E: Consent form.

Appendix F: Serum AMH reference ranges.

Appendix G: WHO 2010/2021 semen parameters reference ranges.

Appendix H: Ovarian stimulation monitoring report.

Appendix I: Data capture sheet.

Appendix J: Data capture sheet questionnaire.

Appendix K: Cleavage stage and blastocyst stage embryo scoring techniques.

Appendix L: Hospital COVID-19 testing sheet and assessment.

Appendix M: ESHRE recommendations on starting ART post-COVID-19.

Appendix N: Expected serum AMH values by age.

Appendix O: Biostatistician letter.

LIST OF ABBREVIATIONS

AFC- Antral Follicle Count

AIDS-acquired immunodeficiency syndrome.

AMH- anti-Müllerian Hormone

ART- Antiretroviral Therapy

ART- Assisted Reproductive Technology

ARV- Antiretroviral drugs

ASRM- American Society for Reproductive Medicine

AZT- Zidovudine

BBV- Blood-Borne Virus

BMI- Body Mass Index

cART- Combined Antiretroviral Therapy

COH- Controlled Ovarian Hyperstimulation

d4T- Stavudine

DDI- Didanosine

DUT- Durban University of Technology

DTG- Dolutegravir

EFV- Efavirenz

ER- Endoplasmic Reticulum/Reticula

ESHRE- European Society of Human Reproduction and Embryology

FF- Follicular Fluid

FSH- Follicle Stimulating Hormone

GnRH- Gonadotropin-Releasing Hormone

GV- Germinal Vesicle

HAART- Highly Active Antiretroviral Therapy

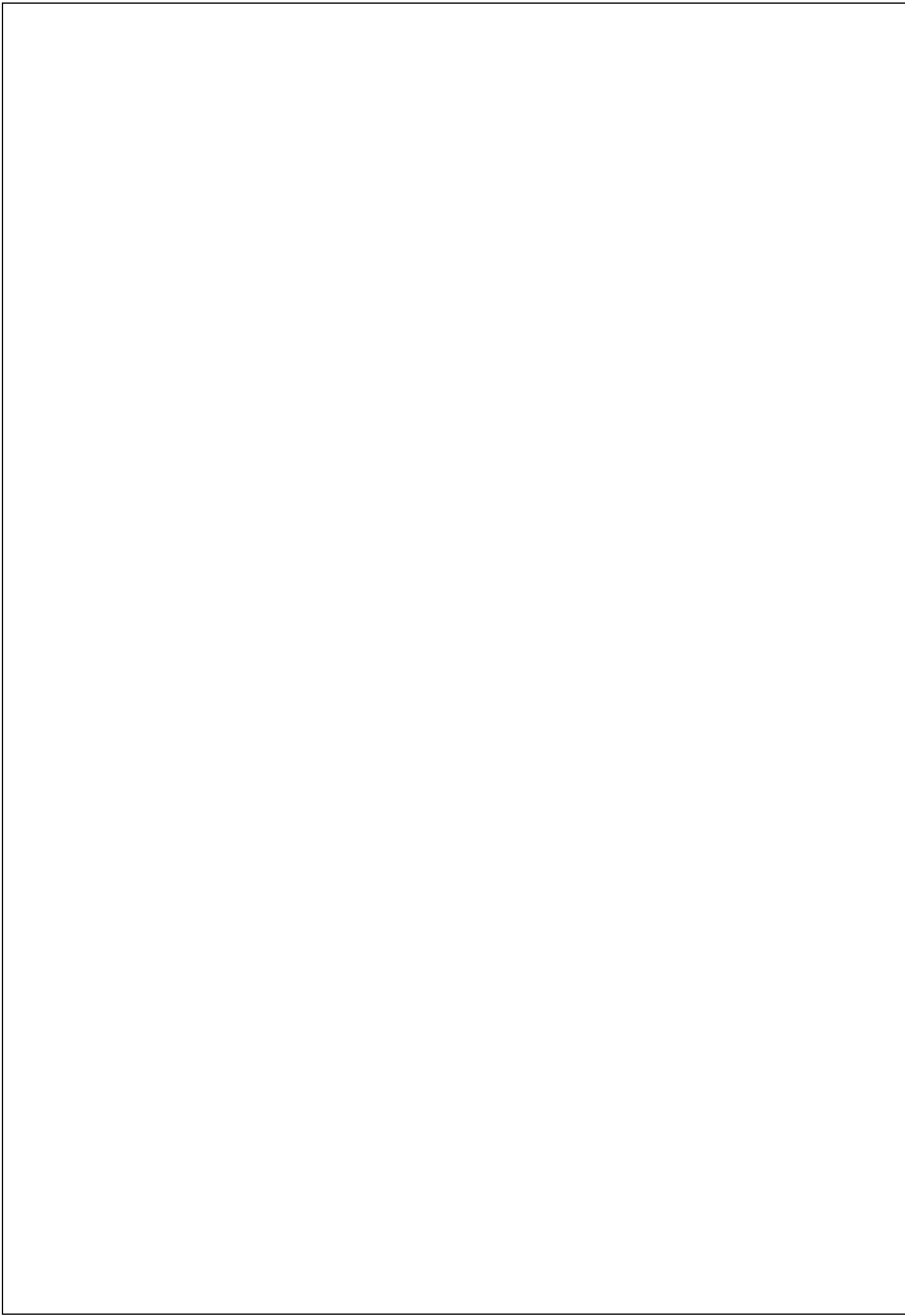
hCG- Human Chorionic gonadotropin

HIV- Human Immunodeficiency Virus

hMG- Human Menopausal Gonadotropin

HPO- Hypothalamic Pituitary Ovarian Axis
HPV- Human Papillomavirus
ICM- Inner cell mass
ICSI- Intracytoplasmic Sperm Injection
IU- International unit
IVF- *In vitro* Fertilisation
LPS- Lipopolysaccharide
LH- Luteinizing Hormone
mcg- microgram
MI- Metaphase I
MII- Metaphase II
ml- Millilitre
mm- Millimetre
MOA- Mechanism of Action
mtDNA- Mitochondrial DNA
ng/ml- nanograms per millilitre
NIH- National Institute of Health
NRTI- Nucleoside Reverse Transcriptase Inhibitor
NNRTI- Non-Nucleoside Reverse Transcriptase Inhibitor
COC- Cumulus Oocyte Complex
OHSS- Ovarian Hyperstimulation Syndrome
OS- Ovarian stimulation
PB- Polar Body
PCOS- Polycystic Ovary Syndrome
PEP- Post-Exposure Prophylaxis
PI- Protease Inhibitor
PLWH- People Living With HIV
PN- Pronuclei
POI- Premature Ovarian Insufficiency

PrEP- Pre-Exposure Prophylaxis
PVS- Perivitelline Space
rFSH- Recombinant Follicle Stimulating Hormone
R-IVM- Rescue *in vitro* maturation
rLH- Recombinant Luteinizing Hormone
ROS- Reactive Oxygen Species
RPL- Repeated Pregnancy Loss
SERC- Smooth Endoplasmic Reticulum/Reticula
STD- Sexually Transmitted Disease
STF- Special Task Force
STI- Sexually Transmitted Infection
TDF- Tenofovir Disoproxil Fumarate
TE- Trophectoderm cells
TFR- Total Fecundity Rate
TLD- TFD, Lamivudine and Dolutegravir
TLE- TFD, Lamivudine and Efavirenz
TVOA- Transvaginal Oocyte Aspiration
WHO- World Health Organization
ZP- Zona Pellucida
°C- Degrees Celsius
et al.- And Others
>- Greater Than
<- Less Than
%- Percentage
μL- microliter



CHAPTER 1

INTRODUCTION

Infertility is a condition that affects 1 in 6 couples and is recognised globally (Farquhar *et al.* 2019:1 and Ingale *et al.* 2019:1). It is defined as unsuccessful repeated and calculated attempts at procreating naturally (without assistance) within 12 months (World Health Organization 2023: n.p. and Carson and Kallen 2021:65). Infertility is further interpreted as the inability to conceive within 1 year in women 35 years and younger, and failure to conceive within 6 months in women older than 35 years (Sharma and Chakrabarti 2019:1). Couples can experience infertility because of factors arising from the male or the female, both male and female, or may be idiopathic in origin (Ogar, Leonard and Bassey 2018:311).

The prevalence of infertility varies with each geographic area; however, global statistics have shown that areas such as South Asia, Sub-Saharan Africa, North and Middle East Africa, and Central and Eastern Europe claim the highest infertility prevalence (Sripad *et al.* 2021:1 and Ombelet and Onofre 2019:65). According to Eldib and Tashani (2018:43), the frequency of infertility ranges geographically, 12%-25.9% in central/south/southeast Asia, 13.7%-25.7% in Sub-Saharan Africa, and 10.5%-25.8% in Latin America and the Caribbean. Furthermore, some commonly observed factors that promote infertility also differ in different geographical areas. In South Asia, records show that infertility is commonly caused by polycystic ovary syndrome (PCOS), diabetes, endometriosis, ethnic factors and varicoceles in males (Pandya 2024:n.p), in Sub-Saharan Africa and the North and Middle East Africa, pelvic inflammatory diseases, tubal occlusions, abortions, varicoceles and poor sperm parameters in males are the most prevalent causes of infertility (Abebe, Afework and Abaynew 2020:1), and in Europe, infertility is linked with advanced maternal age, anatomical and physiological disorders of the male or female reproductive tract, lifestyle choices, some diseases, poor sperm parameters and environmental pollutants and toxins (European Society of Human Reproduction and Embryology 2021:1).

The Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) pandemic is recognised as globally, and South Africa has been identified as the area with the highest infection rate (Udjo 2018:4252). A fifth of the HIV-infected population in South Africa are women of childbearing age (Statistics South Africa 2019:6).

Assisted reproductive techniques are widely used for the management of infertility (Jing *et al.* 2020:1), and the development and successful use of antiretroviral therapies have made it possible for people living with HIV (PLWH) to live a normal life and have children (Zicari *et al.* 2019:1, Cito *et al.* 2019:857 and Sauer 2021:363). However, HIV/AIDS and infertility, among other medically related conditions, are still most frequently the prime reasons people seek Assisted Reproductive Technology (ART), which is fueled by the desire to have healthy offspring at the desired time (Lucas *et al.* 2021:639). This, therefore, drives the importance of addressing these two issues, either interchangeably or independently, as reports have observed that specifically in South Africa, approximately 20% of the infertile community is infected with HIV (Yahya *et al.* 2018:179). While achieving a live birth through ART is

generally not guaranteed, women living with HIV have been found to have even lower chances of falling pregnant even with ART (Santulli *et al.* 2016:1084).

One of the most important steps of ART is ovarian hyperstimulation (OH) which aims to attain as many competent oocytes as possible suitable for insemination that will eventually give rise to a live birth (Haahr, Esteves and Humaidan 2018:1). The mechanism of action (MOA) of OH is the development of multiple follicles in the ovaries in response to ovarian stimulation by medications and hormonal signalling, the goal being to harvest as many oocytes as possible per menstrual cycle (Wu and Wu 2023:2 and Racca *et al.* 2020:973).

After the daily administration of ovarian stimulation medications at various doses for certain periods, the expectation is to attain many good-quality oocytes to bring about a live birth. However, according to Escrich *et al.* (2018:668), approximately 15% of oocyte cohorts contain immature (MI+GV stage) oocytes, and in very rare cases, all the oocytes will be immature on the day of oocyte harvest. Oocyte quality and the number of mature oocytes are important because they are good indicators of the oocyte's fertilisation potential, embryo developmental capability, and can be used to predict the probability of pregnancy (Braga *et al.* 2020:70).

According to Eldib and Tashani (2018:38), there is a paucity of infertility studies conducted and presented in developing continents such as Africa. Eldib and Tashani (2018) did, however, state that in sub-Saharan Africa, infertility prevalence lies somewhere between 13,7% and 25,7%. Furthermore, a report by Yahya *et al.* (2018:178) claims that HIV infection statistics on adult women are mostly built on data gathered from antenatal clinics and seldom on entire population groups. This means there is a gap that exists both in the illumination of infertility in developing countries and in the reporting of the influence of HIV infection on the infertile community. Information on these variables would be of benefit to the infertile community, especially those living with HIV, since it has been observed that this population wishes to have healthy children (Van Ommen *et al.* 2023:770).

A large cross-sectional, population-based household survey- the Sixth South African National HIV Prevalence, Incidence, and Behavior Survey (SABSSMVI) by the Human Sciences Research Council (HSRC) and partners conducted between January 2022- April 2023 revealed that South Africa still faces challenges with tackling HIV which is most prevalent among the black community, women, and young adults (Human Sciences Research Council 2023: n.p).

In summary, infertility is a major problem in and around the world (Lorzadeh *et al.* 2020:447 and Nik-Halzina *et al.* 2022:4). Still, the prevalence of infertility seems to be higher in low- and middle-income countries (Eldib and Tashani 2018:43). 20-60% of couples suffer from infertility in Africa (Lucas *et al.* 2021:635), and according to Yahya *et al.* (2018:179), a significant number of the infertile population are living with HIV. Global HIV statistics in the year 2024 reported that 40.8 million people were living with HIV around the world, 1.3 million new infections were reported, and 31.6 million people were receiving antiretroviral treatments (UNAIDS 2025: n.p.). Investigating and addressing the role of HIV infection on population groups and reproduction (including in ART) provides benefit to the population

infected with HIV and adds to the global body of knowledge regarding the population (Cohen 2016: n.p).

This study aimed to provide a comparative analysis of the mature and immature oocyte yields in oocyte cohorts obtained from HIV-infected females against HIV-uninfected women of reproductive age, COH. Additionally, the study examined the potential influence of factors associated with HIV infection, infertility, and ART, such as age, BMI, viral load, CD4+ lymphocyte counts, antiretroviral medication intake, ovarian stimulation protocols and ovulation trigger injections on oocyte maturation outcomes.

Observations made in practice prompted the desire to gather evidence-based data to address the role of HIV infection on reproduction in the ART setting. Reporting the outcome of such investigations provides useful insights into the knowledge and management of the HIV infected population.

A single-centre prospective study was carried out, embedded within the positivist paradigm, using a longitudinal study approach. A total population of 80 women, compared by HIV serostatus, all attending a fertility clinic for fertility-related complications and interventions, were included in this study. The total population was subdivided into two sets: 40 women who were HIV positive and 40 women who were HIV negative. Data from their COH-ICSI cycle outcomes between the years 2021-2023 were collected, statistically analysed and interpreted.

The data included information on follicular growth, and the number and maturity status of oocytes retrieved following ovarian stimulation from each group, the type of ovarian stimulation protocol and ovulation trigger injection used and data on patient age, BMI, viral load, CD4+ lymphocyte counts and antiretroviral medication intake. To highlight and only assess the female association on HIV and infertility, all the male partners included in this study were HIV negative and presented with normal sperm parameters according to the World Health Organization criteria (Appendix G). The statistical analysis presented the data as descriptive statistics, where tables and graphs were used to document the raw data, and inferential statistics described the relationship between 2 or more variables, and presented the distribution and association between 2 or more variables. A P-value of less than 0.05 on two-sided tests was accepted as statistically significant.

1.1 Structure of the dissertation:

Abstract- Summarises and outlines the entire research study's purpose, methodology and outcomes reached.

Chapter 1: Introduction- Briefly describes the basis on which the research is built and highlights the knowledge gap and how this research study aims to positively add to the current body of literature on the proposed topic.

Chapter 2: Literature review- Unpacks and describes in depth the currently available literature on the proposed research topic, noting any patterns or discrepancies relating to the factors being studied.

Chapter 3: Materials and Methods- Delineates how, when and what type of materials and mechanisms/processes were utilised to conduct the research and allow the collection of raw data for analysis.

Chapter 4: Results- This chapter includes the statistical representation and analysis of all the raw data collected that make up the research.

Chapter 5: Discussion- Addresses all the past and current literature on this present research subject and how this research and its outcomes connect to previously conducted research. In addition, this chapter addresses the research study's aims and objectives.

Chapter 6: Conclusion and recommendations- This chapter concludes the research study by outlining and reviewing the outcomes reached.

Chapter 7: References- This chapter includes all the citations from authors' scholarly works previously published and referenced within this dissertation.

Appendices- Includes all the documents that highlight important information that can be used as a reference.

CHAPTER 2

LITERATURE REVIEW

This chapter delves into the current literature that exists on infertility, female fertility and HIV infection, antiretroviral treatments, controlled ovarian hyperstimulation, ovulation triggers, age, and body mass index (BMI). This section will describe the developments and advancements associated with these categories. For fertility-related publications, electronic information sources included Google Scholar, PubMed, Research Gate, Science Direct, Medline, Cochrane Library, and EPUB Reader, which were searched from the year 2021 to 2024. Keywords used for research included the following: infertility, female reproductive system, HIV/AIDS, immune system, *in-vitro* fertilisation, ovarian reserve, microfollicular environment, controlled ovarian hyperstimulation, female fertility, antiretroviral therapy, age, body mass index, sexually transmitted infections, human oocytes, and assisted reproduction.

2.1 Background

2.1.1 Infertility

A couple is deemed infertile when they have difficulties in successfully procreating following multiple attempts in at least 12 consecutive months of timed unprotected sexual intercourse; this condition affects approximately one in six couples (Farquhar *et al.* 2019:1). The World Health Organization (WHO) and the European Society of Human Reproduction and Embryology (ESHRE) have however, proposed that a couple should be given at least a two-year leeway to conceive and achieve a live birth before being diagnosed as infertile (Ogar, Leonard and Bassey 2018:311). However, the consensus remains that infertility is the failure to reproduce within 12 months of timed unprotected sexual intercourse (World Health Organization 2023).

Individuals can experience either primary or secondary infertility. Primary infertility denounces a woman who has never conceived at any point in her life, and this type of infertility affects approximately 1.9% of the general population; whilst secondary infertility is known to affect at least 10.5% of women, and describes a woman who has fallen pregnant before but for whatever reason, subsequently fails to conceive again at a later stage (Sripad *et al.* 2021:1 and Nik-Hazlina *et al.* 2022:1). Globally, approximately 18% of the general population at one-point experiences either form of infertility (Lorzadeh *et al.* 2020:447), while in Africa, approximately 49.9% of the population is prone specifically to primary infertility (Nik-Hazlina *et al.* 2022:4). Interestingly, secondary infertility is the commonest type of infertility observed in 81% of the HIV seropositive men and women in and around the world (Yahya *et al.* 2018:180).

Developing regions such as Sub-Saharan Africa and Asia bear the greatest burden of infertility, with 20-60% of couples suffering infertility in Africa alone (Lucas *et al.* 2021:635). While in South Africa, approximately 15-20% of the population currently experiences or has at one stage experienced infertility (Mabweazara 2025:180). Additionally, according to

Sripad *et al.* (2021:1) and Ombelet and Onofre (2019:65), Central and Southern Africa are referred to as the “infertility belt”, as they greatly surpass the global 9% prevalence of infertility.

Infertility is a multifactorial issue that has been associated with several pathologies. A series of studies (Deshpande and Gupta 2019:289, Nik-Hazlina *et al.* 2022:1, Lino *et al.* 2022:1-6 and Lorzadeh *et al.* 2020:447-448) have linked an individual’s likelihood to experience infertility with factors such as extreme weight gain or weight loss, certain diets, certain lifestyle practices, anatomical and physiological disorders of the female gonads, hormonal imbalances, age, some medical disorders, contraceptive use, stress, male factor contributions, genital infections, menstrual cycle irregularities, endometriosis, polycystic ovary syndrome (PCOS); or in a small number of cases, infertility may be idiopathic.

While all the aforementioned factors significantly affect female fertility potential to varying degrees, female age remains the most inevitable factor that greatly plays a role in conception (Moghadam *et al.* 2022:105). According to Kalem, Şimşir and Var (2019:227), a 14% chance of failure to conceive exists in women by the age of 35 years, this probability increases to 19% between the ages 35-37 years, the prevalence jumps to 25% between ages 37-40, and mounts to a staggering 40% after the age of 40 years. Generally, though, 35% of factors causing infertility are from the female, while 30% are male factor contributions, 20% are combined female and male, and 15% are idiopathic (Ogar, Leonard and Bassey 2018:311). Additionally, according to infertility statistics, in sub-Saharan Africa, 30% of infertility cases are due to female contributions (Abebe, Afework & Abaynew 2020:6 and Ogar, Leonard & Bassey 2018:311).

Records show that Assisted Reproductive Technology (ART) shifted to Africa in the early 1980s and has been on the rise since (Botha, Shamley, and Dyer 2018:2). Apart from Egypt and South Africa, ART in Africa gained popularity circa 2001 when WHO arranged a meeting including African leaders in Geneva to address infertility; ART later gained even more power around the year 2006 when ESHRE devised a specialized task force “Special Task Force (STF)” to tackle infertility in the setting of low- and middle-income countries (Ombelet and Onofre 2019:66-67). In South Africa, in 1982, two ART units were developed in Pretoria and Cape Town, marking the beginning of a new era (Mabweazara 2025:181). However, in Sub-Saharan Africa and Africa as a whole, there are still too few ART facilities available, making easy access to ART for the masses difficult (Lucas *et al.* 2021:638).

According to an observation by Nik-Hazlina *et al.* (2022:1), infertility statistics are generally not well reported; as a result, most infertility rates are usually population estimates rather than precise direct measurements. Sub-Saharan Africa houses forty-eight countries and global ART statistics obtained from the IFFS Surveillance and the ICMART world report revealed that approximately 80% of African countries do not provide any infertility statistics, and at least 21 countries (Table 1) in Africa in 2019 had a recognized ART facility (Botha, Shamley, and Dyer 2018:9 and Ombelet and Onofre 2019:68).

Table 1: List of African countries and the number of registered IVF units within each country (Ombelet and Onofre 2019:68).

Country	Number of IVF units
Algeria	2
Burkina Faso	1

Cameroon	1
Cameroon	1
Ethiopia	1
Ghana	8
Libyan Arab Jamahiriya	2
Morocco	2
Nigeria	12
Namibia	1
Sudan	2
Togo	2
Uganda	4
Egypt	52
Gabon	1
Kenya	3
Mauritius	1
Niger	2
South Africa	37
Tanzania	1
Tunisia	2
Zimbabwe	1

N.B. Due to the growth in the knowledge and need for ART, the number of IVF centres in each country may have increased by the time of submitting this dissertation.

Infertility is a major concern, and although this condition is recognised globally, its prevalence seems to be higher in low- and middle-income countries (Eldib and Tashani 2018:43), which calls for the condition and its interventions to be scrutinised. Infertility investigations are also necessitated by the fact that infertility comes with multiple adversities, anxiety, marital dissolutions, and financial strains, and the infertile community is prone to experiencing depression at thresholds threefold than citizens not experiencing infertility issues (Sripad *et al.* 2021:2 and Nik-Hazlina *et al.* 2022:1). Therefore, further investigations could potentially help lighten the burdens associated with infertility.

2.2 Human Immunodeficiency Virus (HIV) and Female Fertility

For women to be able to reproduce, they need to have normal working ovaries, fallopian tubes, and a uterus; some infections may threaten the normal functioning mechanisms of the female reproductive system, thus leading to infertility (Carbone *et al.* 2021:83 and Lucas *et al.* 2021:640).

Among other blood-borne viruses (BBVs), infection with the Human Immunodeficiency Virus (HIV) affects the female ovarian reserve more aggressively (Bourdon *et al.* 2021:1073).

2.2.1 Background

Since the emergence of HIV, multiple scientific attempts (Figure 1) have been made to convert this virally debilitating infection into a chronically manageable condition; however,

even with a little over four decades of its lifespan, HIV continues to be the leading cause of human demise in Sub-Saharan Africa (Dwyer-Lindgren *et al.* 2019:189).

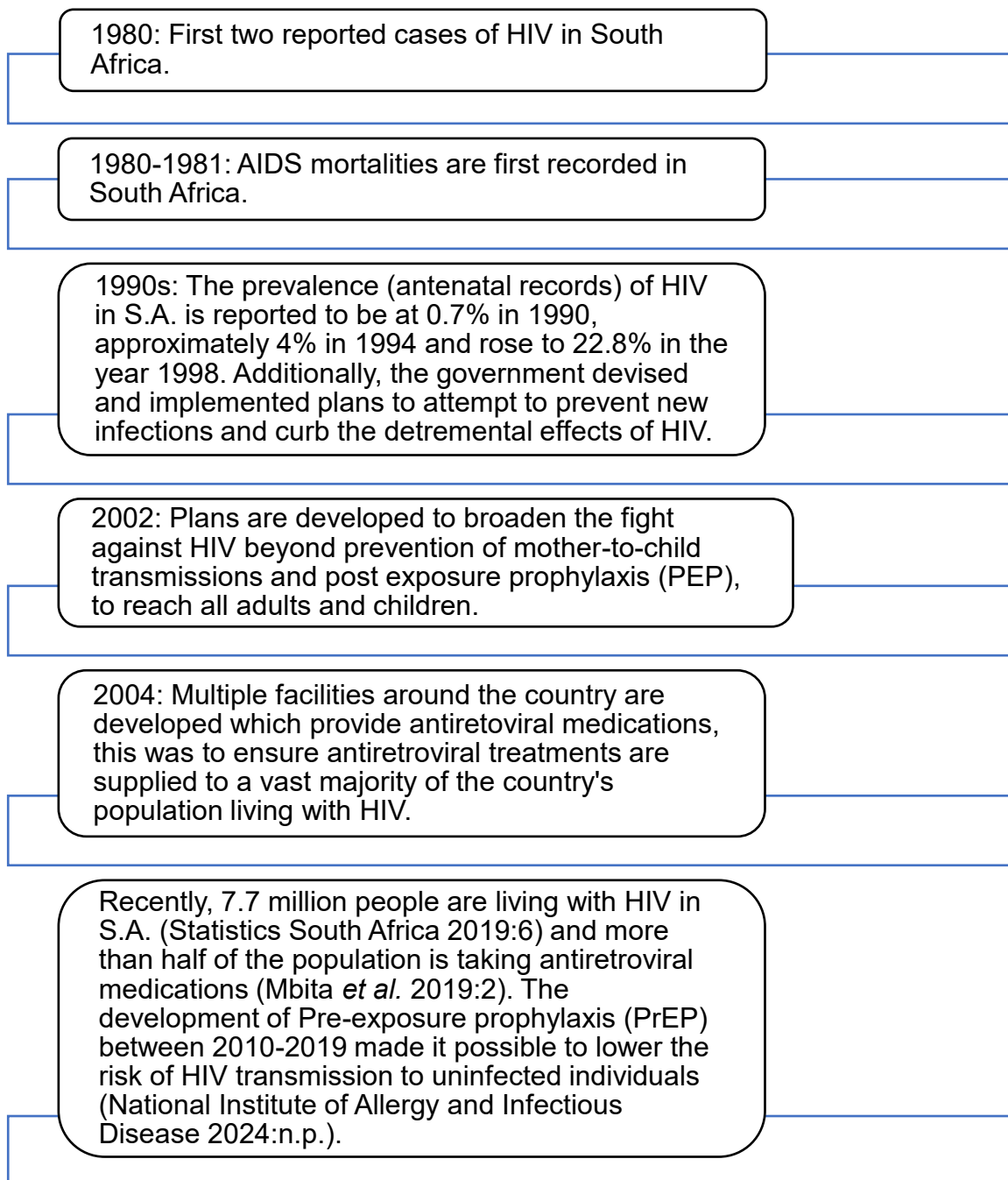


Figure 1: Key features in the fight against HIV in South Africa (Simelela *et al.* 2015:257).

Most of the work on HIV and its treatment initially largely focused on the individual's health status and mother-to-child transmission, and poorly on viral transmission among couples attempting to procreate (Iyer *et al.* 2019:48. e1). This is why there is a paucity of literature on HIV and reproduction, and even poorer documentation in developing regions such as Africa.

One of the most proactive strategies developed in 2014 by the Joint United Nations (UNAIDS) initiative on HIV/AIDS was the 90-90-90 strategy which aimed to end the HIV/AIDS epidemic by the year 2030, this was proposed to be achieved via 3 ways; “90% of all people living with HIV know their status; 90% of all people diagnosed with HIV continuously receive antiretroviral therapy (ART); and 90% of all people on ART should be virally suppressed (Huerga *et al.* 2018:2 and Marsh *et al.* 2019:s213). This strategy has since been updated to the 95-95-95 policy, which encompasses the same vision as the 90-90-90 strategy, which is to have 95% of the total HIV infected population be aware of their status, 95% of PLWH with confirmed diagnoses have initiated and are adherent to antiretroviral medications, and 95% have sufficiently achieved viral suppression (Nyasulu and Maposa 2024:45).

The HIV epidemic prevalence in Sub-Saharan African countries is largely diversified (Figure 2), and according to Dwyer-Lindgren *et al.* (2019:190); ranging from the highest to the lowest in rankings, Botswana has a national prevalence of 22.8%, 3.9% in Tanzania, and 0.7% in the Democratic Republic of the Congo, whilst at country level ranking in 2017 Swaziland was at the top with a prevalence of approximately 27.2%. However, according to Medina-Marino *et al.* (2020:1) and Huerga *et al.* (2018:2), approximately 14% of South Africa’s total population is HIV infected. Globally, this is the country with the highest prevalence of HIV and has the highest rate of new infections. In the 2024 epidemiological estimates, it was noted that Eastern and Southern Africa have a combined population of 21.1 million PLWH, 240,000 in the Middle East and North Africa, and in Western and Central Africa, 5.2 million of the combined population is infected with HIV (UNAIDS 2025).

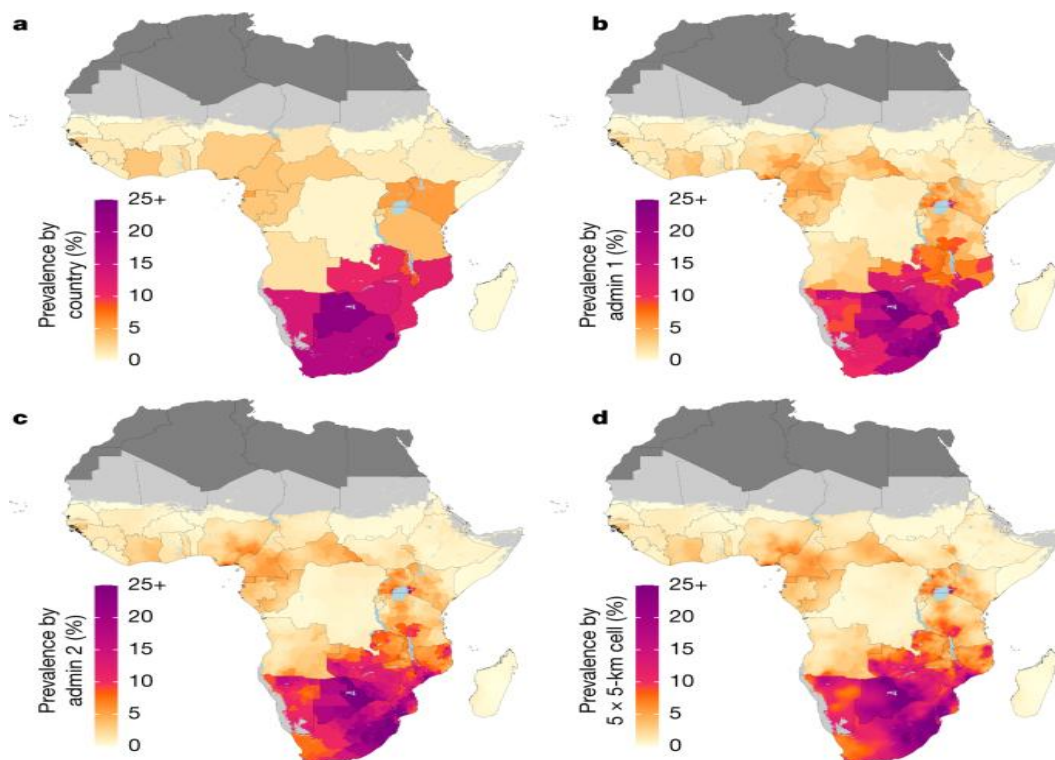


Figure 2: Prevalence of HIV in adults aged 15-49 years in 2017 in sub-Saharan countries. N.B. The analysis did not include countries in dark grey (Dwyer-Lindgren 2019:190).

Approximately 7.7 million people are living with HIV in South Africa, and a fifth of this population constitutes females (15-49years of age) in their child-bearing age (Statistics South Africa 2019:6). Young adult females aged between 15-24 years of age are at higher risk of contracting the virus than males of the same age, reaching a peak between ages 30-34 years (Cohen 2016: n.p) (Figure 3); an estimated 80% of all new infections rise from young females aged between 15-19 years old (Abdool-Karim and Baxter 2019:1470 and Rodrigues-Garcia 2020:1). Moreover, most of the newly diagnosed cases in South Africa are at an advanced stage of infection (CD4 cell count <200 cell/mm³) (Freer and Mudaly 2022:1) and fewer than 50% of the adult population living with HIV have achieved viral suppression (Zanoni *et al.* 2020:2). Additionally, according to Barnabas *et al.* (2020: e1306), globally, 53% of the total population of people living with HIV have achieved viral suppression, and 54% in South Africa.

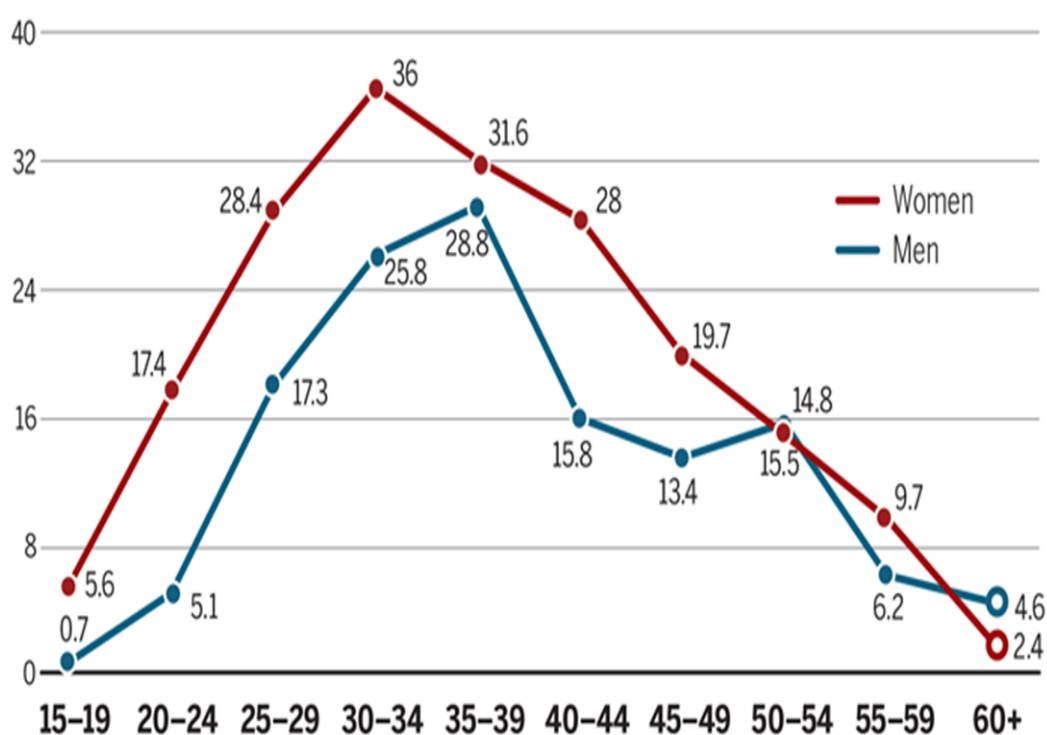


Figure 3: HIV prevalence in South Africa among males and females in different age groups (Cohen 2016: n.p).

2.2.2 Current literature on HIV infection and Female Fertility

In 2018, Udjo (2018:4252) found that following Mauritius, South Africa held the second position in Sub-Saharan Africa with the lowest total fecundity rate (TFR). However, according to Chin and Wilson (2018:449), after applying the instrumental variable (IV) regression on national household survey data gathered from 14 Sub-Saharan countries, they found that the HIV/AIDS epidemic increased the total fecundity rate (TFR) in Sub-Saharan Africa; the epidemiology of this phenomenon was unknown.

HIV-infected women have been described to be more prone to infertility when compared to the HIV-negative females of a similar age group; not only has this lowered fecundity been recognised in natural conception, but it has also been identified in cases of Assisted Reproduction as well (Santulli *et al.* 2016:1084). The likelihood of women living with HIV experiencing ovarian impairments is an idiopathic phenomenon. Still, some past studies have associated this spontaneous ovarian dysfunction with the direct effect of the viral invasion, combined antiretroviral therapy (cART) and chronic inflammation which develops from interminable immune activation observed in people living with HIV (Merlini *et al.* 2021:1). In addition, according to Snider and Wood (2019:81), ovarian dysfunction in women living with HIV has been linked with the elevated permeation of the ovary by macrophages. Women living with HIV are also more susceptible to pelvic inflammatory diseases, anovulation, and tubal factor infertility (Liu *et al.* 2020:3).

There is no unanimity in the literature regarding the HIV-infected population, as past studies and trials have conflicting theories and conclusions. Albeit Udjo (2018:4258) did not find any statistically significant differences in fertility rates between women living with HIV and HIV uninfected women, Marston *et al.* (2017:s69) concluded that it is the period of infection that negatively affects fecundity in HIV-infected females, while in the ART setting, Vianna *et al.* (2021:7) found that this female population showed considerably lower clinical pregnancy; implantation and live-birth rates, had decreased oocyte quantities post COH, lower fertilization rates, higher cycle cancellation rates, longer duration of stimulation and required overall elevated doses of gonadotropins. Furthermore, Vankerkem *et al.* (2017-1493-1494) also observed lower clinical pregnancy rates in HIV-infected women. The lower clinical pregnancy rates may be affiliated with poor oocyte quality observed in this population (Mataro *et al.* 2017:6), or the low oocyte yields observed in HIV-infected females undergoing ART, which ultimately reduces the live birth rate (Vianna *et al.* 2021:7 and Vankerkem *et al.* 2017:1493-1494).

HIV infection has been associated with consistently elevated follicle-stimulating hormone basal levels (FSH), which cause premature ovarian insufficiency (POI), mirroring a constant menopausal state (Yahya *et al.* 2018:181).

Another important fertility-related hormone known to be affected by the viral invasion is anti-Müllerian hormone (AMH). AMH is used to measure and portray a female's ovarian reserve status and is the preferred ovarian reserve biomarker as it is constant throughout the entire menstrual cycle; AMH is also used to predict ovarian stimulation response (Asada *et al.* 2019:275). Basal FSH also assists with assessing and predicting ovarian stimulation response, with elevated levels associated with poorer IVF success rates (Kalem, Şimşir and Var 2019:227).

Merlini *et al.* (2021:6) observed lower than normal levels of circulating AMH in combined antiretroviral therapy (cART) treated women living with HIV; however, their observation could not be firmly associated with immune activation, duration of treatment intake or the time between HIV infection diagnosis and cART initiation. Saxena and Ghumman (2020:40) found that factors such as age, BMI, CD4+ cell counts, and viremia levels negatively affect AMH levels and reduce oocyte quantity (Figure 4).

Reactive oxygen species (ROS) are molecules or compounds that are byproducts produced by a cell-oxygen interaction (Aitken 2020: R189). If there is an increased production of ROS, then the body's oxidative defence system can be reduced and the cells experience oxidative stress; in females, this can alter normal oocyte development, and the ability of the oocytes to fertilise and develop into embryos for implantation (Smits *et al.* 2018:579).

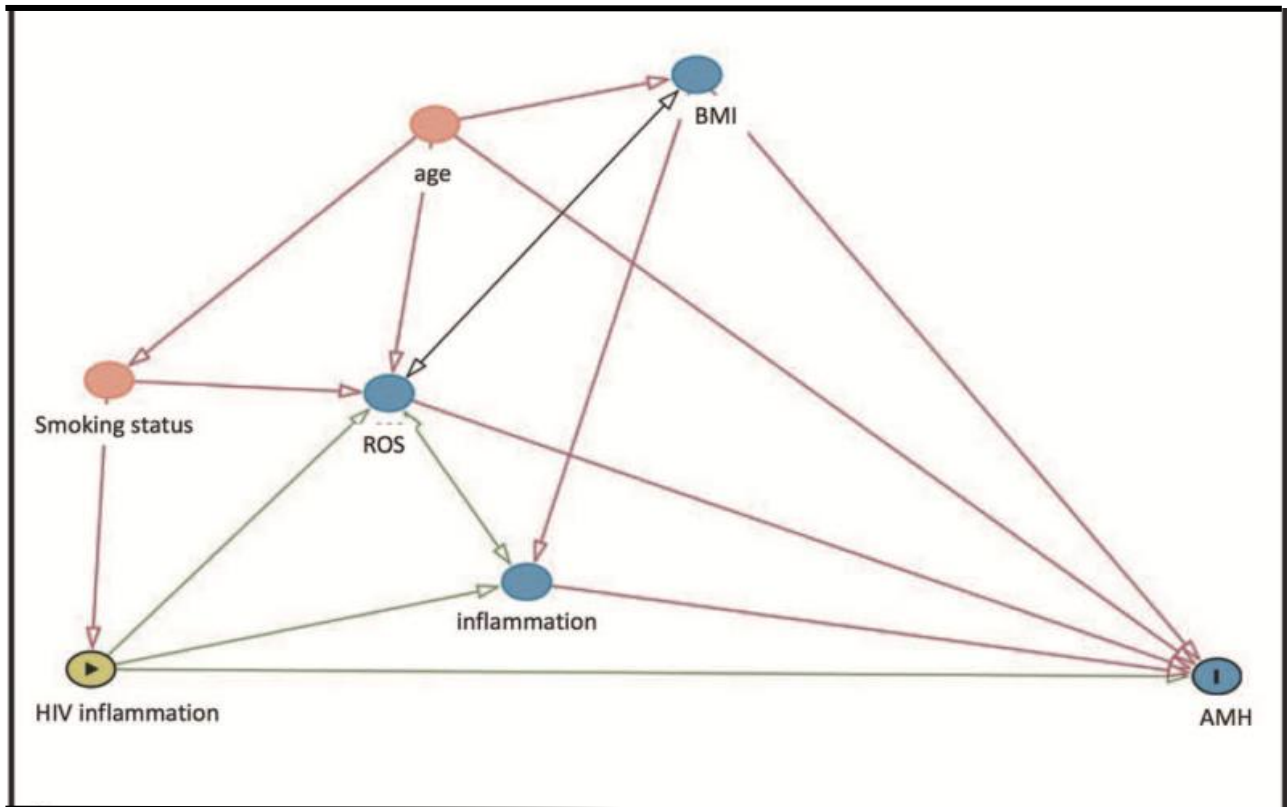


Figure 4: Factors which affect circulating anti-Müllerian hormone levels (Merlini *et al.* 2021:5). (BMI- Body Mass Index, ROS- Reactive Oxygen Species, AMH- Anti-Mullerian Hormone).

HIV-infected females are predisposed to an earlier onset of menopause, depicted by a sharp decrease in AMH levels, which has been noted to be remedied by an increase in CD4+ cell counts (Scherzer *et al.* 2017:46. e1-e6). However, according to Zanni *et al.* (2020: s21), some women living with HIV simply suffer from prolonged amenorrhea rather than the complete premature cessation of menses. Furthermore, while Cejtin *et al.* (2018:np) claimed that this phenomenon is unexplained and should not be immediately presumed to be the early onset of menopause; King *et al.* (2020:493) concluded that prolonged secondary amenorrhea in women living with HIV is linked with opioid use and tobacco smoking, and loosely associated with extremely low levels of CD4+ cell counts (<35 cells/mm³) and according to Zanni *et al.* (2020:s21) prolonged amenorrhea may be mediated by the effects of certain medications acting on the hypothalamic-pituitary hormones. Amenorrhea has also been associated with HIV-linked BMI fluctuations and low CD4+ cell counts (Saxena and Ghumman 2020:37-38).

People living with HIV are prone to metabolic disorders (Zicari *et al.* 2019:6) and low bone mineral density (King *et al.* 2020:486-493) when compared to the general population.

“Human Immunodeficiency virus (HIV) infection promotes microbial translocation and inflammation” (Monnig *et al.* 2019:1126). According to Nasi *et al.* (2017:45), the advancement of HIV affects the gut microbiome and other organs and tissues, resulting in the influx of bacterial products into the circulation, thus igniting the innate immune system, which in turn causes a persistent inflammatory state. Even with proper and timely initiation of antiretroviral medication, people living with HIV continue to show consistent chronic inflammation, which results in the raised production of pro-inflammatory cytokines, thymus dysfunction, affects lymphoid tissue and causes premature ageing (Zicarri *et al.* 2019:1-10). Interestingly, Nasi *et al.* (2017:44) identified markers of premature ageing in young HIV-infected females, which were not consistent with their age.

In an Australian study by Lliberos *et al.* (2021:10) in mice, it was observed that chronic inflammatory body processes are associated with follicle reserve depletion, and this is generally observed in ageing females. A chronic state of inflammation also results in the depletion of the Tcell reservoir, however while the use of antiretrovirals recovers the Tcell reservoir, it does not guarantee the complete resumption of functionality in the Tcells (Nasi *et al.* 2017:45). Moreover, Goldman and Budhram (2020:502) state that chronic inflammation and health deterioration, common in people living with HIV, may form part of the factors which predispose this population to experience abnormal intrauterine fetal growths.

Among other co-morbidities, women living with HIV are susceptible to sexually transmitted diseases (STDs) such as candida, herpes simplex virus-2 and human papillomavirus (HPV) (Saxena and Ghumman 2020:37). Hepatitis C virus hinders follicular activation and impairs AMH production (Scherzer *et al.* 2017:46. e1-6). Sexually transmitted infections (STIs) also alter proper endocrine signalling, reduce the uterus’ ability to maintain gestation, and impair oocyte developmental competence, which subsequently hinders oocyte fertilisation and decreases embryo cell division (Da Broi *et al.* 2018:743).

Apart from ovarian dysfunctions, women living with HIV display obstetric problems such as giving birth to low birthweight babies and have an overall lower cumulative live birth rate (Bourdon *et al.* 2021:1073). Higher miscarriage rates are also quite common in women living with HIV (Liu *et al.* 2020:3). Furthermore, a population-based cohort study in rural KwaZulu-Natal found a correlation between high viremia levels and pregnancy loss, which was defined as either a miscarriage or a stillbirth (Moodley *et al.* 2021:829).

According to the American Society for Reproductive Medicine (ASRM) and the French and International experts, if either partner is HIV infected, timed intercourse (natural conception) may be attempted provided that the viral load of the infected partner is undetectable; they have been on antiretroviral therapy for at least 6 months prior, and the HIV uninfected partner is encouraged to start pre-exposure prophylaxis (PrEP) treatment to further reduce the risk of contracting HIV (Ethics Committee of the ASRM 2021:861 and Vianna *et al.* 2021:8).

One breakthrough in the attempts to combat the toxic effect of the virus must be the development of antiretroviral treatments that basically 'turn off' the virus's functionality, therefore halting viral replication and alleviating the infection's symptoms (Kiweewa *et al.* 2019:2).

2.3 Antiretroviral Therapy and Female Fertility

Breakthroughs in HIV treatments have resulted in minimal-to-no vertical and horizontal transmissions of the virus (Shah *et al.* 2018:86). Even though the virus cannot be annihilated from the body post-invasion, the advent of antiretroviral therapy has enhanced the standard of living of HIV infected individuals and has drastically minimized the morbidity and mortality rates and in turn, has significantly improved the life expectancy of people living with HIV (Zicari *et al.* 2019:1 and Shah *et al.* 2018:86). Currently, people living with HIV have a life expectancy that is only slightly lower than non-infected individuals (Nasi *et al.* 2017:44). Furthermore, following effective aims at ending the global HIV/AIDS epidemic with the vast distribution of antiretroviral medication and increasing education on HIV and its prevention methods, a 23% reduction in new infections has been observed between the years 2010 to 2019 (Pathfinder International 2022: n.p).

In South Africa, antiretroviral (ARV) therapy was not universally accessible until around 2004, with its inception came much backlash and minimal understanding of its mechanism of action; ARV treatment was outlawed in the early years of its availability and the result was the death of approximately 330 000 people and over 400 000 mother-to-child viral transmissions, this proved that ARV treatment was very beneficial to the health of the infected individuals and all other advancements in treatment modalities and interventions (including assisted reproduction) should promote ARV intake (Satoh and Boyer 2019:467 and Zanoni *et al.* 2020:2).

According to Harris and Yudin (2020:1716), an individual should take antiretrovirals and have an undetectable viral load before attempting to conceive. Moreover, the introduction of pre-exposure prophylaxis (PrEP) for timed intercourse and intrauterine insemination (IUI) cycles has made it possible for HIV sero-different couples to successfully have children free from the BBV whilst also preventing inter-partner transmission of the virus, and when other gynaecological issues exist that cause infertility, an optimal OH protocol together with the 'sperm wash' technique for semen coupled with the Intracytoplasmic sperm injection (ICSI) insemination method, can produce the same desired outcome (Cito *et al.* 2019:857 and Sauer 2021:363).

The positive effect of antiretroviral medication in decreasing the viral load, allowing regeneration of the CD4+ T-cell reservoir, and improving the health status and life expectancy came with the desire for more individuals of reproductive age to conceive. Ultimately, this increased wave of desire in people living with HIV to reproduce came with an influx of HIV-infected patients to assisted reproductive technology (ART) units, and over the past three decades, a series of recommendations (Figure 5) have been put forward and published in attempts to assist *in vitro* (IVF) laboratories when dealing with patients who are either both HIV seropositive (both male and female) or when they are serodiscordant (one partner is HIV positive) (Shah *et al.* 2018:86).

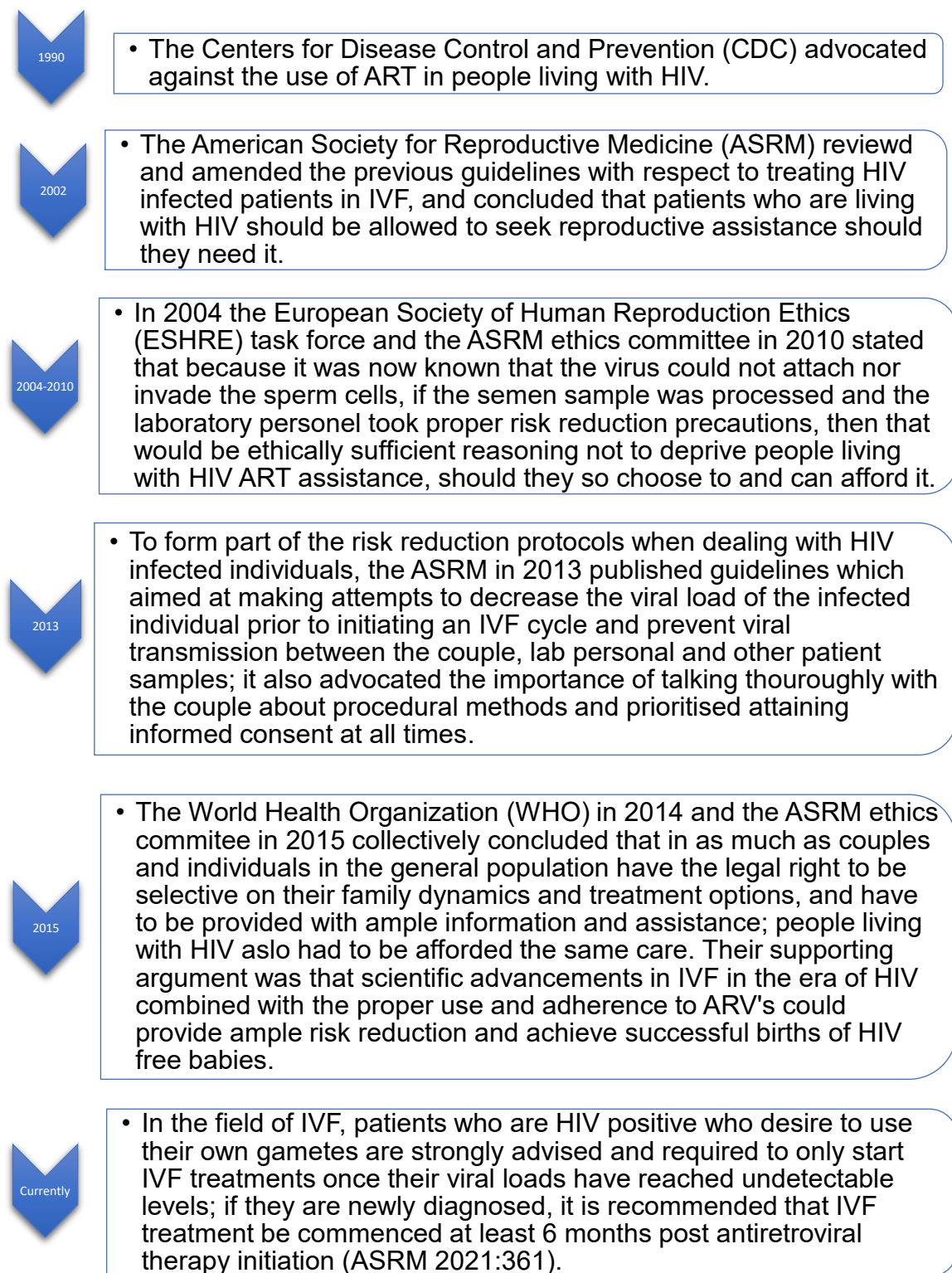


Figure 5: Flow chart of ART recommendations in the era of HIV (Shah *et al.* 2018).

While praised for their positive benefits in improving patient health and altering a once-debilitating viral disease into a chronic condition that can be well managed, antiretroviral

therapies have been associated with a multitude of unpleasant effects on fertility. In this section, we will focus on female fertility.

In a study by Iyer *et al.* (2019:48. e9), conducted at a clinic in Johannesburg, South Africa, in the pre-and post-HAART era, the HIV-infected population suffered from many infertility issues; the infertility rate was higher (78%) when both the male and female were seropositive when compared to the serodiscordant couple.

It is widely known that female age is an important factor that contributes to female fertility potential. According to Lucas *et al.* (2021:639), at least up to the age of 35 years, natural or assisted conception is expected to occur with ease, provided no other gynaecological or habitual issues hinder the process. Mbita *et al.* (2019:10) noted that when compared to older women living with HIV and HIV uninfected women, the youth living with HIV exhibit higher fertility rates, whether they do or do not receive antiretroviral treatments.

A possible direct/indirect proportional relation has been observed between the use of antiretroviral therapy and the premature depletion of the ovarian reserve, noted by the drastic decrease in the major ovarian reserve biomarker: circulating anti-Müllerian hormone (Wessman *et al.* 2018:127; Cassim and Mohamed 2018:1 and Merlini *et al.* 2021:6). However, none of these authors could firmly state that the observation did indeed stem from using antiretrovirals as there was no way of explicitly proving this. Furthermore, in the era of antiretroviral treatments, there has been an increase in the number of HIV-infected females presenting with a classic STI, more prevalent in pregnant women (Cohen *et al.* 2019:28 and Davey *et al.* 2019:11).

Awodele *et al.* (2018:61) found significant reductions in sex hormones after exposing mice to the same quantity and class of antiretroviral drugs ingested by humans. Later, Kitsou *et al.* (2020:2273), who also primarily focused on the effects of antiretroviral treatments (particularly lamivudine) on mice, found ovarian anatomical defects coupled with a drastic reduction in the quantity of primary oocytes. The results of these studies reveal a physiological and anatomical negative correlation between antiretroviral drugs and the female reproductive system. Furthermore, according to Ohinion *et al.* (2019:33), the duration of treatment intake negatively affects follicular development by acting on the circulating level of follicle-stimulating hormone (FSH). In addition, studies by Tang *et al.* (2018) and Kitsou *et al.* (2020) both revealed a time-dependent interaction between reverse nucleoside transcriptase inhibitors and female fecundity. According to Tang *et al.* (2018:3895), this interaction observed between nucleoside reverse transcriptase inhibitors and the ability to conceive manifested as impaired oocyte maturation in rats, both *in vivo* and *in vitro*; reduced embryo cleavage and blastulation rates, and an increase in the level of ROS production. However, it is important to note that many of these remarkable studies and their breakthroughs in the association between ARV treatments and fertility in females are mostly animal studies, and there is a paucity of such studies on humans. Thus, larger population randomised clinical trials are still needed to confirm this same interaction conclusion on the human body. For this reason, not many study outcomes can be generalised to the entire population of people living with HIV.

Exposure to antiretroviral drugs such as zidovudine (AZT), didanosine (DDI), stavudine (d4T), and efavirenz (EFV) has been strongly associated with mitochondrial toxicity (Pasvol *et al.* 2021:343). Mitochondrial toxicity reduces the oocyte pool, affects oocyte quality, lowers normal fertilisation rates *in vivo* and *in vitro*, and significantly reduces pregnancy rates (Tang *et al.* 2018:3895 and Vianna *et al.* 2021:8).

In terms of gestation in the era of antiretroviral therapy, the studies that exist have highlighted a few unpleasant phenomena that HIV-infected women must bear. The phenomenon of preterm parturition and the delivery of low-birth-weight infants has been identified in HIV-positive females who are 1. not on any antiretroviral medication, and/or 2. Individuals who only start treatment just before conception (Gibango, Mda and Ntuli 2018:44 and Uthman *et al.* 2017: e26) and rare in females who have long been on ARV treatment (Goldman and Budhram 2020:504). The prevalence, however, of this phenomenon in this population is not completely known (Anderson *et al.* 2021:263). Furthermore, according to Sebitloane and Moodley (2017:126), preterm birth and the delivery of low-birth-weight babies are a result of the toxic effects of highly active antiretroviral therapy (HAART) regimes.

It is well known that once the virus enters the body, it rapidly proliferates within the body's defence cells, and with the assistance of antiretroviral medications, the spread of the virus can cease (Brenna and McMichael 2022:38). Kudesia *et al.* (2018:4) observed high levels of infertile women living with HIV having the desire to have children. Assisted reproductive techniques exist to assist the infertile population to attain offspring by stimulating the females' ovaries with exogenous gonadotropins to achieve multi-follicular growth and harvest many oocytes to create preimplantation embryos that can help achieve a live birth (Haahr, Esteves, and Humaidan 2018:1).

2.4 Controlled Ovarian Hyperstimulation and Ovulation Trigger Injections.

A collective number of studies have asserted that the better the quality of oocytes harvested, the greater the chance exists to achieving a live birth (Esteves *et al.* 2020:2).

"The process of *in vitro* fertilization/Intracytoplasmic sperm injection (IVF/ICSI) consists of five sequential steps, which are the actual ovarian stimulation (OS), to induce multi-follicular development, the ovulation trigger, aiming to induce final oocyte maturation, the oocyte retrieval, aiming to harvest the matured oocytes, the laboratory procedure, aiming to create embryo's, and the embryo transfer, which aims to transfer the available embryo(s) inside the uterus" (Figure 6) (Racca *et al.* 2020:973).

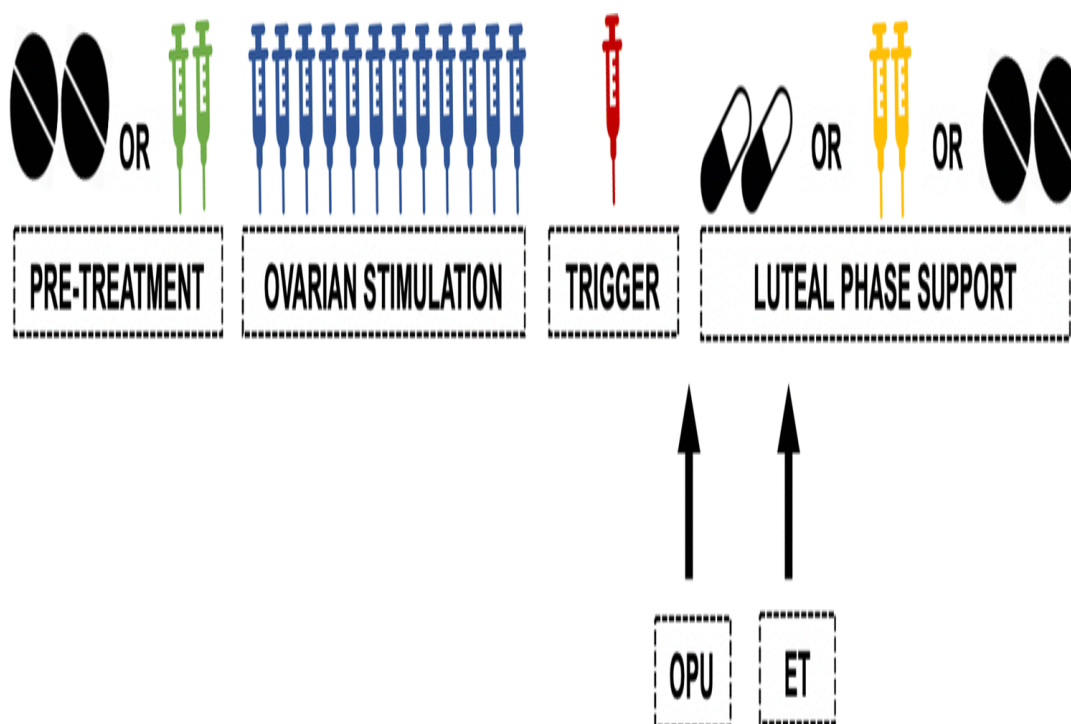


Figure 6: The process of IVF/ICSI: From ovarian hyperstimulation-Ovulation Trigger-Transvaginal oocyte aspiration, to luteal phase support-Embryo transfer (Racca *et al.* 2020:974). (OPU- Oocyte Pick-Up, ET- Embryo Transfer).

Ovarian hyperstimulation (OH) is a prescribed course of *in vitro* fertilisation (IVF) medical treatment, which is utilised to trigger the development of multiple follicles to ultimately attain enough good-quality oocytes required for the success of IVF/ICSI; OH also promotes endometrial development sufficient to allow embryo implantation (Minareci and Ozcan 2019:196).

According to Mostinckx *et al.* (2019:1596) and Racca *et al.* (2020:974), the benefits of ovarian stimulation over natural cycles include increased pregnancy and live birth rates, shorter time to conception, more oocytes can be retrieved with higher numbers of mature oocytes and therefore more preimplantation embryos for selection to transfer to the recipient female.

To maximise treatment efficacy, a stimulation regimen is selected based on the patient's age, clinical history, and ovarian reserve status (Racca *et al.* 2020:973). According to a European study by Paul, Atilan and Tulay (2019:347), the number of stimulation cycles an individual undergoes does not harshly impact the ovarian reserve; in addition, oocyte quality and embryo development are not altered by repeated OH.

In ovarian hyperstimulation, highly purified (hp) hMG and recombinant FSH (rFSH) are the most used exogenous gonadotropins; they both exhibit comparable gestational and live birth rates; however, rFSH gives rise to more oocytes at retrieval (Racca *et al.* 2020:975).

According to Ali-Abed, Abbod and Ali Jwad (2020:7128) and Yang *et al.* (2021:2), the medication for OH serves the following purposes: the exogenous gonadotropins provide the ovarian stimulation needed for stimulating multi-follicular growth, they induce oocyte

maturation and restart the process of meiosis to provide competent oocytes suitable for insemination and suppress pituitary function to inhibit premature ovulation.

Following OH, oocyte heterogeneity is a common phenomenon, with an estimated 10-30% of the oocyte cohort being immature (metaphase I or Germinal vesicle oocytes) (Braga *et al.* 2020:70). This may be a result of multiple factors including transmission failures, hormone deficiencies, resistance to ovarian stimulation, and unexplained oocyte inherent factors (Tantitham *et al.* 2020:133 and Escrich *et al.* 2018:668). In some cases, a patient's batch of oocytes will exhibit an unexpectedly larger number of immature oocytes, and in rare cases, all the oocytes aspirated will be immature (Escrich *et al.* 2018:668).

There is no consensus on the utilisation of immature oocytes harvested following ovarian hyperstimulation. It is eventually at the laboratory's discretion whether to give these oocytes more time to mature *in vitro* or to simply discard them. Giving the oocytes additional development time increases the patient's embryo pool, giving rise to more embryos for selection for an embryo transfer or cryopreservation for future embryo transfer cycles. Escrich *et al.* (2018:638) recommends a 6-21-hour interval for oocyte maturation *in vitro*.

During the period of OH, the patients' response to stimulation is continuously monitored via transvaginal ultrasonography. COH monitoring provides continuous growth assessment, directs proper timing for when the ovulation trigger should be taken to induce final oocyte maturation and serves to provide continuous risk assessment (Robertson, Chmiel, and Cheong 2021:100).

One of the most critical aspects to control during ovarian hyperstimulation is the level of Luteinising hormone (LH). LH is initially suppressed and then reintroduced at a specific time to promote final oocyte maturation in a process called ovulation triggering (Shakerian *et al.* 2021:294). Suppression of LH allows the clinician to regulate the LH peak. Failure to control the peak level of LH can lead to the untimely release of oocytes from the follicles, resulting in fewer oocytes being collected at harvest (Guan *et al.* 2021:2). Thirty years ago, Human Chorionic Gonadotropin (hCG) and gonadotropin-releasing hormone (GnRH) agonists were developed to stimulate final oocyte maturation in GnRH-antagonist stimulation cycles, which remains the most widely used stimulation protocol (Don *et al.* 2022:2). The type of ovulation trigger used has a distinguishable effect on oocyte quantity, oocyte and embryo quality, and embryo development (Ali-Abed, Abbod and Ali Jwad 2020:7131).

The ovarian hyperstimulation syndrome (OHSS) is a medical condition and a complication of OH that manifests as hyperstimulation to OH resulting in superabundant follicular growth at a single time (Braam *et al.* 2020:2). OHSS can cause mild-severe discomfort possibly requiring high care admittance, thromboembolism and even lead to a patient's demise (Magnusson *et al.* 2018:59). Independently, hCG and GnRH- Agonists have been found to have some adverse effects that cannot be overlooked, such as an increased risk to ovarian hyperstimulation syndrome (OHSS) and subject patients to significantly lower IVF outcomes coupled with higher miscarriage rates; however, when combined, multiple studies found conflicting results in terms of little-to-better oocyte maturation rates and total oocyte numbers, a minimal to none risk of OHSS, and similar to better IVF outcomes (Shakerian *et al.* 2021:297 and Dong *et al.* 2022:7). These studies may not have reached a consensus on the subject possibly owing to not enough study population numbers and because the studies would have been done on a variety of patients with varying types and degrees of infertility.

Essentially, ovarian hyperstimulation allows the physician to manipulate the production and harvest of a female's oocytes within a specific cycle using medications and hormonal signaling, the goal being to provide the ability for the infertile population to bear children.

The literature presented in this section describe the role of ovarian hyperstimulation and ovulation triggers in promoting fertility potential in ART by making a way to obtain a large number of competent oocytes suitable to achieve a live birth. However, it is well known that while much effort can be placed into achieving a specified goal, sometimes there are factors that may exist that would threaten the goal being achieved. The most common factor that has been associated with HIV and reproductive potential in females is Age (Kiweewa 2019:2 and Esbert *et al.* 2024: para.1 line 1).

2.5 Age and Female Fertility

Age is a crucial factor in ART; often used to direct the choice of the ART procedure and to predict ART outcomes (Asada *et al.* 2019:275). According to Hosseinzadeh *et al.* (2022:420), at the approximate age of 37 years old, a woman's fertility potential begins to decrease at a rapid rate. Lucas *et al.* (2021:639), however, claim that female fertility starts to markedly drop by the age of 35 years, while Sun *et al.* (2020:1) has noted that at as early as 32 years of age, a woman's reproductive potential starts to thin significantly. This means that on average, a woman's biological clock begins to hasten in her mid-thirties.

Interestingly, however, Zhang *et al.* (2019:7) reported on a portion of females who are older and yet have high serum AMH levels, depicting a normal ovarian reserve. These women had comparable oocyte retrieval rates to the younger women and only differed in clinical pregnancy rates. This phenomenon is idiopathic and does not occur in all women of advanced age. In ART, the recommended cut-off age is 45 years, and beyond this age, oocyte donation is strongly recommended (Van Opstal *et al.* 2021:108).

The number and quality of eggs a woman can produce per menstrual cycle steadily decreases with advancing maternal age (Zhang *et al.* 2019:2). Advancing maternal age is also associated with poor responses to ovarian stimulation, high embryo chromosomal abnormalities, high miscarriage rates, and low clinical and live birth rates (Hosseinzadeh *et al.* 2022:420 and Zhao *et al.* 2020:6). Furthermore, advancing maternal age has been found to closely relate with a rise in ROS elements formation in the body which negatively pressures the ovarian microenvironment thus damages oocyte quality by irreparably damaging oocytes spindles and giving rise to abnormal looking oocytes; this hinders fertilisation (Sun *et al.* 2020:5). Moreover, to some extent, ageing affects the production and functioning of some fertility hormones such as AMH and FSH, and a pattern of lower serum AMH levels and higher FSH levels is often noted with advancing maternal age (Hsieh *et al.* 2020:1).

Advancing age is the most unavoidable factor that strongly affects a woman's ability to have offspring. Evidence suggests that with rising age in years, a woman begins to have trouble with producing good-quality oocytes caused by a reduction in the oocyte pool. Additionally, hormone production also decreases with advancing maternal age. In ART, while synthetic

versions of fertility and sex hormones are available, there is no way to refill the oocyte pool once it has been depleted. Owing to the age effect on oocytes and the power of replacement hormone therapies, older women with diminished ovarian reserves can still achieve a live birth; however, this can only be done with the use of donated oocytes.

Another aspect of ART that is not frequently reported on but has considerable influence on ART outcomes is the paternal age effect. According to Van Opstal *et al.* (2021:111), male age (like in women) has a negative impact on IVF outcomes, affecting embryo development and subsequent clinical pregnancy outcomes.

In conclusion, since advancing maternal age is associated with a rising prevalence of infertility, and Scheffer *et al.* (2018:215) and Moghadam *et al.* (2022:105) have observed a rising frequency of early middle-aged women seeking ART, it is important to investigate and address the age impact on oocyte quality. Furthermore, with maternal age (Wu and Wu 2023:9), ovarian stimulation (Tso *et al.* 2021:205-209) and antiretroviral treatments (Koethe *et al.* 2020:2) having been investigated and associated with body mass index (BMI), it would be of relevance and beneficial in this study to assess the relationship between BMI and fertility because of the population's characteristics- HIV infected women of reproductive age and undergoing ART.

2.6 Body Mass Index (BMI) and Female Fertility

An individual's body mass index (BMI) calculates their body's fat percentage relative to their weight and height (Wu and Wu 2023:1). The WHO and the National Institute of Health (NIH) classify underweight as having a BMI <18.5, normal weight- BMI 18.5-24.9, overweight- BMI 25-29.9, and obese as having a BMI>30 (Al-Obaidi *et al.* 2018:1920).

According to Koethe *et al.* (2020:2), women living with HIV who are on antiretroviral therapies have weight gain that is generally centralised on the torso. This fat deposition has been associated with antiretroviral treatment initiation and extended use and adherence to the treatments.

Andreas, Winstanley and Robker (2021:152), Al-Obaidi, Mahdi and Alwasiti (2018:1920), and Wu and Wu (2023:1) have all noted an increased likelihood of individuals experiencing infertility and some pregnancy complications due to extreme weight gain or weight loss.

In natural conception, severely underweight and overweight females take longer periods to fall pregnant (Wu and Wu 2023:1). While, in assisted reproduction, Sawal *et al.* (2020:23) after reviewing numerous studies on the subject, found no significant differences in the number of oocytes retrieved from neither weight category, however, Wu and Wu (2023:2-8) observed an association between raised BMI and fewer oocyte yields, lower oocyte maturity rates, and poor responses to stimulation, presented by low endometrial lining measurements.

Furthermore, a clear link has also been made between obesity and advancing maternal age, and high levels of oxidative stress and inflammation; this stresses the ovarian reserve leading to lower clinical pregnancies and live birth rates (Snider and Wood 2019:86 and Zhao *et al.* 2020:6). However, there is no correlation between advancing maternal age and

an increasing BMI (Wu and Wu 2023:9). Figure 7 shows the effects of oxidative stress and inflammation, which can arise from changes in gut microbiota, on female fertility potential (Snider and Wood 2019:86).

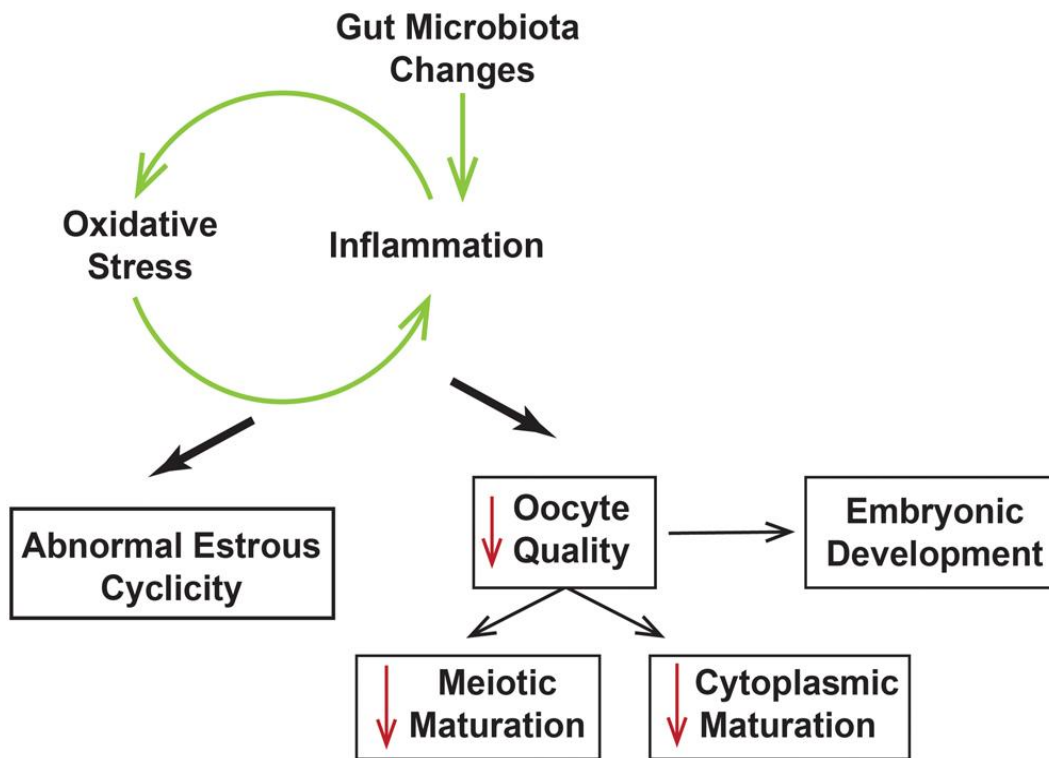


Figure 7: Effects of changes in the gut microbiota, inflammation, and oxidative stress on the ovarian oestrous cyclicity and oocyte quality (Snider and Wood 2019:86).

In some animal and human studies, a high BMI has been linked with alterations in follicular fluid composition and granulosa cell signalling, increased granulosa cell apoptosis, lower primordial follicle pool, anovulation, reduced fertilisation and maturation rates, oocytes presenting with smaller mean diameters (<120 microns), abnormal spindle and chromosome formation, abnormal oocyte mitochondria, stagnant embryo cell division with associated poor embryo quality, resistance to ovarian stimulation and high IVF cancellation rates (Atzmon *et al.* 2017:1145-1149; Andreas, Winstanley and Robker 2021:152, Al-Obaidi, Mahdi and Alwasiti 2018:1922-1923 and REVIVA Fertility and IVF clinic 2020-23). While some of these studies noted results on the infertile population, there is a paucity of information on the association between BMI or dietary plans and ART outcomes, particularly in women living with HIV attending ART.

Extreme weight gain and/or weight loss are associated with many health issues, both in the general population and in the infertile community. In ART, a high BMI has been associated with changes to the ovarian microenvironment, particularly the concentration levels of the follicular fluid. The ovarian-follicular microenvironment is important because the follicles store the arrested oocytes, which have the potential to mature and develop into an embryo (Telfer and Anderson 2021:1117), and the follicular fluid and hormones contain the materials required to control follicle development from within the ovary (Piccinni *et al.* 2021: e722).

2.7 Follicular Milieu and the Oocyte

The follicular fluid (FF) and cumulus cells form part of the major components of the follicular microenvironment and play a key role in regulating oocyte growth and development (Liu *et al.* 2020:515). Aided by hormones and other factors inherent to the ovary, the follicular milieu constantly undergoes a series of adjustments and changes during growth and maturation; ovulation and the generation of the corpus luteum (Harada *et al.* 2021:1). One of the major roles of the FF is mediating communication between the oocyte and its surrounding follicular environment which is a key component for the oocyte's developmental competence (Pizarro *et al.* 2020:2 and Robker *et al.* 2018:3216). Therefore, any changes in the follicular microenvironment will have an impact on the oocyte's quality and subsequent embryo quality.

Follicular fluid hormone concentrations and certain systemic conditions can have an impact on oocyte developmental competence at varying degrees (Da Broi *et al.* 2018:738). Another independent factor that alters the follicular microenvironment is age (Razi *et al.* 2020:588; Babayev and Duncan 2022:359, and Da Broi *et al.* 2018:739). Babayev and Duncan (2022:359) reviewed literature from IVF studies with the intent of reviewing age-related alterations in the oocyte milieu of antral follicles and ageing was associated with a multitude of unique nuclear and mitochondrial genomic alterations; epigenomic, transcriptomic, proteomic and metabolic changes; and also conflicting hormonal composition changes that are somehow interconnected but cannot be generalized, furthermore, the possible effects of external factors such as individual lifestyle, co-morbidities, dietary intake, and medications could not be ruled out as possible causes of the findings. Ageing has also been identified as one of the factors that indirectly cause disruptions in follicular fluid component levels (such as homocysteine), which negatively affects oocyte maturation (Razi *et al.* 2020:588).

A study by Monnig *et al.* (2019:1126) in Rhode Island, USA, which studied substance use in HIV-infected individuals using HIV-uninfected people as the controls, revealed that when compared to the HIV negative population, people living with HIV show elevated concentrations of lipopolysaccharides (microbial translocation biomarkers) and lipopolysaccharide-binding proteins (immune activation biomarkers). The invasion of the uterus by gram-positive bacteria promotes plasma and follicular fluid lipopolysaccharide (LPS) production, a study by Magata (2020:311-321) in Japan, in cattle, revealed that in high doses, LPS acts directly on the follicular milieu, the oocyte and the hypothalamic-pituitary-ovarian (HPO) axis, thus causing a cascade of events such as, inhibiting the secretion of gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH), reduces the production of follicular estradiol (E2), impairs follicular growth, affects meiotic division and mitochondrial distribution; and alters normal oocyte nuclear and cytoplasmic maturation.

Furthermore, accumulated LPS in follicular fluid causes oxidative stress in cells, plays a role in the production of reactive oxygen species, disrupts oocyte maturation thus reducing the maturation rates, leads to fertilisation failures, causes aberrant chromosome and spindle alignment, and induces cell apoptosis (Yi *et al.* 2021:165-167 and Rasekhi *et al.* 2020:70). Moreover, oxidative stress and inflammation tend to induce activated endoplasmic reticulum

(ER) stress, this, in turn, impedes follicular growth, maturation, and ovulation; and reduces oocyte quality (Harada *et al.* 2021:2-9). Additionally, at minimal quantities, ROS regulates normal cell metabolic activities, at high doses however, the build-up of these molecules has been associated with decreased oocyte quantity and quality, negatively impacts the production of ovarian steroid hormones; and impairs oocyte developmental competence, blastulation, implantation and the maintenance of hormones that support pregnancy (Lai *et al.* 2018:518-519; Merlini *et al.* 2021:7 and Pizarro *et al.* 2020:2).

2.8 Conclusion and Rationale

The literature provided and dissected in this chapter undeniably reveals that both the HIV/AIDS and infertility epidemics significantly affect females at varying degrees (Ogar, Leonard and Bassey 2018:311 and Cohen 2016:n.p); these epidemics are most prevalent in Sub-Saharan Africa (Lucas *et al.* 2021:635), and South Africa stands as the hotspot of the HIV/AIDS epidemic (Medina-Marino *et al.* 2020:1 and Hueriga *et al.* 2018:2). It is also evident that these regions still largely struggle with addressing these epidemics although efforts are continuously being made (Hueriga *et al.* 2018:2 and Marsh *et al.* 2019: s213).

In the South African context, the association between HIV and female fertility is an important distinction to make and address since, according to the literature supplied in this chapter, HIV is prevalent among young adult women, who form part of reproductive-aged women that are widely accepted as the most fertile group (Statistics South Africa 2019:6 and Cohen 2016: n.p). The literature also reveals that there is a gap in knowledge on this association particularly in the Sub-Saharan African context owing to limited infertility statistics and limited research on the impact of HIV infection on female fertility (Nik-Hazlina *et al.* (2022:1). What should also be noted and thus promotes the need for more randomized control studies on the subject is the fact that from the literature, the studies that do exist (Baza *et al.* 2019, Lucas *et al.* 2021, Marston *et al.* 2017, Mataro *et al.* 2017, Vankerkem *et al.* 2017, Cassim and Mohamed 2018, Snider and Wood 2019, Liu *et al.* 2020 and Vianna *et al.* 2021) cannot reach a consensus on this association or effect of HIV infection on female fertility, thus making it difficult to generalize treatment and surgical interventions for women living with HIV experiencing infertility.

However, studies conducted previously (Baza *et al.* 2019, Lucas *et al.* 2021, Marston *et al.* 2017, Mataro *et al.* 2017, Vankerkem *et al.* 2017, Cassim and Mohamed 2018, Snider and Wood 2019, Liu *et al.* 2020 and Vianna *et al.* 2021) have assumed that either a direct impact by the viral invasion or viral infection associated factors play a role in female infertility, and factors such as CD4+ lymphocyte counts, female age, female BMI, antiretroviral therapy regimen type and intake, ovarian stimulation protocol type and dosing, and ovulation trigger injection type and dosing all can be associated with HIV infection and have the ability to alter female fertility potential.

While assisted reproductive techniques continuously undergo advancements over the years to assist infertile couples in having children, there still exist some factors that have the potential to alter the outcome expected from ART and leave couples with an even greater

financial, psychological, and emotional burden (Sripad *et al.* 2021:2 and Nik-Hazlina *et al.* 2022:1). The continued development of research studies on infertility, ART and HIV infection impact on female fertility possess the power to assist in conquering the battle of infertility.

This study aimed to provide a comparative analysis of mature and immature oocyte yields in oocyte cohorts obtained from HIV-infected females against HIV-uninfected women of reproductive age, post-controlled ovarian hyperstimulation. Additionally, the study examined the potential influence of factors associated with HIV infection, infertility, and ART, such as age, BMI, viral load, CD4+ lymphocyte counts, antiretroviral medication intake, ovarian stimulation protocols and ovulation trigger injections on oocyte maturation outcomes.

CHAPTER 3

MATERIALS AND METHODOLOGY

This chapter will discuss research study materials and methodology. This chapter will also describe the study setting, population, data collection tool, research process, statistical analysis, and ethical considerations.

3.1 Background

Reports show that infertility statistics are not well presented in Africa, owing to the very few recognized ART facilities (Ombelet and Onofre 2019:68). In addition, Africa is one of the continents with a high prevalence of HIV (Eldib and Tashani 2018:43). Women in their reproductive ages are at the peak of the HIV infection prevalence (Statistics South Africa 2019:6). However, it has been observed that HIV infection statistics on adult women are mostly built on data gathered from antenatal clinics and seldom on entire population groups (Yahya *et al.* 2018:178). This means that HIV infection statistics are also not well portrayed, and neither is the prevalence of HIV infection in the infertile community well depicted.

This study aimed to provide a comparative analysis of the mature and immature oocyte yields in oocyte cohorts obtained from HIV-infected females against HIV-uninfected women of reproductive age, post COH. Additionally, the study examined the potential influence of factors associated with HIV infection, infertility, and ART, such as age, BMI, viral load, CD4+ lymphocyte counts, antiretroviral medication intake, ovarian stimulation protocols and ovulation trigger injections on oocyte maturation outcomes. While oocyte heterogeneity is expected and is a normal phenomenon (Escrich *et al.* 2018:668), the expectation following COH is that all or most of the oocytes will be developmentally competent due to the administration of high-dose medication and proper timing. The retrieval of higher numbers of immature oocytes, therefore, threatens the success of ART.

3.2 Study setting and study design.

This is a single-centre prospective randomised study conducted at a private ART facility, FemiCare Fertility clinic, located in one of the capital cities of South Africa, Pretoria. The majority of patients who attend the clinic are from the black community from various geographical areas within the country and other African countries. The clinic offers services to all age groups, single people, and heterosexual and same-sex couples.

The study is based on infertile women of reproductive age and is built on data collected from COH-ICSI cycles from the year 2021 to 2023. The total population size is n=80 women with varying infertility-related complications, varying ethnic backgrounds, races, and socio-economic standing. A data capture sheet (Appendix I) was developed to serve as a collection tool to gather the data on the outcomes from the participants COH-ICSI procedures, and a data capture sheet questionnaire (Appendix J) was used to gather extra

data needed for the purpose of the study which fell outside of the data obtainable from the normal COH-ICSI cycle data. All the raw data were then statistically analysed, interpreted and presented in this dissertation.

Permission to approach potential participants for recruitment purposes (Appendix A) and to access the patient database for clinical history and blood results (when needed) was granted by the patient's clinician and practice management (Appendix B).

The study design was approved by the Durban University of Technology (DUT) Institutional Research Ethics Committee (IREC), ethical clearance number IREC 138/22 (Appendix C).

3.3 Recruitment process

Participants for this present study were selected from patients attending a fertility clinic for infertility-related complications and medical interventions. These are women of child-bearing age, aged between 18-39 years old, with different ethnicities and social and economic standings. These women also had different HIV sero-statuses. Upon providing consent to start the IVF process, the patients were approached and asked if they would be willing to participate in the study, upon receipt of formal written consent of participation, these women were asked to complete a short questionnaire to help collect some raw data, while the bulk of the raw data was collected from the outcomes observed during the COH-ICSI cycle. This data was then statistically analysed and interpreted.

3.4 Study Population

Participants were selected from a group of premenopausal women attending a fertility clinic for infertility-related complications and interventions. Eligibility was only based on the inclusion (premenopausal women aged between 18-39 years undergoing COH-ICSI) and exclusion (post-menopausal, donor-recipient cycles) criteria. These women had, after extensive counselling, provided informed consent to undergo routine IVF procedures and take fertility treatments at the IVF centre. Patients who met the study requirements (based on the inclusion/exclusion criteria) were then approached by the investigator during follow-up appointments to ask for permission to be included in the study. Patients were informed of the nature and purpose of the study and were made aware that they could withdraw participation at any time during the study. All patients approached were allowed to read through the information letter (Appendix D) and discuss the study with their clinician and partners, and when they agreed to participate, they were given the consent form to sign (Appendix E).

Amongst other study outcome measures (Table 2), the study aimed to assess the number of mature oocytes against the number of immature oocytes in oocyte cohorts retrieved from HIV-uninfected and HIV-infected women undergoing COH-ICSI cycles at a fertility clinic. All women included in the study intended to use their oocytes for personal reproductive purposes. The total study population was divided into two categories: 1- the test group, consisting of n=40 premenopausal HIV-infected females between the ages of 18-39 years, and 2- the control group, a group of n=40 premenopausal HIV uninfected females aged between 18-39 years.

Table 2: Study outcome measures.

Primary Outcomes Total number of oocytes retrieved (Oocyte quantity). Number of MII oocytes (Oocyte quality). Number of GV+ MI oocytes. Secondary Outcomes The influence of Age and Body Mass Index. Efficacy of the stimulation protocol and trigger. Relationship and influence of the viral load, antiretroviral treatment intake and CD4+ lymphocyte levels. Relationship between AMH, Follicle Number and Oocyte Number.
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Additional information that this study will provide will include information on fertilisation rates and embryo quality from the investigated oocytes (supplementary statistics).

The inclusion criteria include:

- Premenopausal women undergoing COH-ICSI cycles at the fertility clinic (ICSI was chosen because the clinic predominantly uses this insemination technique-convenience, not patient-related).
- Women between the ages of 18-39 years.
- Women with serum AMH ≥ 1.22 ng/ml (Beckman Coulter Counter- Ampath Laboratories) (Appendix F)
- Women who are HIV seropositive and women who are HIV seronegative.

The exclusion criteria include:

- Postmenopausal women.
- Women on donor egg-recipient cycles.
- Women who experience gonadotropin-resistant ovary syndrome, and women with premature ovarian insufficiency (POI).

The study population included participants who were attending ART and were of reproductive age with serum AMH ≥ 1.22 ng/ml and were placed on the GnRH-antagonist protocol. According to the ESHRE guideline group on ovarian stimulation, based on safety and efficacy measures, the GnRH-antagonist protocol is the most used and is preferred for women with normal ovarian reserve levels (The ESHRE Guideline group on Ovarian Stimulation *et al.* 2020:5). There were no records of the number of cycles previously attempted by each patient.

To potentially reduce bias and specifically highlight the influence of the HIV infection on the female and the potential impact on fertility, sero-different couples were included where the male was HIV uninfected and had normal sperm parameters, based on WHO 2010/2021

reference ranges (Appendix G). All the participants were on antiretroviral treatment and had accomplished viral suppression.

3.5 Data collection tool

The quantitative research approach was used to collect the raw data for this study. Participants raw data was collected from electronic patient IVF-OH monitoring reports (Appendix H) (FemiCare Fertility Clinic laboratory protocol, 2018), and included the following measurements:

- Patient's weight and height.
- Patients age.
- Patient's infertility diagnosis.
- AMH level.
- Follicle numbers, sizes, and anatomical location.
- Type of stimulation, type and dose of exogenous gonadotropin used, and type and dose of ovulation trigger used.
- Number of total oocytes retrieved.
- Maturation status of the total retrieved oocytes.
- Endometrial thickness.
- Fertilisation and embryo development outcomes.
- Semen analysis result.
- HIV serostatus.

Additional raw data were collected using the research study protocols collection tool (Appendix I). Finally, another portion of the raw data was collected from mini questionnaires that the participants were requested to complete to gather more information about the patient's background (Appendix J).

3.6 Research process

3.6.1 Clinical Process

3.6.1.1 Controlled Ovarian Hyperstimulation

Controlled ovarian hyperstimulation is the first step to ART and is a technique that is used to achieve the growth of multiple follicles to attain the maximum number of oocytes per menstrual cycle (Sighinolfi, Sunkara and La Marca 2018:490). There are different types of ovarian hyperstimulation protocols, all developed to initiate and support the development of several follicles and cater to various types of patients (Alper and Fauser 2017:346). According to the ESHRE guideline group on ovarian stimulation, based on safety and efficacy measures, the GnRH-antagonist protocol (Figure 8) is the most used and is preferred for women with normal ovarian reserve levels (The ESHRE Guideline group on Ovarian Stimulation *et al.* 2020:5).

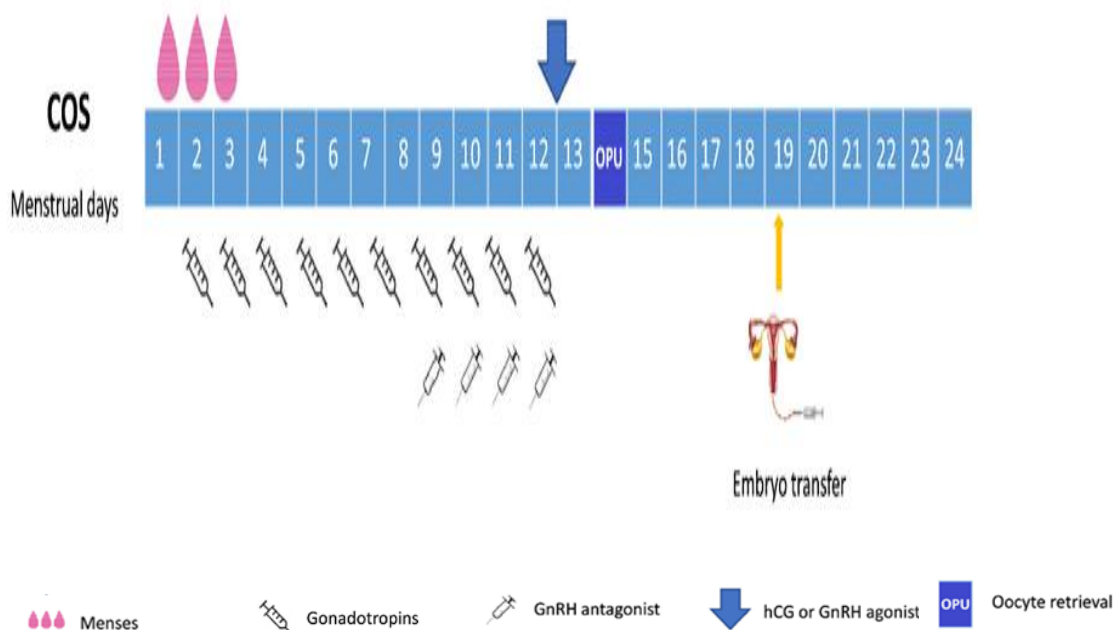


Figure 8: Image depicting Controlled Ovarian Hyperstimulation using the GnRH-antagonist protocol (La Marca and Capuzzo 2019:322). (COS: Controlled Ovarian stimulation, OPU: Oocyte Pick-Up, GnRH: Gonadotropin Releasing Hormone and hCG: Human Chorionic Gonadotropin).

3.6.1.2 Ovarian stimulation protocol:

Gonadotropin Releasing Hormone (GnRH)- Antagonist (using Cetrotide 0.25mg as the antagonist). This sufficiently subdues the pituitary at the start of the menstrual cycle and inhibits early ovulation- the release of the egg from the follicles (Sauerbrun-Cutler *et al.* 2020:1662).

3.6.1.3 Exogenous Gonadotropins for follicular growth

Specific exogenous gonadotropins are preferred over others for certain patient characteristics:

- below 35 years, high ovarian reserve, and high response to stimulation- Gonal-F.
- 35 years and older based on low ovarian reserve and slow OS response- Menopur/Menopur-premix.
- 35 years and older with low ovarian reserve requiring a higher dose of gonadotropins- Pergoveris.

(FemiCare Fertility Clinic laboratory protocol, 2018).

Multi-follicular growth was stimulated by the daily administration of 1 of the following subcutaneous gonadotropin injections (Table 3. a, and 3. b):

- Gonal-F®- 300IU, 450IU, 900IU (Recombinant FSH- rFSH: Follitropin alpha, pre-filled pen at 300IU/0.5ml, 450IU/0.75ml, 900IU/1.5ml. MERCK, N19Z5202F).

- Menopur® 75IU ampoules- (Menotropin with 75IU FSH and 75IU LH. Ferring (Pty) Ltd. A39/21.10/0357)
- Menopur® pre-mix 1200IU (Highly purified menotropin- human menopausal gonadotropin, hMG. 1200IU FSH and 1200IU LH. Ferring (Pty) Ltd. A44/21.10/0383).
- Pergoveris®- 150IU/75IU (rFSH- 150IU Follitropin alfa, and rLH- 75IU Lutropin alfa. MERCK, N7545201F).

Ovarian hyperstimulation using subcutaneous gonadotropins (Table 3) was initiated from day 2/3 of the menstrual cycle (in the follicular stage). The initial dose (ranging between 150IU-300IU) and type of stimulation medication were selected based on age, hormone profiling, antral follicle count (AFC) measurement, BMI, and clinical history.

Table 3. a: Example of patient injection timetable (FemiCare Fertility Clinic laboratory protocol, 2019).

Date	Day of the cycle	06h00-08h00	16h00-18h00	Trigger Injection	Ultrasound scan
	Day 1		Menopur Subcutaneous Powder+ water	Lucrin®/Ovitrelle®	
	Day 2				
	Day 3		3+1		1 st
	Day 4		3+1		
	Day 5		3+1		
	Day 6		3+1		
	Day 7		3+1		
	Day 8		2+1		2 nd
	Day 9	Cetrotide	2+1		
	Day 10	Cetrotide	2+1		
	Day 11	Cetrotide		Trigger @36 hours before TVOA	
	Day 12				
	Day 13	TVOA			
	Day 14				
	Day 15				

Table 3. b

Date	Day of the cycle	06h00-08h00	16h00-18h00	Trigger Injection	Ultrasound scan
	Day 1		Gonal-F Subcutaneous	Lucrin®/Ovitrelle®	
	Day 2				
	Day 3		225IU		1 st
	Day 4		225IU		

	Day 5		225IU		
	Day 6		225IU		
	Day 7		150IU		
	Day 8		150IU		2 nd
	Day 9	Cetrotide	150IU		
	Day 10	Cetrotide	150IU		
	Day 11	Cetrotide		Trigger hours TVOA @36 before	
	Day 12				
	Day 13	TVOA			
	Day 14				
	Day 15				

Follicular monitoring was initially performed on day 3 and successively on day 8 of the ovarian stimulation cycle. This process entailed the use of transvaginal ultrasonography to evaluate the number and size of growing follicles.

When the largest follicle reached ≥ 14 mm in diameter size, the patient was required to administer a GnRH-antagonist: Cetrotide (0.25 mg), daily at 08:00 am, occurring around day 9 of the cycle.

At around day 12 of the cycle, the patient had to administer an ovulation trigger to induce ovulation and provide final oocyte maturation. Two types of ovulation trigger injections were used: Ovitrelle or Lucrin. A patient had to administer one or both (dual trigger) ovulation trigger injections. Selection of the ovulation trigger was based on the ovarian response to stimulation, patient age and at the clinician's discretion.

The Transvaginal Oocyte Aspiration (TVOA) procedure was performed 36 hours post-ovulation trigger injection administration in the ART facility's procedure room by the clinician.

3.6.1.4 Ovulation triggering

An ovulation trigger was given to the patient 36 hours before the transvaginal oocyte aspiration (TVOA) procedure when at least 3 or more follicles measured 17-18mm in mean diameter. The trigger injection prompts final oocyte maturation and assists in developing the corpus luteum, which provides hormones required for endometrial growth to prepare for embryo implantation (Don *et al.* 2022:2). The patients received one of the following ovulation trigger injections.

Types of Ovulation Triggers:

Lucrin ® injection (Leuprolide acetate 5mg/ml, AbbVie (Pty) Ltd, 84904TB39).

Ovitrelle® injection-recombinant human chorionic gonadotropin-rhCG (250µg/0.5ml-6500IU Choriogonadotropin alfa, MERCK, N54G5203E).

Lucrin® was given at a dose of either 40IU or 80IU, and Ovitrelle® was standardly given at 250mcg. The type and dose of the trigger were selected based on follicular growth- the number and size of follicles.

To prevent the oocytes from being released too early from their follicular encapsulation in the ovary (premature ovulation), the patient, days before (from day 8/9 of the menstrual cycle) administering the ovulation trigger injection, was required to take an antagonist injection of Cetrotide 0.25mg powder and solvent.

3.6.1.5 Ovarian Stimulation Monitoring

Approximately 2-3 transvaginal ultrasound-guided scans (using the SonoAce X8 ultrasound machine from Samsung) were performed by the attending physician. The number of times a patient had to return for ovulation monitoring was at the clinician's discretion and was based on the rate of follicular growth. However, the most common scans were the first scan, which was done on day 2 or 3 of the menstrual cycle to assess the ovarian environment and measure the AFC, the results assisted with the selection, timing, and dosing of OH medication. The second transvaginal ultrasound scan was done around day 8 of the stimulation cycle to assess follicular development (size and number of growing follicles) in response to ovarian hyperstimulation (Figure 9) and for medication dose adjustments if there was a need. The final scan was done between days 10,11, and 12 to determine when the ovulation trigger injection would be given, and the patient was prepared for the TVOA procedure. The sizes of all the follicles were measured and noted in the patient's file at every consultation.

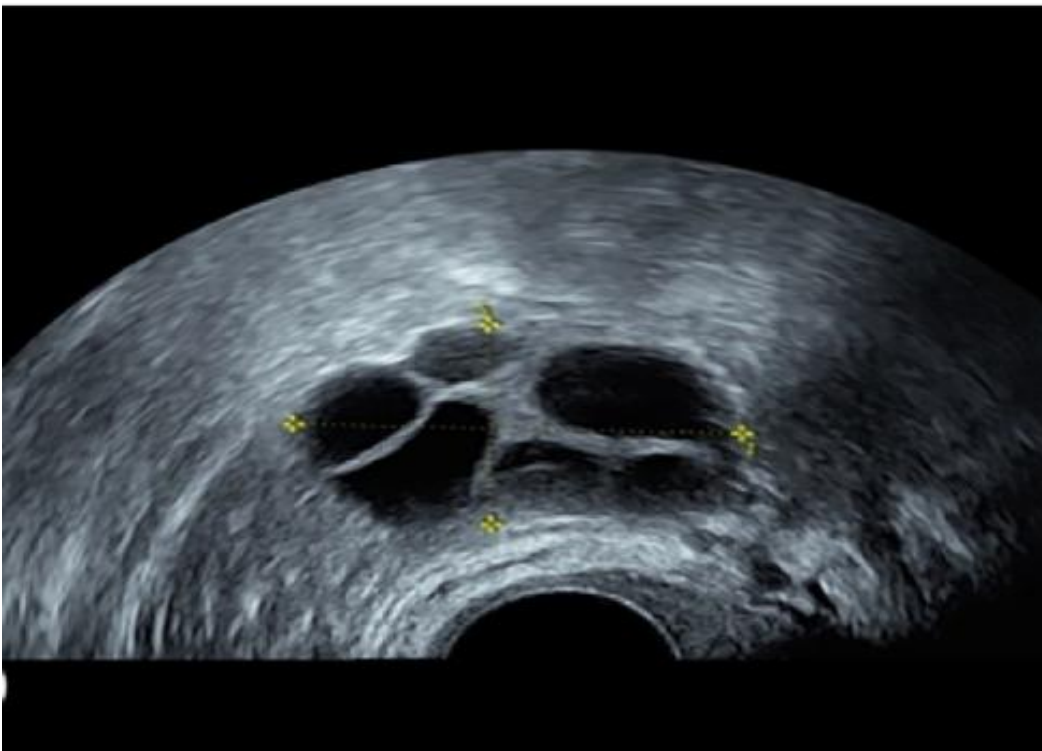


Figure 9: Sonographic image portraying multi-follicular growth in the ovaries, often observed during ovarian stimulation monitoring (Liang *et al.* 2020:3128).

3.6.2 Laboratory Process

After the clinician notified the patient when the ovulation trigger would be administered, the process that followed is depicted in Figure 10. The patient had a procedure called

Transvaginal Oocyte Aspiration (TVOA), where the clinician aspirated all the oocytes from the accurately grown follicles using an oocyte recovery needle. These oocytes were aspirated within a fluid called follicular fluid. The follicular fluid was collected into tubes and handed over to the embryologist, who would then search for, gather, and store all the oocytes for clinical use- insemination. The embryologist was the principal researcher. Insemination was done using the ICSI technique. The selection of an insemination technique is usually based on the quality of the semen sample, diagnosis of male factor infertility or previous fertilisation failure with conventional IVF (Sauerbrun-Cutler *et al.* 2020:1661). In this study, all the male partners had normal semen parameters, so the insemination method was chosen at the clinic's discretion and not based on the criteria for ICSI use nor because the ICSI method yielded better IVF outcomes than the traditional IVF technique (Sciorio and Fleming 2023:3). Following insemination, the oocytes-zygotes, were then cultured in single-drops culture media (covered in oil) in a benchtop incubator until the embryos had developed and were ready to be transferred into the recipient female or cryopreserved.

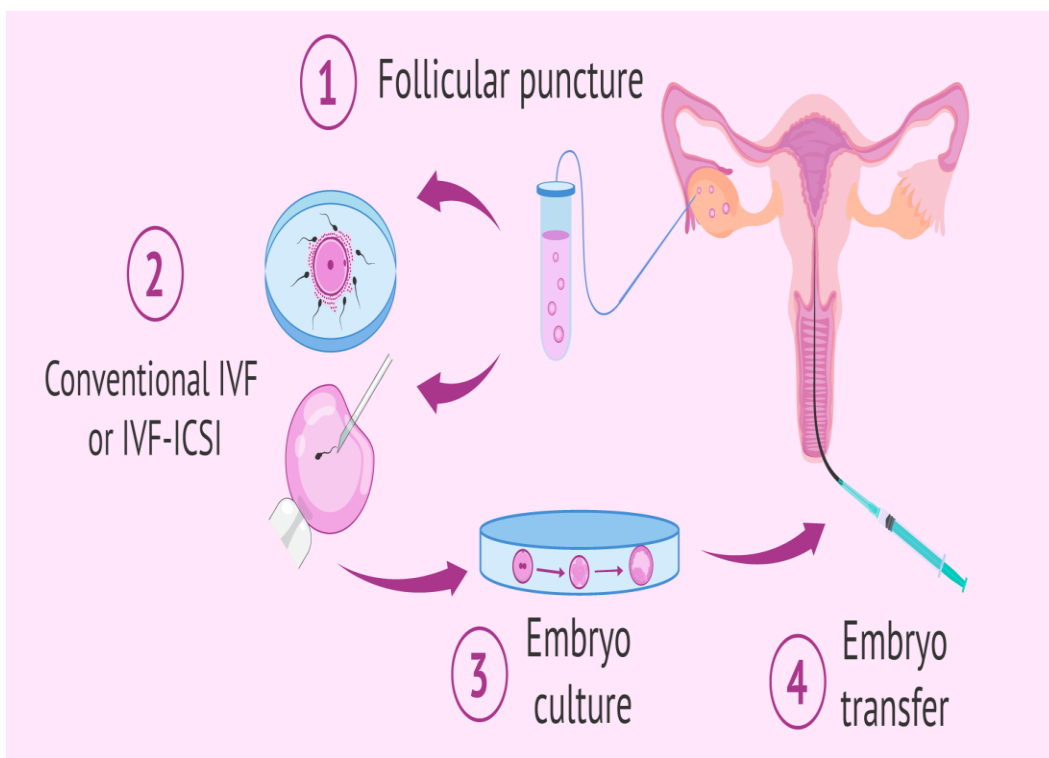


Figure 10: Image describing the laboratory process that follows ovarian stimulation, from oocyte aspiration-oocyte insemination-embryo culture-embryo transfer (Rodrigo *et al.* 2022).

3.6.2.1 Transvaginal Oocyte Aspiration (TVOA)

Materials:

- Follicle puncture needle 17G 35cm (Birr Biosciences B.V. #220762, Netherlands).
- Disposable (12ml) culture tubes (Ps) with dual-position cap printing graduation (IVF Gen #GEN82341, Netherlands).
- Individually wrapped (3ml) Pasteur pipettes (Labchem #18433, Netherlands).
- K System (R65) Warming table (CooperSurgical Inc., United States of America).

- Sterile gynaecological theatre pack (Femina Women's Hospital, Pretoria).
- SONOACE X8 Ultrasound machine (Samsung), abdominal/vaginal probe, and ultrasound guide (Samsung).
- Pre-warmed GMOPS (Vitrolife #510841, Sweden) “flushing media” aliquoted into a 12ml disposable culture tube (warmed at 37°C overnight).

Method:

The TVOA procedure was conducted at approximately 36 hours post-administration of the ovulation trigger, under conscious sedation. The procedure was done in a small theatre room at the clinic under transvaginal ultrasound guidance.

While the anaesthetist administered the anaesthesia, the doctor and nurse sterilised their hands by doing a thorough hand wash and started preparing for the procedure.

Once the patient was fully sedated, the doctor used the pre-warmed materials (cotton swabs, sterile water, swab holding forceps, vaginal speculum) in the sterile gynaecological theatre pack to clean in and around the vaginal area of the patient. As an additional measure to prevent and treat pain resulting from the procedure, the doctor injected the patient around the cervix with a local anaesthesia called Lignocaine HCl Fresenius.

At the start of the procedure, to maintain sterility and have better vision, the doctor put a condom with ultrasound gel at the tip and then put the condom on the probe. The doctor then attached an ultrasound guide to the covered probe and inserted the probe into the vagina and first started with a short assessment to locate all the follicles on either side of the ovaries.

Once the follicles had been located on both ovaries, the follicle puncture needle 17G 35cm, which was connected to a foot pump on the K system (R65) warming table, was then inserted into the ultrasound guide attached to the probe. The nurse and doctor first flushed and “rinsed” the needle with an equilibrated GMOPS medium; this was done to prime the needle for easy fluid flow through it. The doctor then proceeded to puncture and aspirate fluid from all visible and accessible follicles. The procedure is done in a warm, dark theatre room to try and minimise the negative effects of light on the sensitive oocytes.

Inside the K system (R65) warming table, there were pre-warmed empty 12 ml disposable culture tubes. The nurse used these disposable culture tubes for the collection of all the follicular fluid aspirated from all visible and accessible follicles, typically measuring equal to or greater than 10mm in size. For the duration of the procedure, once the doctor had punctured and aspirated follicular fluid from the follicles, the tubes containing follicular fluid were all put into a warm tube rack. The tube rack had to be warm to prevent unnecessary stress on the oocytes, which would result in damaging defects. All the tubes and their contents were handed over to the embryologist (the principal researcher) in the adjacent room.

N.B. The entire process of conception *in vivo* happens in a dark space with no exposure to light, and the temperature is constantly regulated by the body to suit the process; therefore, *in vitro* culture strives to ensure the oocytes and embryos are exposed to a similar *in vivo* environment. The wavelength, intensity and duration of light exposure can cause cellular damage in eggs and embryos, leading to lower fertilisation, development and blastulation rates (Khodavirdilou *et al.* 2021:3161). Temperature control is important because

temperature variations outside of the normal range disrupt the process of meiosis, disrupt spindle formation, destroy the cell structure, leading to cell (oocyte) death, and disrupt normal cell gene expression (Khan *et al.* 2020:7).

(Reference: Revised ESHRE guidelines for good practice in IVF laboratories 2015: n.p).

3.6.2.2 Laboratory analysis

Materials:

- Disposable (12ml) culture tubes (Ps) with dual-position cap printing graduation (IVF GEN #GEN82341, Netherlands).
- Individually wrapped (3ml) Pasteur pipettes (Labchem #18433, Netherlands).
- IVF Pasteur pipets 5,75" (14.6cm) (Origio 272866, Denmark).
- FALCON® Tissue culture dish 100x20mm (FALCON® #2041003, United States of America).
- Oosafe® 35mm dish, High wall (SparMED APS #07993, Denmark).
- Culture dish (5-Well) (Vitrolife 13444, Sweden).
- Oosafe® 60mm Dish (SparMED ApS #08181, Denmark).
- Pre-warmed GMOPS (Vitrolife #510841, Sweden) "flushing media" aliquoted into a 12ml disposable culture tube (warmed at 37°C overnight).
- GMOPS plus (Vitrolife #510982, Sweden).
- Ovoil (Vitrolife #510748, Sweden).
- G-IVF plus (Vitrolife #510742, Sweden).
- Universal IVF medium (Origio #009903, Denmark).
- G-TL (Vitrolife #511288, Sweden)
- K System IVF workstation L426 (CooperSurgical Inc., Denmark).

Method

Preparation- the day before the TVOA procedure:

Media equilibration and dish prep

Per patient, an Oosafe® 35mm dish was prepared with 3ml G-IVF plus medium layered with 1ml Ovoil and stored in a gassed incubator at 37°C overnight for the media to equilibrate (Origio Planer BT 37-IFU bench-top incubators with premixed gases including a combination of 7.26-7.28% CO₂, 4.92-4.97% O₂ and the balance is Nitrogen). The incubators are set at specific levels of CO₂, O₂ and nitrogen to maintain a good pH balance ranging between 7.27-7.34 (Esbert *et al.* 2024:3).

A second dish: A 5 Well culture dish was prepared by aliquoting 1ml of G-IVF plus into the first top two wells of the dish and covering with approximately 0.5ml Ovoil. In between the wells, 3ml of Universal IVF medium was poured to maintain humidity. This dish was then stored in a gassed incubator at 37°C overnight for the media to equilibrate (Origio Planer BT 37-IFU bench-top incubators with premixed gases including a combination of 7.26-7.28% CO₂, 4.92-4.97% O₂, and the balance is Nitrogen).

Three tubes were also prepared, and the media were equilibrated for the following functions:

- G-MOPS (6ml per patient) media for flushing the follicle puncture and aspiration needle.

- G-MOPS plus (7ml per patient) handling media for handling and manipulating oocytes and embryos at ambient temperature.
- Ovoil (2ml per patient) to cover the medium during oocyte screening and denudation.

N.B. All the dishes were labelled with the patient's surname and initials for clear identification.

On the day of the TVOA procedure- Day 0

Once the embryologist (the principal researcher) received the tubes containing follicular fluid, they poured each tube's contents sequentially into a 100x20mm Falcon dish (Figure 11). In the K-Systems IVF workstation, the embryologist examined the follicular fluid for oocytes by individually microscopically screening for oocytes in all the tubes they had been given.

Once located, the cumulus-surrounded oocytes were then temporarily removed ("rinsed"), using a 5,75" (14.6cm) IVF Pasteur pipette, from the follicular fluid into flushing medium GMOPS plus in a 35mm dish overlaid with Ovoil.

Once all the tubes were screened, all the harvested oocytes were then transferred into the pre-prepared 35mm Oosafe dish with G-IVF plus medium and placed back into the gassed incubators at 37°C for at least 2 hours, awaiting denudation and subsequent insemination.

(Reference: Revised ESHRE guidelines for good practice in IVF laboratories. 2015: n.p).

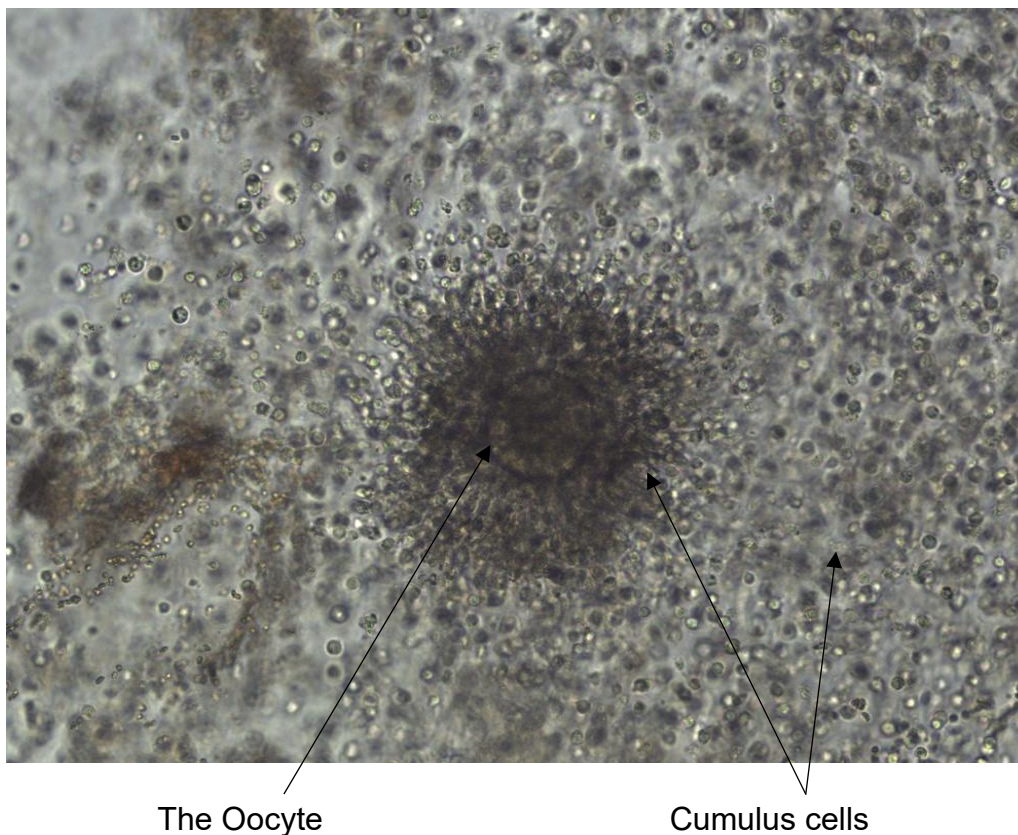


Figure 11: Photomicrograph depicting an enclosed cumulus-oocyte-complex in follicular fluid in a screening dish during the oocyte aspiration procedure. (FemiCare Fertility Clinic

2023). (Photomicrograph taken under 20X objective using an inverted microscope. Dimensions: 1400x928. Actual image size: Height- 9.9cm and width- 13cm).

3.6.2.3 Denudation and Insemination

Materials

- Individually wrapped Pasteur pipettes- 3ml (Labchem #18433, Netherlands).
- Culture dishes- 5 Well (Vitrolife #13444, Sweden).
- IVF Pasteur pipette- 5,75" (14.6cm) (Origio #272866, Denmark).
- Hand stripper pipette (The Stripper®, United States of America).
- Stripper tips (135µm, United States of America).
- GMOPS plus (Vitrolife #510982, Sweden).
- Hyaluronidase 80U/mL SAGE™ (Cooper Surgical Inc #220617-007326, United States of America).

3.6.2.3.1 Oocyte denudation

Oocyte denudation was done after 2 hours of incubation following the TVOA procedure. The incubation period after the TVOA is provided to allow some oocytes that did not mature *in vivo* to mature (Speyer *et al.* 2019:648). Denudation is the process of removing the cumulus-oocyte-complex (COC) that surrounds the oocyte, for the oocyte to be completely exposed, to allow the evaluation of the oocyte's quality and nuclear maturation status. Denudation on day zero is a prerequisite for ICSI-inseminated oocytes or oocytes that will be cryopreserved.

In a Vitrolife 5-Well culture dish, oocyte denudation (in one well of the 5-well culture dish) was achieved by enzymatically exposing the cumulus intact oocytes for a short period to a 100µL hyaluronidase solution (80 U/ml in HEPES-HTF with 5mg/ml HAS, SAGE™ USA) that was diluted with 100µL handling media under oil. The oocytes were then mechanically pipetted gradually through a 135µm stripper tip attached to a hand stripper pipette. Once the cumulus cells had detached from around the oocytes, the oocytes were then sequentially rinsed in a series (2-3) of 1ml handling media aliquots covered in oil in the other 4 wells of the Vitrolife 5-Well culture dish. This was done to remove the excess hyaluronidase solution.

All the oocytes were then transferred into the pre-warmed Vitrolife 5-Well culture dish with G-IVF plus fertilisation medium and put into the gassed incubator, awaiting insemination.

(FemiCare Fertility Clinic laboratory protocol, 2018).

3.6.2.3.2 Oocyte maturation assessment

One of the ways to assess oocyte quality and maturation is to use the non-invasive route of morphologically analysing the oocyte's cellular components and immediate surroundings (Figure 12. a and Figure 12. b) (Lemseffer *et al.* 2022:1). Important factors to observe during oocyte morphological assessments include cytoplasm appearance, first polar body extrusion, the zona pellucida (ZP) thickness, and the size of the perivitelline space (PVS) (Pandit and Sharma 2022:9).

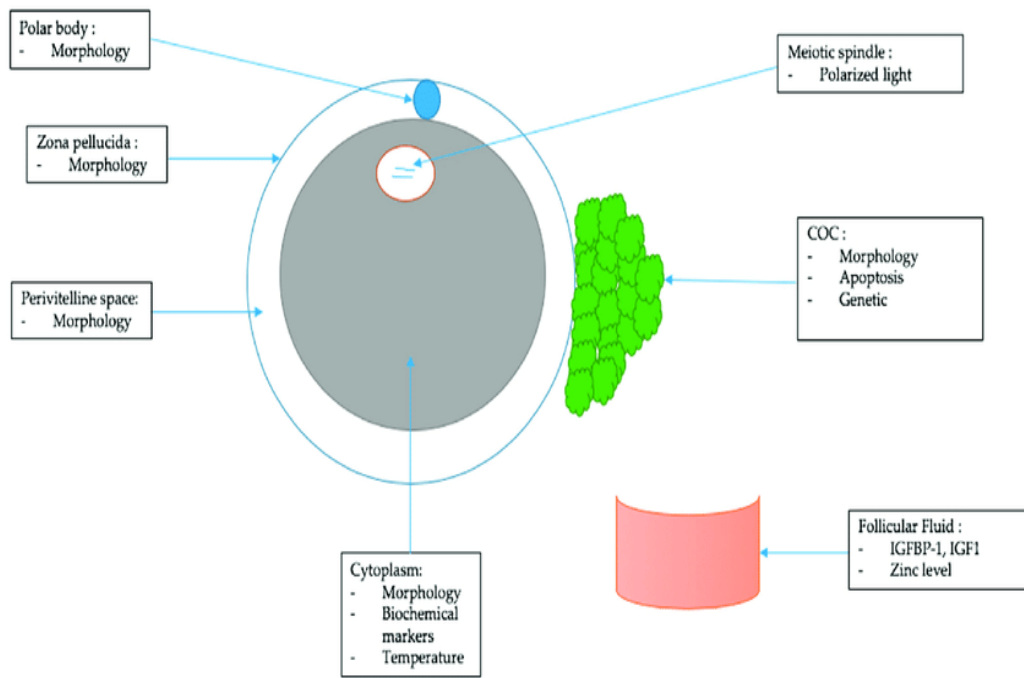
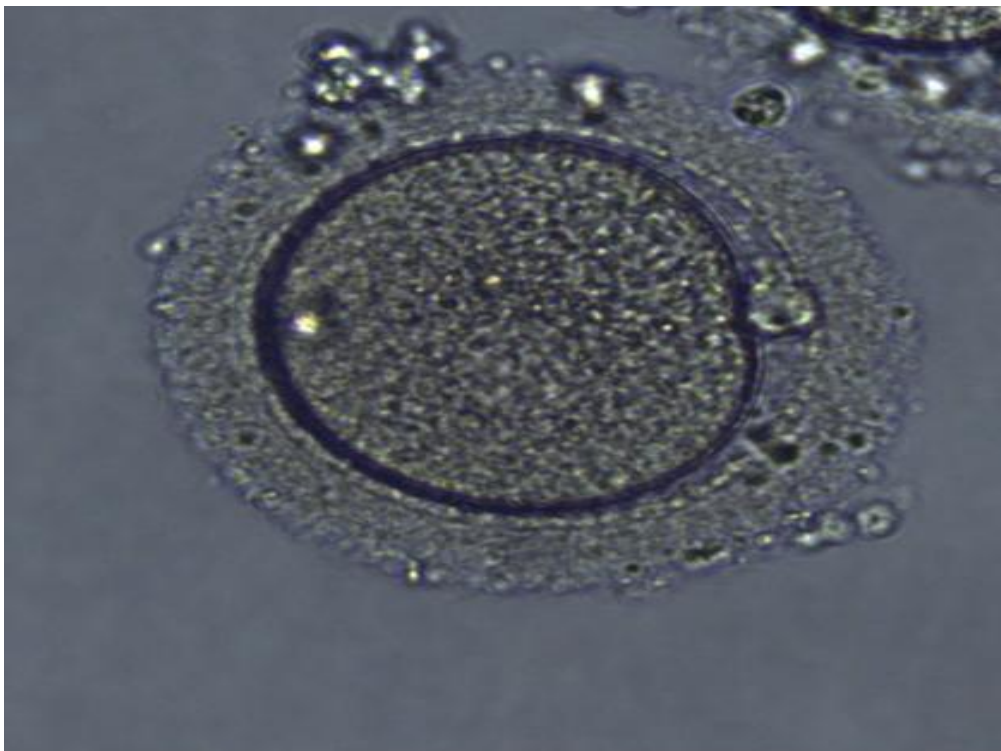


Figure 12. a: Criteria used to assess oocyte quality as presented in a study by Lemseffer *et al.* (2022:1).



III OOCYTE (FEMICARE FERTILITY)

Figure 12. b: Oocyte Photomicrograph depicting Lemseffer *et al.* (2022:1) oocyte quality assessment characteristics (FemiCare Fertility Clinic 2023). (Taken under 20X objective using an inverted microscope. Dimensions: 1400x928. Actual image size: Height- 9.9cm and width- 13cm).

Under an inverted microscope (Zeiss- Stemi 508), the oocyte morphology and nuclear maturity (Figure 13) were assessed and typically fell under one of the following categories:

- Germinal Vesicle (GV) oocyte: Organelles are condensed centrally within the cytoplasm, so there is a germinal vesicle sack visible, and the first polar body (PB) is absent.
- Metaphase I (MI) oocyte: No germinal vesicle and no polar body (PB) are visible in the perivitelline space.
- Metaphase II (MII): Exhibits the first extruded polar body (PB), has uniform cytoplasmic material, adequate zona thickness, and normal size perivitelline space.

(Ali-Abed, Abbod and Ali Jwad 2020:7128-7129).

In every cohort of eggs, not all will fall perfectly into either one of the above categories; some eggs will be abnormal or degenerated. Abnormal oocytes describe oocytes that have a thicker (>25 microns) or thinner (<17-18 microns) zona pellucida, are overly bigger in mean diameter (>120 microns) than what is considered normal, will have larger than normal-sized or fragmented polar bodies, have vacuoles or many smooth endoplasmic reticula (SERC), or the ooplasm will be darker or not be uniform and be granular (Ali-Abed, Abbod and Ali Jwad *et al.* 2020:7129 and REVIVA Fertility and IVF clinic 2020-23). None of these types of oocytes were inseminated nor included in the study analysis.

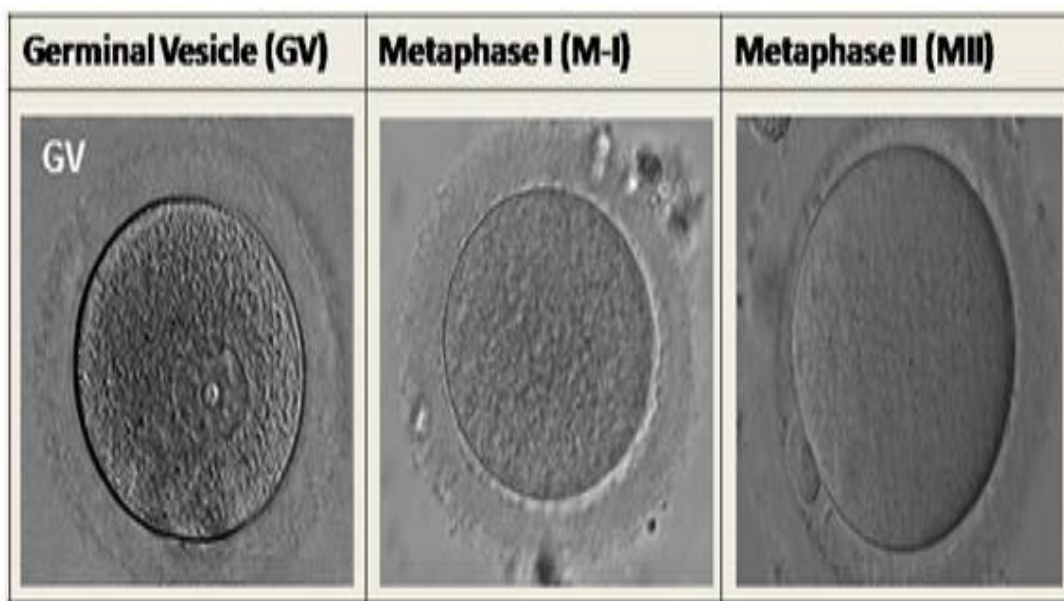


Figure 13: Photomicrograph of Oocytes at different stages of maturity (REVIVA Fertility and IVF clinic 2020-23).

All the immature eggs (GV+ MI) were co-cultured in one well of the Vitrolife 5-well culture dish with equilibrated G-IVF plus medium layered with Ovoil prepared the previous day. All the mature oocytes were also group cultured in a separate well, prepared the same way. The immature oocytes were evaluated between 6 hours and approximately 24 hours post-TVOA procedure to allow possible *in vitro* maturation (Escrich *et al.* 2018:638).

The mature (MII) oocytes on day 0 were either cryopreserved or inseminated at the patient's and doctor's discretion. On day 1, all the previously immature oocytes that were then mature were either cryopreserved or inseminated, also at the patient's and doctors' discretion.

3.6.2.3.3 Insemination

Sperm preparation (Figure 14) was done using the 20-minute (at 2000rpm) density gradient centrifugation sperm wash method- 1ml 40% gradient wash medium layered over 1ml 80% gradient wash medium in a conical tube and topped with 1ml of the semen sample. An additional 10-minute (at 1500rpm) wash of the resultant pellet with handling media was then carried out. Sperm preparation was done to separate the dead and non-usable sperm and other cells from the viable sperm capable of fertilising an oocyte.

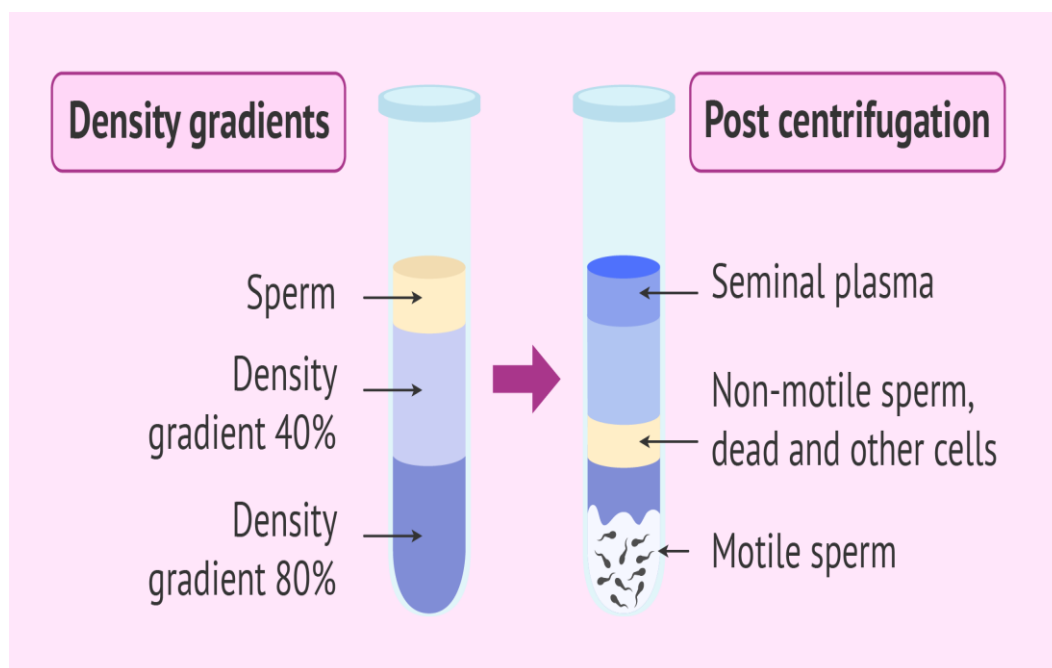


Figure 14: Sperm selection technique for IVF/ICSI (Rodrigo *et al.* 2022).

Oocyte insemination was performed after 2 hours of oocyte denudation. The insemination procedure chosen for all patients was intracytoplasmic sperm injection (ICSI). While the ICSI method is indicated for cases of poor semen parameters or previous IVF cycle failures (Balli *et al.* 2022:2), some IVF centres use this method even in non-male factor infertility-related cases, and according to Speyer *et al.* (2019:652), ICSI and conventional IVF produce comparable ART outcomes when the sperm concentration is $\geq 1 \times 10^6$. The use of the ICSI technique for oocyte insemination was not patient-related but a clinic choice.

Only the mature (MII) oocytes (day 0 or day 1) were inseminated.

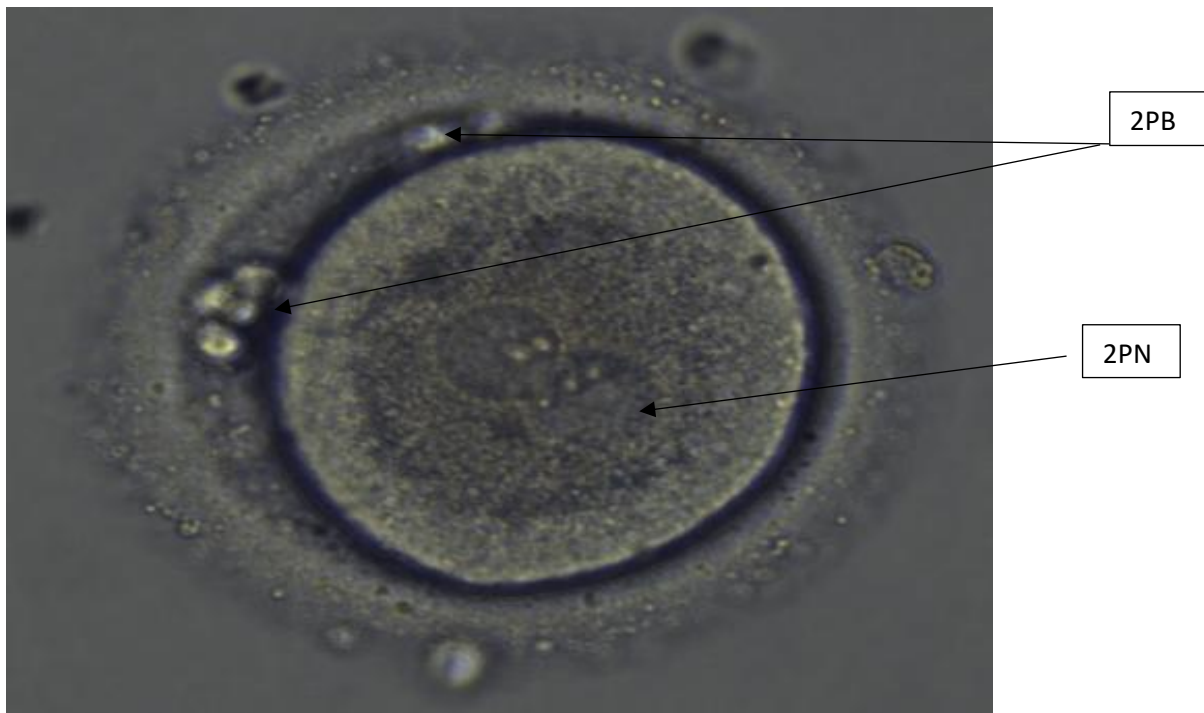
Post insemination, all the oocytes were co-cultured in one well in the Vitrolife 5-well culture dish and then placed in Origio Planer BT 37-IFU bench-top incubators with premixed gases including a combination of 7.26-7.28% CO₂, 4.92-4.97% O₂, and the balance is Nitrogen, for ± 17 hours. This allowed for the complete process of fertilisation to take place.

(FemiCare Fertility Clinic laboratory protocol, 2018).

3.6.2.4 Fertilisation and embryo development

Fertilisation assessments were done between 16-18 hours post-ICSI.

At this time, the presence of two pronuclei (PN) and two polar bodies (PB) signified normal oocyte fertilisation (Figure 15). The oocytes (zygotes) were then individually cultured in single droplets of 1-step 100µL GTL culture medium and covered with oil in an Oosafe 60mm culture dish for the next 3-5 days.



2PN-2PB ZYGOTES (FEMICARE FERTILITY)

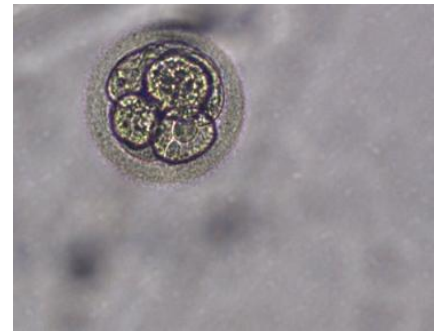
Figure 15: Fertilised oocyte (Zygote) photomicrograph showing 2 Pronuclei (2PN) and 2 Polar bodies (2PB) depicting normal fertilisation (FemiCare Fertility Clinic 2022). (Photomicrograph taken under 20X objective using an inverted microscope. Dimensions: 1400x928. Actual image size: height- 9cm, width: 13cm).

Embryo evaluation (Figure 16) and selection were performed traditionally by the embryologist only on the embryo transfer day or the day of oocyte/embryo cryopreservation for non-transfer cycles. Embryo quality (blastocyst stage) was scored according to the blastocyst grading criteria developed and described by Professor D. Gardner (Gardner and Schoolcraft 1999:385) (Appendix K).

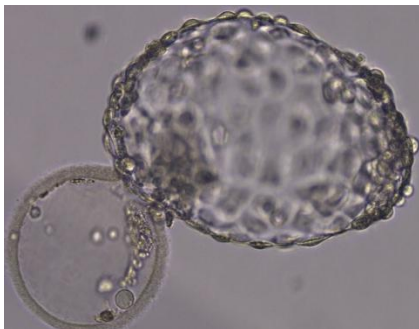
Cleavage stage (day 3) embryos were scored based on cell number, cell size, level of fragmentation, and nuclear appearance and blastocyst (day 5) embryos were scored by assessing blastocoel enlargement, and the distinction and characteristics of the inner cell mass (ICM) and trophectoderm (TE) cells (Shukla *et al.* 2022:9-14). The length of embryo culture and subsequent day of embryo transfer and/or cryopreservation at the cleavage stage (day 3) or blastocyst stage (day 5) was determined by the doctor and the embryologist. This was based on the number of oocytes that showed signs of fertilisation, the day of the week the embryo transfer would fall on, and previous cycle outcomes.



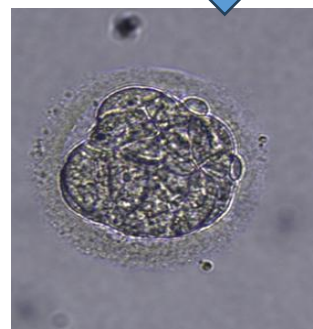
1: 2-CELL (FEMICARE FERTILITY)



2: 6-8-CELL (FEMICARE FERTILITY)



4: BLASTOCYST (FEMICARE FERTILITY)



3: MORULA (FEMICARE FERTILITY)

Figure 16: Photomicrograph of embryo developmental stages from the 2-cell stage to the blastocyst stage (FemiCare Fertility Clinic 2021). (Photomicrographs taken under 20X objective using an inverted microscope. Dimensions: 1400x928. Actual image size: height-4.3cm and width- 5.5cm).

3.7 Statistical Analysis

The raw data analysis and interpretation of the results were done with the assistance of the institutional biostatistician (Appendix O).

3.7.1 Software and statistical representation

The statistical significance of the data was assessed and evaluated using the IBM SPSS version 19.

The study statistics were presented as:

1. Descriptive statistics

Counts and proportions (expressed in percentages) were used to present categorical variables. Minimum, maximum, mean and standard deviation values were used to describe the normally distributed measured data. The results were presented in tabular and/or graphical formats.

2. Inferential statistics

Independent- Samples Wilcoxon Mann-Whitney U test was used to describe the frequency distribution of patients' oocyte cohorts, focusing on oocyte retrieval rates, mature-MII

oocytes, immature-MI oocytes and immature-GV oocytes. This test was also used to assess the population's follicular growth and R-IVM oocytes.

Independent-Samples Median test was used to test whether the means of metaphase 2, metaphase 1 and germinal vesicle oocytes were equal. Essentially, this test was used to evaluate the ratio of mature to immature oocyte levels between the two groups by testing the means of the oocytes.

An independent samples T-test was used to test the association between different exogenous gonadotropins used for ovarian stimulation, ovulation triggers, age and BMI on follicle number, oocyte retrieval rates and MII oocytes. In addition, this test also measured the association between female age and AMH and analysed the fertilisation rates between the HIV-infected and HIV-uninfected women, by testing the equality of means.

Pearson correlation coefficient was used to measure the strength of the relationship between AMH, follicle number and MII oocyte number. A correlation coefficient can take on a value between -1 and +1. It is +1 if there is a perfect positive correlation and -1 if there is a perfect negative correlation. The correlation is significant if the associated p-value is <0.05.

The Fisher-Freeman-Halton Exact test was used to assess the distribution of patients' oocyte cohorts, after matching patients by age. Emphasis was placed on oocyte retrieval rates and mature-MII oocyte rates among the entire population and within each group.

The interpretation of results was performed at a 95% confidence limit (2-sided).

A P-value less than 0.05 on two-sided tests was accepted as statistically significant.

3.7.2 Ethical considerations

The facility where this research was conducted is part of a hospital and as part of the newly revised hospital protocol post COVID-19, all the patients entering the hospital for any length of stay are required to complete an assessment and go for SARS-CoV-2 testing (~48 hours before their stay) at Lancet laboratories and would only be allowed entry upon receipt of a negative result (Appendix L). This precautionary measure is supported by and stems from the ESHRE guidelines recommendation that all patients should undergo a preliminary assessment two weeks before initiating ovarian stimulation to assess the risk of infection with SARS-CoV-2 (The ESHRE COVID-19 Working Group *et al.* 2021:277). This is achieved by COVID-19 testing with a negative result and completing a questionnaire relating to the patient's current health status (Appendix M).

Patients were selected from a group of women attending a fertility clinic and were selected into the study based on the inclusion and exclusion criteria without bias. All patients, as part of the facility's rules and recommendations, were required to undergo at least two mandatory sessions with a psychologist to mentally prepare the patient for the entire process and address patient expectations and possible realistic outcomes. Gatekeeper permission was requested and obtained from the patient's physician and facility management to approach patients to partake in this research study (Appendix A and Appendix B), and the request to attain permission to conduct the study was submitted to a professional ethics board- The Institutional Research Ethics Committee (IREC). Full ethics approval was attained for the study design before initiating the study- IREC 138/22 (Appendix C).

No additional procedure(s) were done specifically for the purpose of the study; all data were collected from IVF cycles that the patients intended to undergo when attending the clinic. Therefore, no harm to patient health or gametes was inflicted during the period of the study. Furthermore, no emotional, financial, or psychological strains were imposed on the patients.

It was made known to patients that this study is voluntary, neither the patient nor the researcher would obtain any monetary gains, and that the patient could at any point withdraw consent without compromising their ART success.

3.7.3 Confidentiality and the POPI Act

Concerning the POPI Act and overall patient information confidentiality requirements (The Presidency of the Republic of South Africa 2013:2), during the period of this study, a strict standard of patient anonymity was maintained. All the participants were assigned a special file number not relating to any of their personal information. No patient-identifying data was put on any of the data collection sheets or questionnaires. All the participants were recruited directly by the principal researcher, and only the researcher knew the patients' identities. All patient records are kept confidential in electronic file versions in a secure server that only the clinician has access to, and during the study, the researcher was allowed access to. The participant was only awarded the title when they had provided informed written consent.

3.7.4 Storage of records

Generally, all patients' data are stored in an electronic file on a secure server at the ART facility. All raw data (documents) collected for this study will be discarded in an appropriate manner that will continue to maintain strict patient confidentiality. Hard copy documents and all other paper trails collected and used during the study were kept in a locked storage cabinet, and data collected electronically was kept in a USB that was also kept in a secure (lockable) cupboard when not in use. Upon completion and submission of the research dissertation, all the study-related data on paper will be kept for five years as per institutional regulations, and thereafter shredded. Furthermore, all data on the study stored in the USB device will also be deleted, and any backup devices will be formatted at the end of the 5 years as stipulated by the university through which this study was conducted.

3.8 Conclusion

Selecting the quantitative research approach was ideal for this study, as this allowed the comparison of measured variables to best conduct this study's investigation. Even though the use of a single centre is limiting, the strength of this study is that it is prospective and therefore creates a good basis on which other studies could potentially develop. The methods, materials, and techniques described in this chapter were sufficient and reliable in providing sound evidence-based data for utilization in accomplishing the aim and objectives of this research study. In this chapter, all established guidelines and procedures were presented accordingly, and certain rules, procedures, and protocols were considered and followed, especially since human specimen samples were handled and described. Lastly, this chapter outlined in detail the entire process of the procedure the participants underwent to procure the raw data, pointed out the roles of every individual that was involved in the research process, described the population type and setting where the research study was

conducted, described the gathering and analysis process of the raw data, highlighted some of the expected outcomes that were directly interrelated to the overall aims and objectives and took into account the ethical considerations and participant confidentiality methods that are observed when research studies are conducted.

CHAPTER 4

RESULTS

4.1 Introduction, aim and objectives.

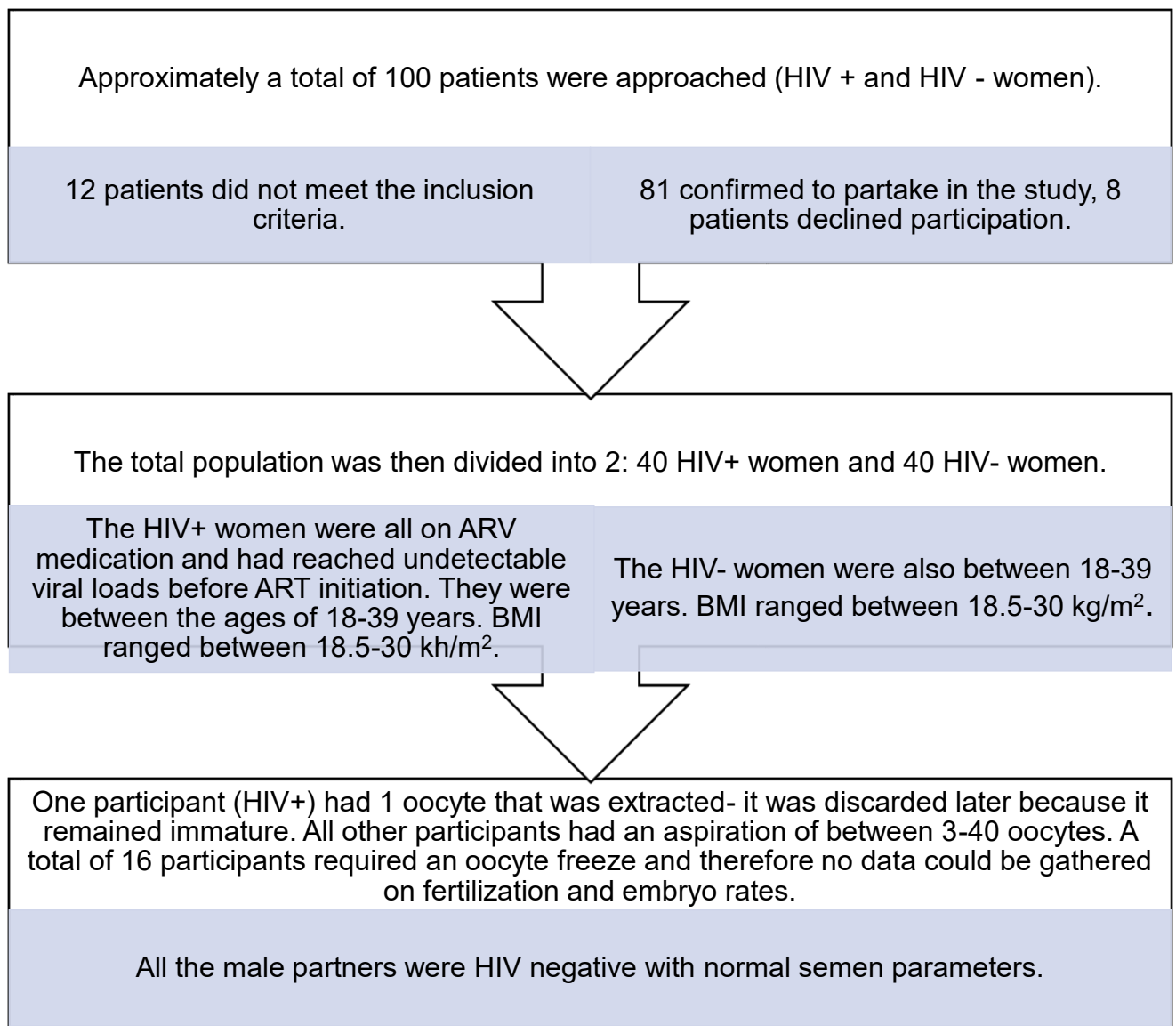
In this study, the researcher aimed to provide a comparative analysis of mature and immature oocyte yields in oocyte cohorts obtained from HIV-infected females against HIV-uninfected women of reproductive age, post-controlled ovarian hyperstimulation. The aim was to observe if oocyte maturation, oocyte retrieval rates and follicular growth post-COH may be impacted by the HIV infection, HIV associated and patient-related factors, thus promoting infertility. To achieve this, oocyte cohorts from a group of HIV seropositive women and HIV non-infected controls in their childbearing years and attending an ART clinic were evaluated, and their COH-ICSI cycle outcomes were recorded. The raw data for the study were mostly collected from electronic patient files at the clinic. A file is created for every patient when they start attending the clinic for infertility or gynaecological interventions. The researcher, the clinical embryologist, conducted the laboratory procedures, recorded all the results as part of normal clinic practice, and gathered the data for utilisation in this research study only from the patients who submitted signed consent forms. Statistical analysis and interpretations were done with the help of a certified biostatistician provided by the institution.

Research Aim and Objective:

This study aimed to provide a comparative analysis of the mature and immature oocyte yields in oocyte cohorts obtained from HIV-infected females against HIV-uninfected women of reproductive age, post-controlled ovarian hyperstimulation (COH). Additionally, the study examined the potential influence of factors associated with HIV infection, infertility, and ART, such as age, BMI, viral load, CD4+ lymphocyte counts, antiretroviral medication intake, ovarian stimulation protocols, and ovulation trigger injections on oocyte maturation outcomes.

4.2 Study population and sample size

This study included a total of 80 patients undergoing IVF, the test group- HIV-infected females (n=40) and the control group- HIV uninfected females (n=40) who met the inclusion criteria. The population's baseline characteristics and reproductive outcome data are presented in Table 4. A total of 16 patients who were included in the study opted for fertility cryopreservation; therefore, their oocyte fertilisation and embryo developmental rates were not included. In one patient in the HIV-infected group, only 1 oocyte was retrieved on the day of oocyte aspiration, and it was immature; it was given an extended 24-hour culture, and it still did not mature. This oocyte was excluded from the fertilisation and embryo development analysis. All the patients had diverse types of causes of infertility. Some of the many reasons couples seek ART assistance not presented in Table 4 include multiple female factors accounting for 10% of the HIV-infected group, recurrent miscarriages- 5% of the HIV-infected group, and genetic testing- 2,5% of the HIV uninfected group.



The male was HIV-negative and had normal semen parameters; the sample was prepared by density gradient centrifugation processing, and the oocytes were inseminated using the ICSI technique. Approximately 15% of the sperm samples used in the cycles with the women living with HIV were either donated or frozen previously due to the male partner struggling to produce the sample the usual way (by masturbation) or not being available at the time of cycle initiation, while 25% of the sperm samples used in the cycles of the HIV uninfected women were from donors or previously cryopreserved, for the same reasons as in the women living with HIV.

The statistical analyses presented data on the ratio of mature to immature oocytes in oocyte cohorts from women living with HIV compared to HIV seronegative women's oocyte cohorts, it also included the statistical analyses of the response to ovarian stimulation, age, BMI, the relationship between serum AMH, follicle number and oocyte number and fertilization and embryo development between the two groups.

Table 4: Study population baseline characteristics and reproductive outcomes table.

Criteria	Test group (HIV infected)	Control group (HIV uninfected)	p-value
Number of IVF cycles (n)	n=40	n=40	
Woman's age (years) mean ($\pm$ SD)	36.65 ($\pm$ 3.19)	34.20 ($\pm$ 2.80)	0.131
Body mass index (BMI) (kg/m ²) mean($\pm$ SD)	30.11 ($\pm$ 6.31)	28.04 ($\pm$ 5.67)	0.145
Infertility aetiology %			
Tubal	57.5%	32.5%	
Endometriosis	5%	5%	
Idiopathic	2.5%	5%	
Polycystic ovary syndrome (PCOS)	2.5%	7.5%	
Recurrent IVF failure	2.5%	2.5%	
Anatomic female factor	-	5%	
Female+ male factor	5%	-	
AMH (ng/ml) (mean)	2.77	2.73	0.92
Viral load value	undetectable	-	
CD4 Lymphocyte counts (Cells/ μ L) (mean)	749.3	-	
Gonadotropins used n (%):			0.062
Gonal-F	16 (40%)	26 (65%)	
Menopur	16 (40%)	6 (15%)	
Menopur-Premix	6 (15%)	7 (17.5%)	
Pergoveries	2 (5%)	1 (2.5%)	
Duration of stimulation (Days)			
Ovulation trigger dose n (%):			0.626
Lucrin	11 (45.8%)	13 (54.2%)	
Ovitrelle	29 (51.8%)	27 (48.2%)	
Endometrial thickness at trigger (mm) mean ($\pm$ SD)	9.73 ($\pm$ 2.68)	10.15 ($\pm$ 2.56)	
Number of follicles (mean)	10.0	10.0	0.881
Number of total oocytes retrieved (mean)	12.0	12.0	0.536
Number of MII oocytes at day 0 mean ($\pm$ SD)	9.08 ($\pm$ 7.89)	9.33 ($\pm$ 5.69)	0.264
Number of MI oocytes at day 0 mean ($\pm$ SD)	1.25 ($\pm$ 1.45)	1.28 ($\pm$ 1.74)	0.352
Number of GV oocytes at day 0 mean ($\pm$ SD)	2.55 ($\pm$ 2.94)	2.15 ($\pm$ 2.19)	0.258
Number of MII oocytes at day 1 (%)	42.11%	60.45%	
Total Fertilisation rate (%)	78.80%	76.67%	
Total Cleavage rate (%)	76.72%	74.60%	

Total Blastocyst formation rate (%)	45.42%	44.44%	
Number of good quality embryos (Day 3) (n)	n=20	n=40	
Number of good quality embryos (Day 5) (n)	n=119	n=132	
Number of transferred embryos (n)	n=30	n=24	
Number of frozen embryos (%)	67.5%	75%	
Number of oocytes cryopreserved (%)	22.5%	17.5%	
Sample type %			
Fresh ejaculate (H)	62.5%	57.5%	
Frozen ejaculate (H)	7.5%	15%	
Frozen ejaculate (D)	7.5%	10%	

Based on the results in Table 4, the mean number of follicles observed during follicular monitoring is slightly lower than the actual number of total oocytes collected. One of the reasons for this is that at the time of calculating and noting the number of follicles, only a certain size (preferably >14mm in size) of the follicles is considered appropriate for aspiration; however, occasionally, there will be follicles measuring below this acceptable size, which may be aspirated and give an oocyte.

4.3 Descriptive statistics

In the HIV-infected group, as a standard IVF clinic procedure, all patients (n=40) living with HIV were required to undergo testing of their T-cell lymphocyte reservoir. All 40 patients' CD4⁺ lymphocyte counts were above 500 cells/ μ L. The mean CD4⁺ lymphocyte count was 749.3 cells/ μ L.

One of the important outcomes of ART is acquiring a good number and quality of mature MII oocytes necessary to achieve a live birth. Figure 17 is a scatter plot of the number of total oocytes and mature (MII) oocytes on the day of harvest, obtained from HIV-infected women against HIV-uninfected women. The scatter graph shows comparable results of total oocyte retrieval rates and the number of mature oocytes on the day of oocyte aspiration between the two groups.

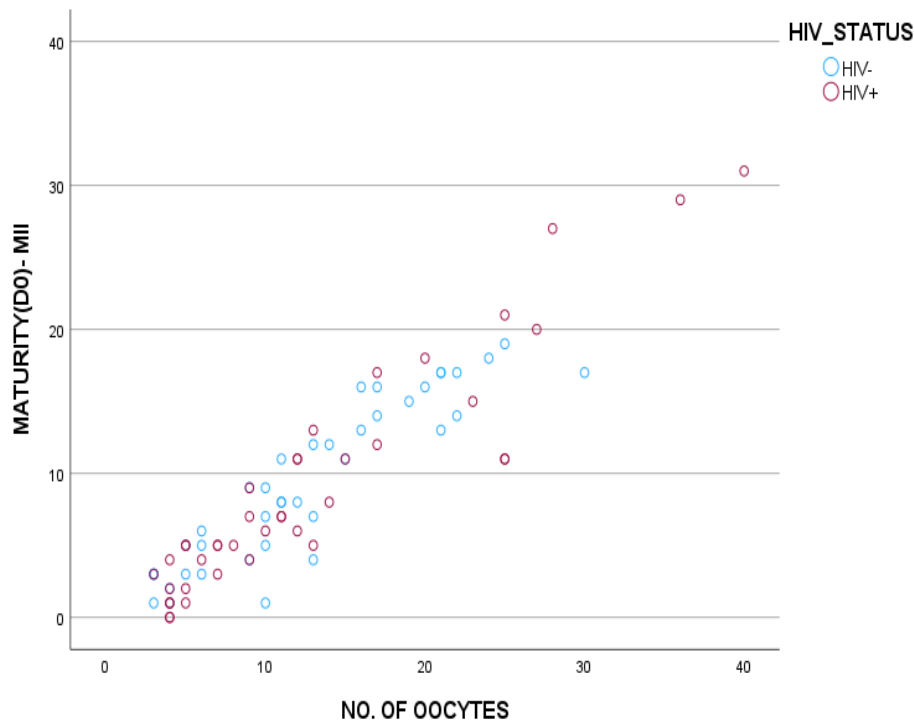


Figure 17: Scatter plot diagram of oocyte retrieval rates and maturation rates in the HIV-infected and HIV-uninfected groups. The diagram represents the quantity and heterogeneity of oocyte cohorts from each patient group.

Table 5 presents the number of MII oocytes per patient in the HIV-infected and HIV-uninfected controls. The retrieval of at least 6-15 oocytes is associated with an increased chance of achieving a live birth; lower oocyte numbers decrease pregnancy outcomes; and harvesting at least 20 oocytes promotes higher cumulative birth rates (Magnusson *et al.* 2018:59-61). A study by Zhang *et al.* (2016:1) categorized MII oocytes as minimal: 1-2, fair: 3-6 and excessive: ≥ 7 in a set of patients undergoing mild ovarian stimulation, and according to Esteves *et al.* (2020:12) for women in their mid-30s, at least 7-10 MII oocytes are required to obtain one chromosomally normal blastocyst. In this present study all the participants used the GnRH-antagonist protocol for ovarian hyperstimulation and based on the oocyte quantifications used in other studies and this study's population characteristics, for the purpose of this study, the MII oocytes were categorized as follows: low: 0-5, intermediate: 6-15, high: 16-20 and excessive: ≥ 21 .

At least 45% of the HIV-infected group had lower rates (0-5) of MII oocyte numbers, 37.5% had intermediate numbers (6-15) of MII oocytes and only 7.5% had higher levels of 16-20 MII oocyte numbers and 10% had an excessive number of MII oocytes; as opposed to the HIV uninfected group that had 35% low MII numbers (0-5), 42.5% had intermediate (6-15) numbers and 22.5% had high numbers (16-20). None of the HIV uninfected patients had excessive MII oocyte numbers.

Table 5: Frequency distribution of MII oocytes among the HIV-positive and HIV-negative populations oocyte cohorts.

HIV STATUS	Number of oocytes	Number of patients	Percentage (%)
HIV infected women	0-5	18	45.0%
	6-10	7	17.5%
	11-15	8	20.0%
	16-20	3	7.5%
	21-25	1	2.5%
	26-31	3	7.5%
HIV uninfected controls	0-5	14	35.0%
	6-10	8	20.0%
	11-15	9	22.5%
	16-20	9	22.5%

Based on the results in Table 6, in the test group, the mean for MII oocytes was 9.08 (± 7.89), for MI oocytes it was 1.25 (± 1.45) and the mean for GV oocytes was 2.55 (± 2.94); in the control group, the MII oocyte mean was 9.33 (± 5.69), the mean for MI oocytes was 1.28 (± 1.74) and the mean for GV oocytes was 2.15 (± 2.19). In the entire study population, the mean values were 9,2 (± 6.83), 1.26 (± 1.59) and 2.35 (± 2.56) for MII, MI and GV oocytes, respectively. This statistically proves that the ratio of MII to immature-MI+GV oocytes between the women living with HIV against the HIV-uninfected women did not differ significantly.

Table 6: Representation of the average number of mature-MII and immature (MI+GV) oocytes in oocyte cohorts aspirated from HIV-positive women and HIV-negative women.

HIV status: HIV+					
	Total	Mean	Std. Dev.	Minimum	Maximum
Maturity: MII	n=40	9.075	7.885031	0	31
Maturity: MI	n=40	1.25	1.445595	0	5
Maturity: GV	n=40	2.55	2.943485	1.22	9.11
HIV status: HIV-					
Maturity: MII	n=40	9.325	5.690241	1	19
Maturity: MI	n=40	1.275	1.739253	0	7
Maturity: GV	n=40	2.15	2.118902	0	8

Exogenous gonadotropins induce the development of many follicles, the goal being to harvest high quantities of good-quality oocytes. Exogenous gonadotropins used in OS vary in composition and MOA. In this study, 42 (52.5%) patients used Gonal-F®, 22 (27.5%) patients used Menopur®, 13 (16.25%) patients used Menopur® pre-mix and only 3 (3.75%) patients used Pergoveris® (Table 7). More patients used the exogenous gonadotropin Gonal-F® (52.5%) because of patients' characteristics (patient age (younger) and ovarian reserve quantity).

Table 7: Distribution of patients across different types and doses of exogenous gonadotropins used for ovarian stimulation.

Exogenous gonadotropin	DOSE (IU)	Gonal-F® (n)	Menopur® (n)	Menopur®-Premix (n)	Pergoveris® (n)	Total (n)
	150IU	23 (28.75%)	1 (1.25%)	0	0	24 (30%)
	2Powder+1 Water (150IU)	0	0	2 (2.5%)	0	2 (2.5%)
	2Powder+1 Water (150IU)	0	0	2 (2.5%)	0	2 (2.5%)
	2Powder+1 Water (150IU)	0	0	0	2 (2.5%)	2 (2.5%)
	225IU	12 (15%)	14 (17.5%)	0	0	26 (32.5%)
	225IU+150IU	1 (1.25%)	0	0	0	1 (1.25%)
	225IU+150IU	1 (1.25%)	0	0	0	1 (1.25%)
	225IU+150IU	1 (1.25%)	0	0	0	1 (1.25%)
	225IU+150IU	2 (2.5%)	0	0	0	2 (2.5%)
	225IU+150IU	1 (1.25%)	0	0	0	1 (1.25%)
	225IU+150IU	1 (1.25%)	0	0	0	1 (1.25%)

	3Powder+1 Water (225IU)	0	0	4 (5%)	0	4 (5%)
	3Powder+1 Water (225IU)	0	0	1 (1.25%)	0	1 (1.25%)
	3Powder+1 Water (225IU)	0	0	1 (1.25%)	0	1 (1.25%)
	300IU	0	6 (7.5%)	0	1 (1.25%)	7 (8.75%)
	300IU+225IU	0	1 (1.25%)	0	0	1 (1.25%)
	3Powder+1 Water (225IU)	0	0	1 (1.25%)	0	1 (1.25%)
	4Powder+1 Water (300IU)	0	0	1 (1.25%)	0	1 (1.25%)
	4Powder+1 Water (300IU)	0	0	1 (1.25%)	0	1 (1.25%)
	Total	n=42 (52.5%)	n=22 (27.5%)	n=13 (16.25%)	n=3 (3.75%)	n=80 (100%)
	Pearson chi2(54)	172.9115				

Table 8 shows that in this study, 56 (70%) patients used 250mcg Ovitrelle® ovulation trigger injection, 15 (18.75%) patients used Lucrin® ovulation trigger injection at a dose of 40IU and only 9 (11.25%) patients used the 80IU Lucrin® ovulation trigger injection. Each trigger type and dose depended on the follicle number and sizes measured during OH monitoring.

Table 8: Distribution of patients across different types and doses of ovulation trigger injections.

	Ovulation trigger injection		
Dose	Lucrin® (n)	Ovitrelle® (n)	Total (n)
250mcg	0	56 (70%)	56 (70%)
40IU	15 (18.75%)	0	15 (18.75%)
80IU	9 (11.25%)	0	9 (11.25%)
Total	24 (30%)	56 (70%)	80 (100%)

Table 9 depicts the ovarian response outcome to exogenous gonadotropin stimulation through the number and size of the follicles seen before the TVOA procedure. Ovarian monitoring is done on both ovaries if they are functional; hence, the table has “R”-right and “L”-left locations of the follicles, along with the size and quantity observed. At face value, when compared by HIV serostatus, the mean values for the number and size of either ovarian follicle seem similar. This suggests that patterns of ovarian stimulation response in women living with HIV follow a similar pattern as observed in HIV-uninfected women.

Table 9: Follicular growth (number and size) measurements observed during ovulation monitoring, before the transvaginal oocyte aspiration procedure.

HIV_STATUS		N	Minimum	Maximum	Mean	Std. Deviation
HIV-	R no follicles	40	0.00	16.00	6.1500	3.64129
	R follicle size	40	0.00	19.00	15.2250	3.81285
	L no follicles	40	0.00	14.00	5.8750	3.53871
	L follicle size	40	0.00	21.00	15.5500	4.08845
HIV+	R no follicles	40	0.00	14.00	5.6500	3.01747
	R follicle size	40	0.00	25.00	15.5250	3.32039
	L no follicles	40	0.00	14.00	5.8500	3.36307
	L follicle size	40	0.00	18.00	14.6250	4.45886

The endometrial thickness during OH is measured at every follicle growth assessment session. Endometrial growth is associated with follicular growth, thus providing valuable information to the clinician regarding the hormonal-follicular relationship. Endometrial lining measurements used for analysis were taken around the time the ovulation trigger was administered, and the results are presented in Table 10. The mean endometrial thickness (in mm) in the test group was 9.73 (± 2.68), and the mean endometrial thickness (in mm) in the control group was 10.16 (± 2.56). Overall total population mean endometrial thickness (in mm) was 9.94 (± 2.61), meaning that neither the test nor the control group's endometrial thickness deviated too far from the total population average. An endometrial lining of less than 9mm at the timing of the LH peak is associated with poorer pregnancy rates (Onogi *et al.* 2020:6). The endometrial thickness measurements seen in this study's population fell within this acceptable value that is sufficient to attain a clinical pregnancy.

Table 10: Mean endometrial thickness measurements observed in the women living with HIV and HIV uninfected females.

HIV status: HIV +					
	Total (n)	Mean	Std. Dev.	Minimum	Maximum
Endometrial thickness (mm)	n=40	9.7325	2.678536	6	17
HIV status: HIV -					
Endometrial thickness (mm)	n=40	10.1575	2.56324	6	16,1
	Total (n)	Mean	Std. Dev.	Minimum	Maximum
Total population					
Endometrial thickness (mm)	n=80	9.945	2.61364	6	17

Women of various age groups seek ART assistance for a variety of reasons. In this study, in the entire population, approximately 52.25% of the population included women 35 years and younger, and 48.75% were early middle-aged (36–39-year-old) women (p-value 0.131) (Table 11). The p-value > 0.05 shows no statistically significant differences (by age) in the spread of patients amongst the entire study population.

Table 11: Frequency distribution of patients across different age categories.

Age (Years)	Frequency (n)	Percentage (%)	Cumulative percentage (%)	p-value
26-30	8	10.00	10.00	
31-35	33	41.25	51.25	
36-39	39	48.75	100.00	
Total	80	100.00		0.131

This study also investigated the role BMI plays in oocyte retrieval and maturation rates. The entire study population was matched by BMI category, and then a comparative analysis was conducted. The goal was to see if patients in the same weight category can achieve similar ART outcomes despite being HIV sero-different.

In the entire study population, only 1(1.25%) patient (living with HIV) was underweight (BMI<18.5), 20 (25%) patients were categorised as being of normal weight (BMI 18.5-24.9), 21 (26.25%) patients were classified as overweight (BMI 25.0-29.9), and compared to other BMI categories, 38 (47.50%) patients were categorised as obese- BMI ≥30 (Table 12). This

result shows the spread of the entire study population by BMI category, and no statistically significant differences (p-value 0.145) were found in the distribution of patients across the different BMI categories.

Table 12: Frequency distribution of patients across different BMI categories.

Body Mass Index-BMI (kg/m²)	Frequency (n)	Percentage (%)	Cumulative Percentage (%)	p-value
Underweight <18.5	1	1.25	1.25	
Normal weight 18.5-24.9	20	25.00	26.25	
Overweight 25.0-29.9	21	26.25	52.50	
Obese ≥30	38	47.0	100.00	
Total	80	100.00		0.145

The mean patient age was 35.65 years (± 3.19) in the HIV-infected group and 34.2 years (± 2.80) in the HIV-uninfected group, and the mean patient BMI was 30.11 kg/m² (± 6.31) in the HIV infected group and 28.04 kg/m² (± 5.67) in the HIV uninfected group (Table 13).

Table 13: Representation of average population age and body mass index (BMI) by HIV serostatus.

HIV status:					
HIV +					
	Total (n)	Mean	Std. Dev.	Minimum	Maximum
Age (Years)	n=40	35.65	3.190933	27	39
BMI (kg/m ²)	n=40	30.10825	6.308734	17.53	46.67
HIV status:					
HIV -					
Age (Years)	n=40	34.2	2.802929	28	39
BMI (kg/m ²)	n=40	28.04525	5.673467	19.29	43

4.4 Inferential Statistics

4.4.1 Independent-Samples Wilcoxon Mann-Whitney U Test

An Independent-Samples Wilcoxon Mann-Whitney U Test was used to assess the distribution of the patient's follicles post-ovarian stimulation, total oocyte retrieval rates and mature (MII) oocyte numbers on the TVOA day. The graphs (Figures 18, 19 and 20) are results showing how many patients in each group had low, good, or excessive rates in follicular growth, oocyte retrieval and maturation rates.

Follicle growth is low at 0-5, sufficient or good at 6-15, high at 16-20 and excessive at ≥ 21 (FemiCare Fertility clinic laboratory protocol). Oocyte retrieval rates were categorised as: ≤ 5 reduced-normal oocyte retrieval, 6-20 normal-high oocyte retrieval and ≥ 21 excessive oocyte retrieval rates (Ampath Laboratories, Appendix F). The distribution of patients' follicular growth response and oocyte cohorts shows there were no significant differences in outcomes between the HIV-infected patients and HIV-uninfected patients in terms of follicular growth (p-value 0.794), total oocyte numbers (p-value 0.686) and mature-MII oocyte numbers (p-value 0.429).

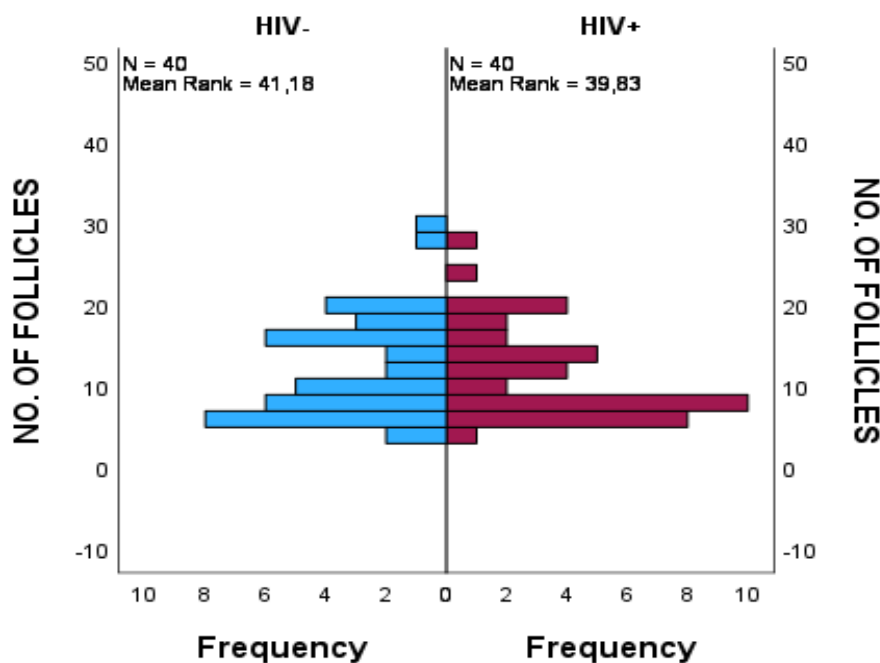


Figure 18: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the follicular growth rates observed between the HIV-infected and HIV-uninfected females. P-value 0.794.

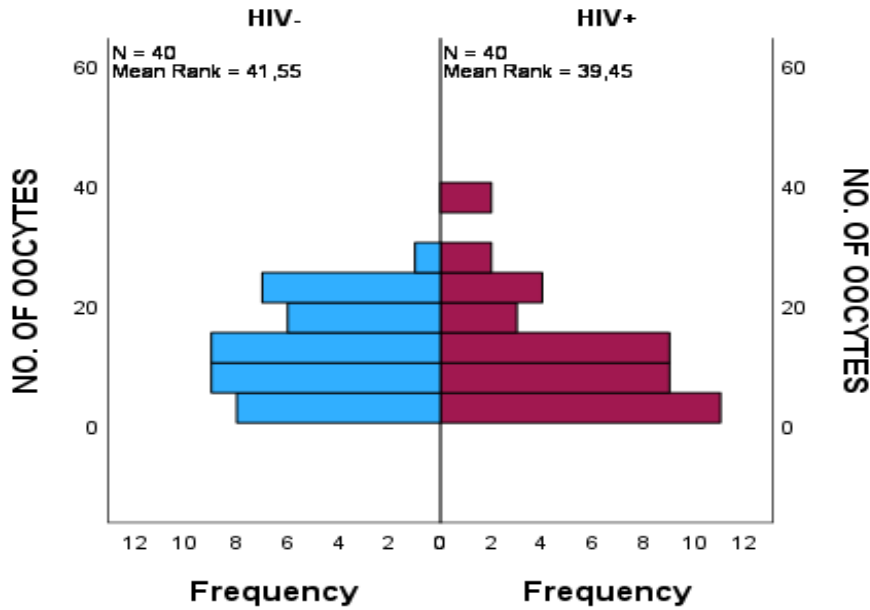


Figure 19: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the total number of oocytes aspirated between HIV-infected and HIV-uninfected females. P-value: 0.686.

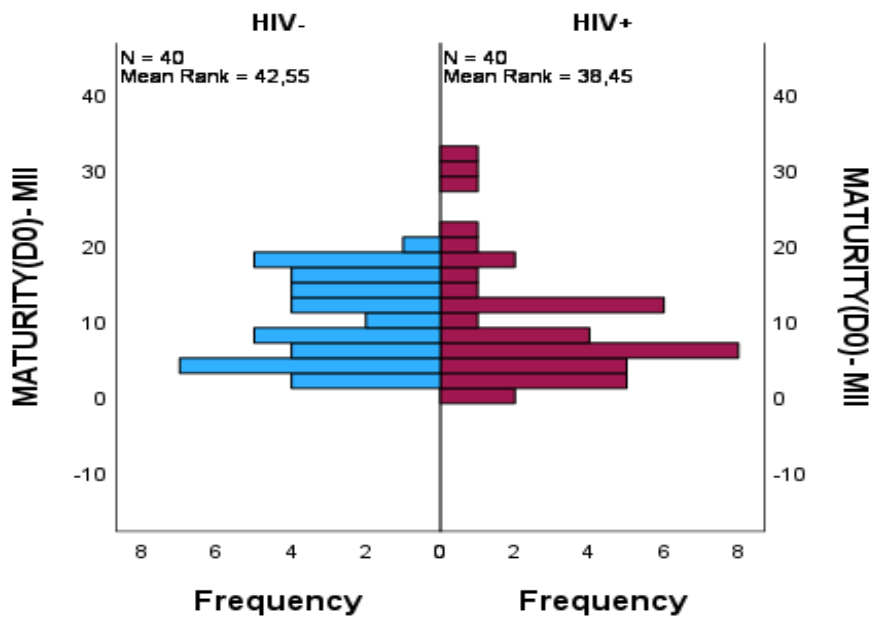


Figure 20: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the total number of MII oocytes aspirated between HIV-infected and HIV-uninfected females. P-value: 0.429.

In addition, Figures 21 and 22 depict the distribution of the immature (MI+GV) oocytes aspirated from the HIV-infected females against the HIV-uninfected females on the TVOA

day. The results show that there were no significant differences (MI: p-value 0.759 and GV: p-value 0.753) in the distribution of these immature oocytes observed in these patients' oocyte cohorts.

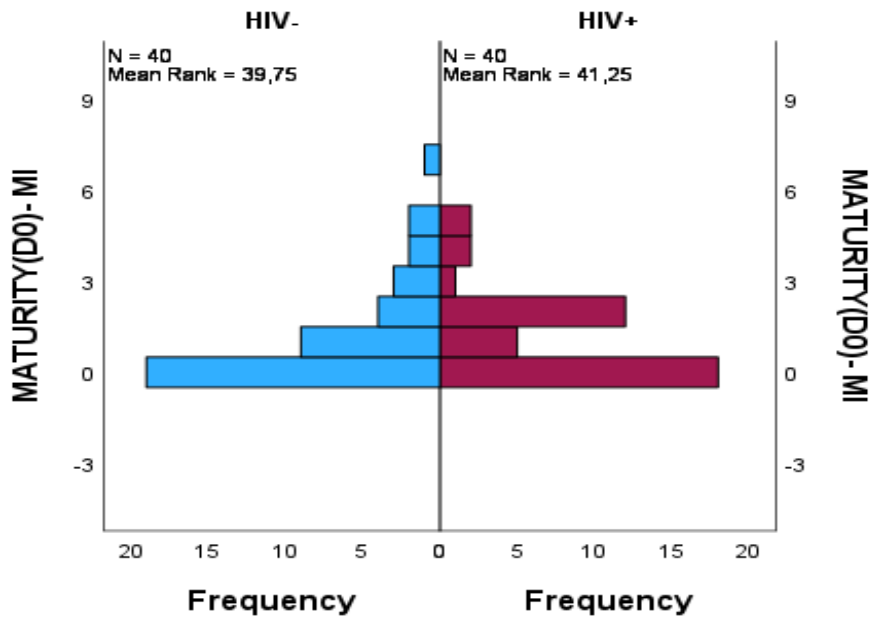


Figure 21: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the number of MI oocytes aspirated between HIV-infected and HIV-uninfected females. P-value 0.759.

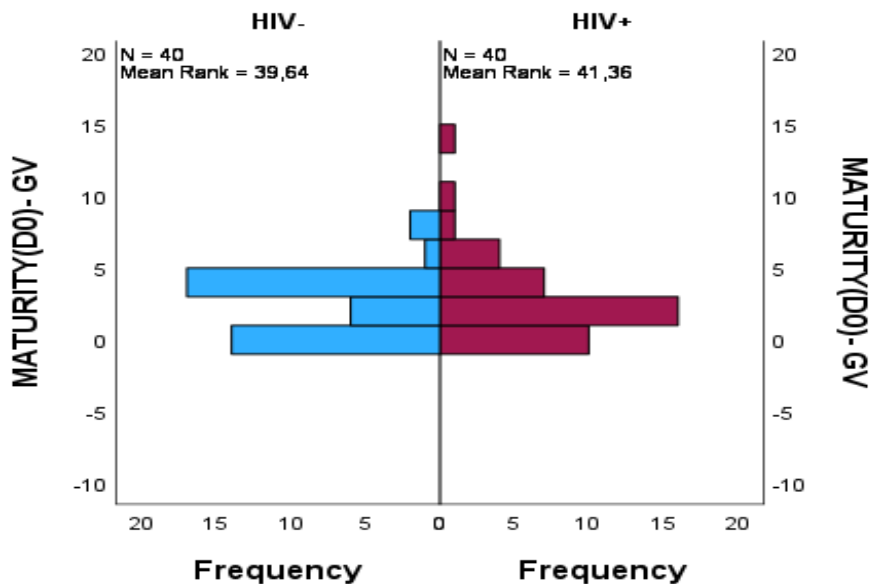


Figure 22: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the number of GV oocytes aspirated between HIV-infected and HIV-uninfected females. P-value 0.753.

4.4.2 Independent-Samples Median Test

The Independent-Samples Median test was used to evaluate the ratio of mature (MII) to immature (MI+GV) oocytes in the patient's oocyte cohorts. The aim was to observe if there was a linear pattern in the quantities of mature (MII) and immature (MI+GV) oocytes within each group. All the p-values > 0.05 show no significant variations in maturity in oocyte cohorts from HIV-infected women against HIV-uninfected women.

Independent-samples median test (Table 14) analysed the number of MII oocytes on the TVOA day by HIV status. Yates's Continuity Correction Chi-Square value was 1.250, Degree of Freedom- 1 and p-value 0.264 (p-value > 0.05). The p-value showed no significant difference in the medians of MII oocytes obtained from the HIV-infected and HIV-uninfected females. This means no one group had oocyte cohorts presenting with less mature MII oocytes than expected.

Table 14: Independent-Samples Median analysis depicting the comparison in means of mature MII oocytes between the HIV-infected females and HIV-uninfected females.

Total number		80
Median		7.500
Test Statistic		1.800 ^a
Degree of Freedom		1
Asymptotic Sig. (2-sided test)		0.180
Yates's Continuity Correction	Chi-Square	1.250
	Degree of Freedom	1
	Asymptotic Sig. (2-sided test)	0.264

a. Multiple comparisons are not performed because there are less than three test fields.

Independent-Samples Median test (Table 15) was used to quantify the number of immature MI oocytes in oocyte cohorts sampled from the HIV-infected female and the HIV-uninfected female on the TVOA day. Yates's Continuity Correction Chi-Square value was 0.865, Degree of Freedom- 1, and p-value 0.352 (p-value > 0.05). The p-value indicated there were no significant differences in the medians of MI oocytes across the entire study population.

Table 15: Independent-Samples Median analysis depicting the comparison in means of immature-MI oocytes between the HIV-infected females and HIV-uninfected females.

Total number		80
Median		1.000
Test Statistic		1.352 ^a
Degree of Freedom		1
Asymptotic Sig. (2-sided test)		0.245
Yates's Continuity Correction	Chi-Square	0.865
	Degree of Freedom	1
	Asymptotic Sig. (2-sided test)	0.352

a. Multiple comparisons are not performed because there are less than three test fields.

An Independent-Samples Median test (Table 16) was used to quantify the number of immature GV oocytes harvested from the HIV-infected and HIV-uninfected groups. Yates's

Continuity Correction Chi-Square value was 1.279, Degree of Freedom-1, and p-value 0.258 (p-value > 0.05). The p-value shows there was no significant difference in the medians of GV oocytes across the entire study population.

Table 16: Independent-Samples Median analysis depicting the comparison in means of immature GV oocytes between the HIV-infected females and HIV-uninfected females.

Total number		80
Median		2,000
Test Statistic		1.841 ^a
Degree of Freedom		1
Asymptotic Sig. (2-sided test)		0.175
Yates's Continuity Correction	Chi-Square	1.279
	Degree of Freedom	1
	Asymptotic Sig. (2-sided test)	0.258

a. Multiple comparisons are not performed because there are less than three test fields.

4.4.3 Independent samples T-test (Two-sample t-test)

Table 17 represents an Independent-samples t-test analysis of the follicular growth (number of follicles), the number of total oocytes and the number of mature MII eggs achieved per exogenous gonadotropin used in the HIV-infected females and HIV seronegative controls. In addition, in Table 18, the Independent-samples t-test showed the association between ovulation trigger and outcomes: follicle number, follicle size, total oocyte counts and MII oocytes. The analysis was based on the different types and doses of ovulation trigger injections. No statistical differences (all p-values > 0.05) in outcomes in the HIV-infected and HIV-uninfected females in both tables were observed. This adds to the result that found no significant differences in ovarian stimulation between the HIV-infected and HIV-uninfected women.

Table 17: Outcomes of follicle number, oocyte number and MII oocyte number based on different exogenous gonadotropins used for ovarian induction.

MENOPUR/GONAL-F		HIV status	N	Mean	Std. Deviation	p-value
GONAL-F	NO. OF FOLLICLES	HIV-	26	12.54	6.544	0.816
		HIV+	16	13.00	5.574	
	R_ no_ follicles	HIV-	26	6.08	3.698	0.704
		HIV+	16	6.50	3.077	
	L_ no_ follicles	HIV-	26	6.50	3.455	0.811
		HIV+	16	6.25	2.933	
	NO. OF OOCYTES	HIV-	26	14.04	7.518	0.200
		HIV+	16	17.63	10.269	
	MATURITY(D0)- MII	HIV-	26	10.54	5.501	0.326
HIV+		16	12.69	8.546		
MENOPUR		HIV-	6	13.00	8.532	0.708

	NO. OF FOLLICLES	HIV+	16	11.69	6.710	
		HIV-	6	8.00	4.604	0.181
	R_ no_ follicles	HIV+	16	5.63	3.160	
		HIV-	6	4.83	4.262	0.518
	L_ no_ follicles	HIV+	16	6.06	3.768	
		HIV-	6	10.33	3.724	0.977
	NO. OF OOCYTES	HIV+	16	10.44	8.294	
		HIV-	6	5.33	3.933	0.427
	MATURITY(D0)- MII	HIV+	16	7.94	7.398	
		HIV-	6	10.00	5.228	0.338
	NO. OF FOLLICLES	HIV+	6	7.67	2.944	
		HIV-	7	5.14	2.478	0.394
MENOPUR (PRE-MIX)	R_ no_ follicles	HIV+	6	4.00	2.098	
		HIV-	7	4.86	3.338	0.451
	L_ no_ follicles	HIV+	6	3.67	2.066	
		HIV-	7	11.00	7.483	0.560
	NO. OF OOCYTES	HIV+	6	8.83	5.037	
		HIV-	7	8.86	6.768	0.180
	MATURITY(D0)- MII	HIV+	6	4.50	3.937	
		HIV-	7	7.00	.	NA
	NO. OF FOLLICLES	HIV+	2	8.50	4.950	
		HIV-	1	4.00	.	NA
	R_ no_ follicles	HIV+	2	4.00	2.828	
		HIV-	1	3.00	.	NA
PERGOVERIS	L_ no_ follicles	HIV+	2	7.50	6.364	
		HIV-	1	6.00	.	NA
	NO. OF OOCYTES	HIV+	2	6.50	3.536	
		HIV-	1	5.00	.	NA
	MATURITY(D0)- MII	HIV+	2	3.00	1.414	
		HIV-	1	3.00	6.364	

Table 18: Outcomes of follicle growth, oocyte number and MII oocyte number based on the different ovulation triggers in the HIV-infected and HIV-uninfected females.

OVULATION TRIGGER	DOSE (IU/MCG)		HIV STATUS	N	Mean	Std. Deviation	p-value
LUCRIN	40IU	NO. OF FOLLICLES	HIV-	5	20.80	5.404	0.222
			HIV+	10	17.50	4.353	

		R_ no_ follicles	HIV-	5	10.40	3.578	0.237
			HIV+	10	8.50	2.369	
		R_ follicle_ size	HIV-	5	16.40	1.140	1.000
			HIV+	10	16.40	1.174	
		L_ no_ follicles	HIV-	5	10.20	2.490	0.371
			HIV+	10	9.00	2.309	
		L_ follicle_ size	HIV-	5	15.60	1.949	0.371
			HIV+	10	16.50	0.850	
		NO. OF OOCYTES	HIV-	5	17.80	9.338	0.245
			HIV+	10	24.30	9.911	0.246
		MATURIT Y(D0)- MII	HIV-	5	12.20	5.975	0.223
			HIV+	10	18.10	9.291	0.164
	80IU	NO. OF FOLLICLES	HIV-	8	16.63	3.249	NA
			HIV+	1	14.00	.	
		R_ no_ follicles	HIV-	8	7.63	1.685	NA
			HIV+	1	6.00	.	
		R_ follicle_ size	HIV-	8	16.13	1.246	NA
			HIV+	1	14.00	.	
		L_ no_ follicles	HIV-	8	9.00	2.000	NA
			HIV+	1	8.00	.	
		L_ follicle_ size	HIV-	8	17.13	1.553	NA
			HIV+	1	13.00	.	
		NO. OF OOCYTES	HIV-	8	19.63	4.627	NA
			HIV+	1	3.00	.	

OVITRELLE	250 mc g	MATURIT Y(D0)- MII	HIV-	8	15.38	3.662	NA
			HIV+	1	3.00	.	
		NO. OF FOLLICLES	HIV-	27	9.04	5.110	0.859
			HIV+	29	9.28	4.898	
		R_ no_ follicles	HIV-	27	4.93	3.385	0.739
			HIV+	29	4.66	2.636	
		R_ follicle_ size	HIV-	27	14.74	4.520	0.633
			HIV+	29	15.28	3.807	
		L_ no_ follicles	HIV-	27	4.15	2.656	0.478
			HIV+	29	4.69	2.989	
		L_ follicle_ size	HIV-	27	15.07	4.779	0.434
			HIV+	29	14.03	5.074	
		NO. OF OOCYTES	HIV-	27	9.78	5.265	0.718
			HIV+	29	9.28	5.091	
		MATURIT Y(D0)- MII	HIV-	27	7.00	4.583	0.496
			HIV+	29	6.17	4.441	

A Two-sample T-test was performed to assess the serum AMH measurements by HIV status (Table 19). A p-value of 0.92 (p-value >0.05) was found, showing no significant differences in mean serum AMH values between the HIV-infected and HIV-uninfected women. The mean serum AMH was 2.77 ng/ml (SD 1.74) in the females living with HIV, 2.73ng/ml (SD 1.38) in the HIV uninfected group and 2.75 ng/ml (SD 1.56) when combined.

Table 19: Two-sample t-test analysis of the serum AMH levels of the HIV-infected test group over the HIV-uninfected control group.

Group	Total (n)	Mean	Standard Error	Standard deviation	95% confidence interval		p-value
HIV +	40	2.77	0.2750368	1.739486	2.213686	3.326314	
HIV -	40	2.73375	0.2185869	1.382465	2.291616	3.175884	
Combined (HIV+ & HIV-)	80	2.751875	0.1745564	1.56128	2.404429	3.099321	0.92
Difference		-0.03625	0.3513196		-0.7356736	0.6631736	

Diff- mean (0)- mean (1)

T- -0.1032

Degrees of Freedom- 78

Age is the one constant factor known to affect serum AMH values across all races and ethnicities (Kotlyar and Seifer 2021:5). A T-test analysis presented in Table 20 shows the mean serum AMH comparisons between the HIV-infected women against the HIV-uninfected women in this study population after matching by age. In the age category 26-30 years, a statistically significant difference (p-value 0.030, p-value< 0.05) was found when comparing the mean serum AMH values in the HIV-infected women (mean 4.61) against the mean serum AMH values in the HIV uninfected group (mean 2.62). The drawback of this result is the small sample size; therefore, this result should be interpreted with caution. Furthermore, in the age categories 31-35 years (p-value 0.374, p-value> 0.05) and 36-39 years (p-value 0.461, p-value> 0.05), all the p-values were greater than 0.05, showing no statistically significant differences in the means of serum AMH values between the HIV infected women and HIV uninfected women in this study.

Any effect on the serum AMH levels will affect ovarian stimulation response, thus altering follicle growth and oocyte number.

Table 20: T-test analysis of the comparison in serum AMH levels between the HIV-infected and HIV-uninfected females matched by age.

Age category (Years)	Variable	HIV status	Number	Mean	Std. Deviation	One-sided p-value
26-30	AMH (ng/ml)	HIV+	3	4.61	0.91162	0.030
		HIV-	5	2.62	1.57662	
31-35	AMH (ng/ml)	HIV +	13	3.3615	1.50632	0.374
		HIV -	20	3.1830	1.56224	
36-39	AMH (ng/ml)	HIV +	24	2.2200	1.06965	0.461
		HIV -	15	2.1727	1.66652	

An independent samples T-test analysis is presented in Table 21. This test quantified follicle number, total eggs obtained and the number of mature (MII) eggs by age in the HIV-infected women against the HIV-uninfected women. Multi-follicular growth that gives rise to mature oocytes is achieved by ovarian stimulation with exogenous gonadotropins, and maturation is induced with ovulation trigger injections. The results found in this statistical analysis (all p-values> 0.05) indicate that when the patients were matched by age, there were no significant differences in follicle number, oocyte retrieval rates and number of MII oocytes between the two groups.

Table 21: T-test analysis comparing the outcomes- follicle number, total oocyte number and MII oocyte number in the HIV-infected females and HIV-uninfected control women, matched by age.

Age category (Years)	Variable	HIV status	Number	Mean	Std. Deviation	One-sided p-value
26-30	NO. OF FOLLICLES	HIV+	3	15.33	6.429	0.316
		HIV-	5	12.00	10.124	
	NO. OF OOCYTES	HIV +	3	3.3615	20.67	0.177
		HIV -	5	3.1830	8.40	
	MATURITY(D0)- MII	HIV +	3	2.2200	16.67	0.077
		HIV -	5	2.1727	6.00	
31-35	NO. OF FOLLICLES	HIV +	13	13.54	4.612	0.230
		HIV -	20	12.15	5.537	
	NO. OF OOCYTES	HIV +	13	17.69		0.116
		HIV -	20	14.35		
	MATURITY(D0)- MII	HIV +	13	12.38	6.826	0.236
		HIV -	20	10.80	5.606	
36-39	NO. OF FOLLICLES	HIV +	24	9.83	6.077	0.171
		HIV -	15	11.87	6.927	
	NO. OF OOCYTES	HIV +	24	9.29	7.184	0.122
		HIV -	15	12.07	7.035	
	MATURITY(D0)- MII	HIV +	24	6.33	6.425	0.153
		HIV -	15	8.47	5.939	

Among other factors, an individual's weight can lead to infertility (WHO 2023:3). Therefore, it is acceptable in ART to note a patient's BMI as it may impact the success of ART. Tables 22a, 22b and 22c display the mean values for follicular growth (number and size), total oocyte number and MII oocyte numbers derived from the HIV seropositive women against the HIV uninfected controls across different BMI categories (underweight- <18.5, normal weight- 18.5-24.99, overweight- 25.0-29.99 and obese- ≥30) after controlled ovarian stimulation. A single HIV positive participant was categorised as underweight and therefore

no comparisons were made in this weight category. No statistically significant differences (all p-values > 0.05) were found in follicular growth, oocyte retrieval rates and MII oocyte numbers between the HIV-infected women and HIV-uninfected women after being matched based on BMI category (normal weight, overweight and obese).

Table 22. a: T-test analysis comparing the outcomes- follicle number, total oocyte number and MII oocyte number observed between the HIV seropositive women and the HIV uninfected control group, matched by BMI category.

Measured variable	HIV STATUS	N	Mean	Std. Deviation	p-value
R_ no_ follicles	HIV-	14	6.57	4.201	0.548
	HIV+	6	5.33	3.983	
L_ no_ follicles	HIV-	14	5.86	3.697	0.704
	HIV+	6	5.17	3.601	
R_ follicle_ size	HIV-	14	14.36	4.483	0.639
	HIV+	6	13.17	6.463	
L_ follicle_ size	HIV-	14	14.14	4.521	0.906
	HIV+	6	13.83	6.853	
NO. OF OOCYTES	HIV-	14	13.71	8.462	0.992
	HIV+	6	13.67	11.690	
MATURITY(D0)- MII	HIV-	14	10.14	6.150	0.925
	HIV+	6	10.50	10.540	
a. BMI category = 18.5 – 24.99 (Normal weight)					

Table 22. b: T-test analysis comparing the outcomes- follicle number, total oocyte number and MII oocyte number observed between the HIV seropositive women and the HIV uninfected control group, matched by BMI category.

Measured Variable	HIV STATUS	N	Mean	Std. Deviation	p-value
R_ no_ follicles	HIV-	9	5.89	2.848	0.152
	HIV+	14	4.36	2.098	
L_ no_ follicles	HIV-	9	4.67	3.082	0.882
	HIV+	14	4.86	2.878	
R_ follicle_ size	HIV-	9	16.67	1.000	0.251
	HIV+	14	15.93	1.685	
L_ follicle_ size	HIV-	9	16.33	1.225	0.309
	HIV+	14	14.71	4.514	
	HIV-	9	11.67	6.305	0.337

NO. OF OOCYTES	HIV+	14	9.14	5.829	
MATURITY(D0)-MII	HIV-	9	8.33	5635	0.366
	HIV+	14	6.36	4.584	
b. BMI category = 25.0 - 29.99 (Overweight)					

Table 22. c: T-test analysis comparing the outcomes- follicle number, total oocyte number and MII oocyte number observed between the HIV seropositive women and the HIV uninfected control group, matched by BMI category.

Measured Variable	HIV STATUS	N	Mean	Std. Deviation	p-value
R_ no_ follicles	HIV-	17	5.94	3.699	0.517
	HIV+	19	6.68	3.110	
L_ no_ follicles	HIV-	17	6.53	3.659	0.763
	HIV+	19	6.89	3.557	
R_ follicle_ size	HIV-	17	15.18	4.096	0.484
	HIV+	19	16.00	2.828	
L_ follicle_ size	HIV-	17	16.29	4.552	0.275
	HIV+	19	14.74	3.871	
NO. OF OOCYTES	HIV-	17	12.53	6.568	0.350
	HIV+	19	15.32	10.409	
MATURITY(D0)-MII	HIV-	17	9.18	5.582	0.553
	HIV+	19	10.68	8.913	
c. BMI category = ≥ 30 (Obese)					

4.4.4 Pearson Correlation Coefficient Test

A Pearson correlation coefficient test was performed to test the strength of the relationship between AMH, follicle number and MII oocyte retrieval rates, by controlling for age (Table 23).

Since a correlation is significant if the associated p-value is <0.05 , in the age category 26-30 years old, irrespective of HIV serostatus, all the p-values obtained were >0.05 , meaning that there was a weak correlation between the measured variables. This may be a result of certain patient characteristics that influenced the raw data collected. In addition, it should be noted that there was a small sample size in the HIV-infected population group, which affects the validity and reliability of the results obtained.

After separating by HIV serostatus and matching by age, in the women living with HIV, a Pearson correlation coefficient value=0.633 (p-value 0.020) was found when assessing the association between AMH and follicle number, a Pearson correlation coefficient value=0.718

(p-value <0.006) was found in the association between AMH and MII oocyte rates, and a Pearson correlation coefficient value=0.824 (p-value <0.001) was found when evaluating the association between follicle number and MII oocyte rates. This means that there was a very strong association between the stated paired variables.

Similarly, in the HIV uninfected controls, in the age category 31-35 years, a Pearson correlation coefficient value=0.748 (p-value <0.001) was found in the association between AMH and follicle number, a Pearson correlation coefficient value=0.797 (p-value <0.001) was found in the association between AMH and MII oocytes, and a Pearson correlation coefficient value=0.886 (p-value <0.01) was found in the association between follicle number and MII oocyte numbers. Again, this means that there was a very strong association between the stated paired variables. A similar trend is seen in both groups in the 36–39-year-old category and seems to be mediated by age. This means that the serum AMH value usually corresponds to the follicular recruitment number and the expected number of MII oocytes, therefore can be employed to predict IVF outcomes.

Table 23: Pearson correlation coefficient analysis of the relationship between AMH, follicle number and MII oocyte number in the HIV-infected females and HIV-uninfected women by age.

	Age (Years)	Variable		NO. OF FOLLICLES	AMH (ng/ml)	MATURITY(D0)- MII
HIV-	26 - 30	NO. OF FOLLICLES	Pearson Correlation	1	0.525	-0.351
			p-value		0.364	0.562
			N	5	5	5
		AMH (ng/ml)	Pearson Correlation	0.525	1	0.568
			p-value	0.364		0.317
			N	5	5	5
		MATURITY(D0)- MII	Pearson Correlation	-0.351	0.568	1
			p-value	0.562	0.317	
			N	5	5	5
	31 - 35	NO. OF FOLLICLES	Pearson Correlation	1	0.748	0.88
			p-value		<0.001	<0.001
			N	20	20	20
		AMH (ng/ml)	Pearson Correlation	0.748	1	0.797
			p-value	<0.001		<0.001
			N	20	20	20
		MATURITY(D0)- MII	Pearson Correlation	0.886	0.797	1

	36 -39	NO. OF FOLLICLES	p-value	<0.001	<0.001	
			N	20	20	20
			Pearson Correlation	1	0.584	0.441
		AMH (ng/ml)	p-value		0.022	0.100
			N	15	15	15
			Pearson Correlation	0.584	1	0.811
		MATURITY(D0)- MII	p-value	0.022		<0.001
			N	15	15	15
			Pearson Correlation	0.441	0.811	1
HIV +	26 - 30	NO. OF FOLLICLES	p-value	0.100	<0.001	
			N	15	15	15
			Pearson Correlation	0.441	0.811	1
		AMH (ng/ml)	p-value	0.100	<0.001	
			N	15	15	15
			Pearson Correlation	0.441	0.811	1
		MATURITY(D0)- MII	p-value	0.100	<0.001	
			N	15	15	15
			Pearson Correlation	0.441	0.811	1
	31 - 35	NO. OF FOLLICLES	p-value	0.100	<0.001	
			N	15	15	15
			Pearson Correlation	0.441	0.811	1
		AMH (ng/ml)	p-value	0.100	<0.001	
			N	15	15	15
			Pearson Correlation	0.441	0.811	1
		MATURITY(D0)- MII	p-value	0.100	<0.001	
			N	15	15	15
			Pearson Correlation	0.441	0.811	1
	36 -39	NO. OF FOLLICLES	p-value	<0.001	<0.001	
			N	20	20	20
			Pearson Correlation	1	0.584	0.441
		AMH (ng/ml)	p-value		0.022	0.100
			N	15	15	15
			Pearson Correlation	0.584	1	0.811
		MATURITY(D0)- MII	p-value	0.022		<0.001
			N	15	15	15
			Pearson Correlation	0.441	0.811	1

		AMH (ng/ml)	Pearson Correlation	0.668	1	0.833
			p-value	<0.001		<0.001
			N	24	24	24
		MATURITY(D0)- MII	Pearson Correlation	0.814	0.833	1
			p-value	<0.001	<0.001	
			N	24	24	24

4.4.5 Fisher-Freeman-Halton Exact Test

Although it is beyond the scope of this research study, as a supplementary measure, a Fisher-Freeman-Halton test was conducted to evaluate oocyte retrieval and oocyte maturation rates among the entire study population and within each group. The results of this analysis are presented in Table 24. a, 24. b and 24. c. In Table 24. a, in the entire study population (HIV-infected and HIV-uninfected women), after matching by age, a statistically significant association was found in the distribution of patients (by age) and total oocytes retrieved (p-value 0.054) and mature-MII oocytes (p-value 0.037) among the entire study population. This means that age was positively associated with oocyte retrieval and mature-MII oocyte rates, and this is widely known and accepted.

In Table 24. b, no statistically significant difference was observed in the distribution of patients (by age) oocyte cohorts when assessing oocyte retrieval rates (p-value 0.452) and mature-MII oocyte rates (p-value 0.376) within the HIV uninfected group only. This means that when analysing the distribution of total oocytes retrieved and mature MII oocytes, regardless of age, similar patterns were observed in this group.

Finally, in Table 24.c, in the HIV-infected group, there was a statistically significant association in the distribution (by age) of patients' oocyte cohorts when assessing oocyte retrieval rates (p-value 0.004) and mature-MII oocyte rates (p-value 0.007). A higher number of older women, aged between 36-39 years old, had more oocyte cohorts that had small numbers (0-5 oocytes). This is an important observation since these women have lower oocyte yields than HIV uninfected women of the same age, and younger women living with HIV.

Table 24.a: A comparative analysis of the total oocyte number and MII oocyte numbers in oocyte cohorts obtained among the HIV-uninfected versus the HIV infected females.

Number of total oocytes	Age (Years)			p-value	Number MII oocytes	Age (Years)			p-value
	26-30	31-35	36-39			26-30	31-35	36-39	
0-5	2 (25%)	2 (6.1%)	15 (38.5 %)	0.054	0-5	4 (50%)	6 (18.2%)	22 (56.4 %)	0.037

6-10	2 (25%)	7 (21.2%)	9 (23.1%)		6-10	1 (12.5%)	7 (21.2%)	7 (17.9%)	
11-15	2 (25%)	10 (30.3%)	6 (15.4%)		11-15	1 (12.5%)	10 (30.3%)	6 (15.4%)	
16-20	1 (12.5%)	4 (12.1%)	4 (10.3%)		16-20	1 (12.5%)	8 (24.2%)	3 (7.7%)	
21-25	0	8 (24.2%)	3 (7.7%)		21-25	0	1 (3%)	0	
≥26	1 (12.5%)	2 (6.1%)	2 (5.1%)		≥26	1 (12.5%)	1 (3%)	1 (2.6%)	
Total (n=80)	8 (100%)	33 (100%)	39 (100%)		Total (n=80)	8 (100%)	33 (100%)	39 (100%)	

Table 24.b: A comparative analysis of the total oocyte number and MII oocyte numbers in oocyte cohorts obtained only among the HIV-uninfected females.

Number of total oocytes	Age (Years) HIV-			p-value	Number MII oocytes	Age (Years) HIV-			p-value
	26-30	31-35	36-39	0.452		26-30	31-35	36-39	0.376
0-5	1 (20%)	2 (10%)	5 (33.3%)		0-5	3 (60%)	4 (20%)	7 (46.7%)	
6-10	2 (40%)	5 (25%)	2 (13.3%)		6-10	1 (20%)	5 (25%)	2 (13.3%)	
11-15	2 (40%)	5 (25%)	2 (13.3%)		11-15	1 (20%)	4 (20%)	4 (26.7%)	
16-20	0	2 (10%)	4 (26.7%)		16-20	0	7 (35%)	2 (13.3%)	
21-25	0	5 (25%)	2 (13.3%)		21-25	0	0	0	
≥26	0	1 (5%)	0		≥26	0	0	0	
Total (n=40)	5 (100%)	20 (100%)	15 (100%)		Total (n=40)	5 (100%)	20 (100%)	15 (100%)	

Table 24.c: A comparative analysis of the total oocyte number and MII oocyte numbers in oocyte cohorts obtained only among the HIV-infected females.

Number of total oocytes	Age (Years) HIV+			p-value	Number MII oocytes	Age (Years) HIV+			p-value
	26-30	31-35	36-39	0.004		26-30	31-35	36-39	0.007
0-5	1 (33.3%)	0	10 (41.7%)		0-5	1 (33.3%)	2 (15.4%)	15 (62.5%)	
6-10	0	2 (15.4%)	7 (29.7%)		6-10	0	2 (15.4%)	5 (20.8%)	
11-15	0	5 (38.5%)	4 (16.7%)		11-15	0	6 (46.2%)	2 (8.3%)	
16-20	1 (33.3%)	2 (15.4%)	0		16-20	1 (33.3%)	1 (7.7%)	1 (4.2%)	
21-25	0	3 (23.1%)	1 (4.2%)		21-25	0	1 (7.7%)	0	
≥26	1 (33.3%)	1 (7.7%)	2 (8.3%)		≥26	1 (33.3%)	1 (7.7%)	1 (4.2%)	
Total (n=40)	3 (100%)	13 (100%)	24 (100%)		Total (n=40)	3 (100%)	13(100%)	24 (100%)	

4.5 Conclusion

This chapter presented the findings of the statistical analysis, patterns and trends of the study data. In summary, the test group- HIV-infected women had comparable ART results to the control group- HIV uninfected women. The groups were matched by age, BMI, exogenous gonadotropin and ovulation trigger injections, and ovulation response was found to be similar. These results are discussed in depth in Chapter 5 of this dissertation.

4.6 Supplementary statistics

4.6.1 Outcomes When the Diagnosis is the Same- Independent-Samples Wilcoxon Mann-Whitney U Test

All the patients were attending a fertility clinic for infertility-related reasons. Infertility can be caused by many factors, one of which is the tubal factor, with a prevalence of 20% (Madziyire *et al.* 2021:2). In this study, 57.5% of the HIV-infected group presented with tubal factor caused infertility, and 32.5% of the HIV uninfected women presented with tubal factor caused infertility.

Figures 23, 24 and 25 are Independent-Samples Mann-Whitney U test analysis that exhibits the distribution of the follicular growth rates, total oocytes and mature-MII oocytes between the HIV-infected and HIV uninfected women when they both suffer tubal infertility. This was done to evaluate the patient's ART outcomes without confounders such as diagnoses that significantly alter the way a patient responds to stimulation.

Follicle growth is low at 0-5, sufficient or good at 6-15, high at 16-20 and excessive at ≥ 21 (FemiCare Fertility clinic laboratory protocol). Oocyte retrieval rates are ≤ 5 reduced-normal oocyte retrieval, 6-20 normal-high oocyte retrieval and ≥ 21 excessive oocyte retrieval rates (Ampath Laboratories, Appendix F). The distribution of patients' follicular growth response and oocyte cohorts shows there were no significant differences in outcomes between the HIV-infected patients and HIV-uninfected patients in terms of follicular growth (p-value 0.537), total oocyte retrieval rates (p-value 0.745) and mature-MII oocyte numbers (p-value 0.580).

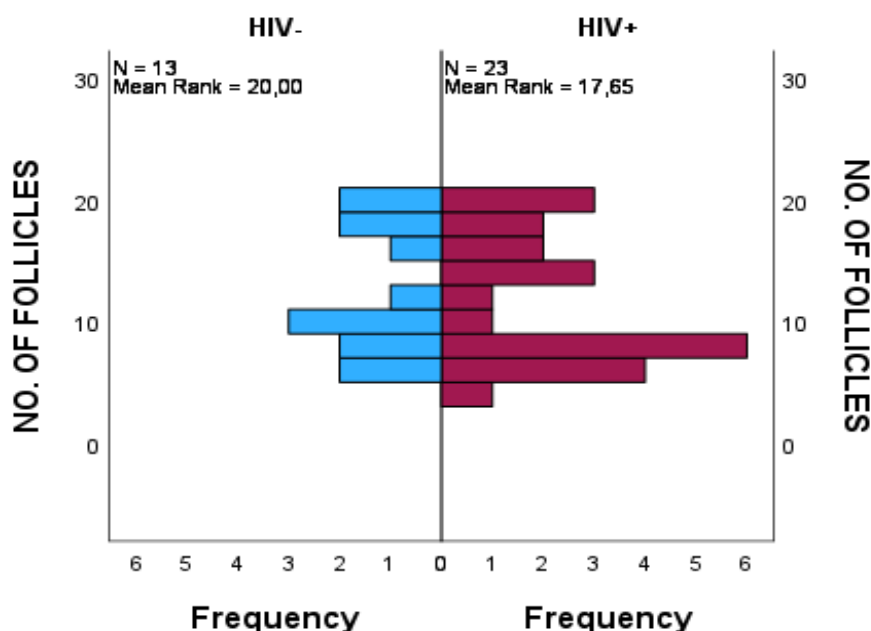


Figure 23: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the follicular growth rates observed between HIV-infected and HIV-uninfected females with the same infertility diagnosis. P-value 0.537.

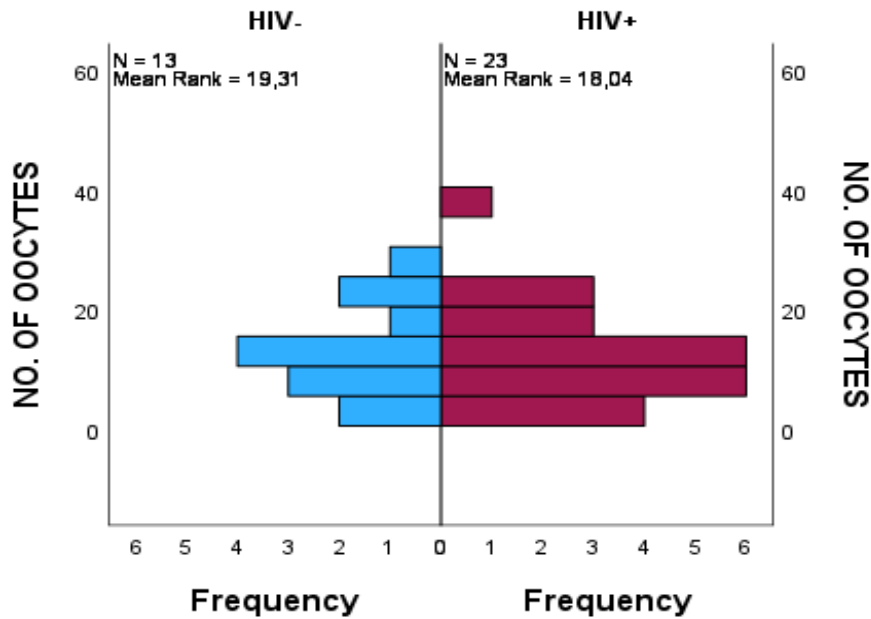


Figure 24: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution of the total number of oocytes aspirated between HIV-infected and HIV-uninfected females with the same infertility diagnosis. P-value 0.745.

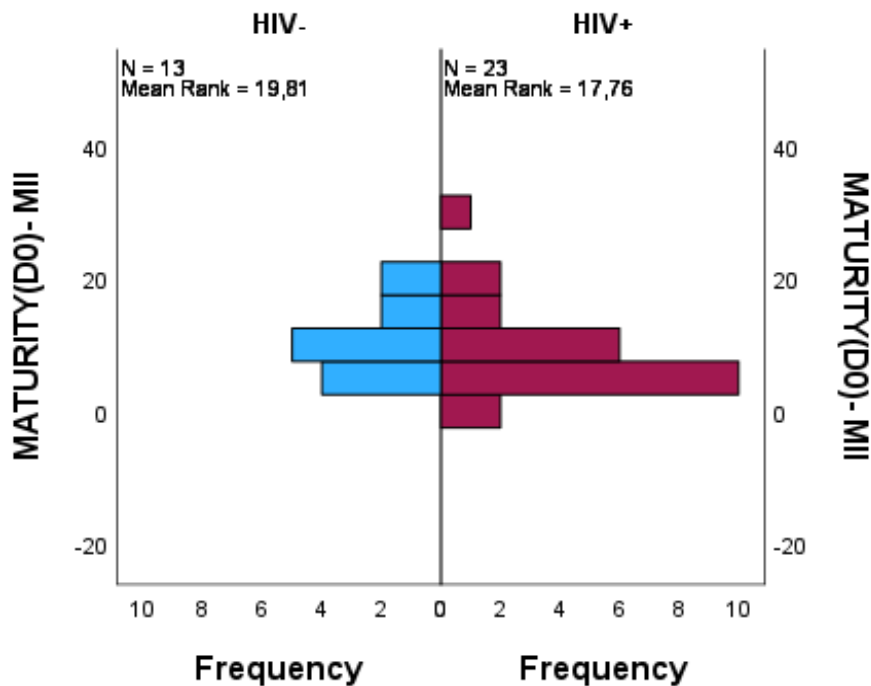


Figure 25: Independent-Samples Wilcoxon Mann-Whitney U Test illustrating the frequency distribution patterns of the number of MII oocytes aspirated between HIV-infected and HIV-uninfected females with the same infertility diagnosis. P-value 0.580.

4.6.2 Outcomes in Rescue *in vitro* Matured (R-IVM) Oocytes- Independent-Samples Wilcoxon Mann-Whitney U Test

A portion of the oocytes attained through ovarian stimulation will be immature- MI or GV oocytes on the day of TVOA. These oocytes are in some facilities discarded; however, in other facilities, extended culture for these oocytes is provided, thus increasing the patient's preimplantation embryo pool, a process called rescue *in vitro* maturation (R-IVM) (Qin *et al.* 2022:9). Traditional incubators can provide sufficient culture (~24 hours) for immature-GV oocytes to reach the MII stage, and these fertilise well (Escrich, Pellicer and Meseguer 2018:638). The facility at which this study was conducted cultured GV and MI oocytes for an additional 24 hours after these oocytes failed to mature *in vivo*.

Since the clinic routinely provided extended (~24 hours) culture of all immature (MI+GV) oocytes for all patients, it was interesting to evaluate how many of these oocytes reached maturity (MII) following this period. The following figures (Figure 26 and Figure 27) depict the spread of patients' oocyte cohorts (by HIV status) that convert to MII following culture.

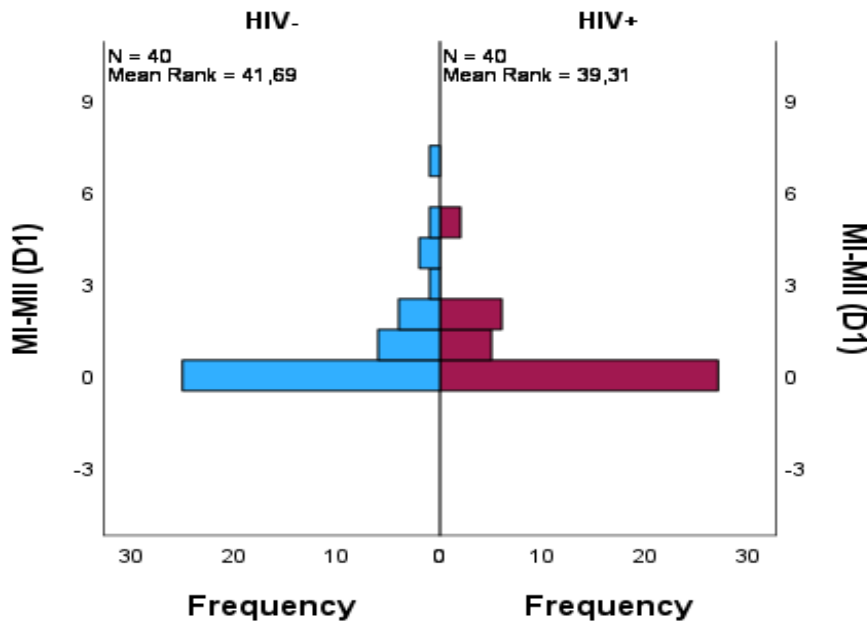


Figure 26: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the number of rescue-IVM oocytes that were initially immature (MI oocytes) on the day of oocyte retrieval, but matured following extended culture, between HIV-infected and HIV-uninfected women. P-value 0.590.

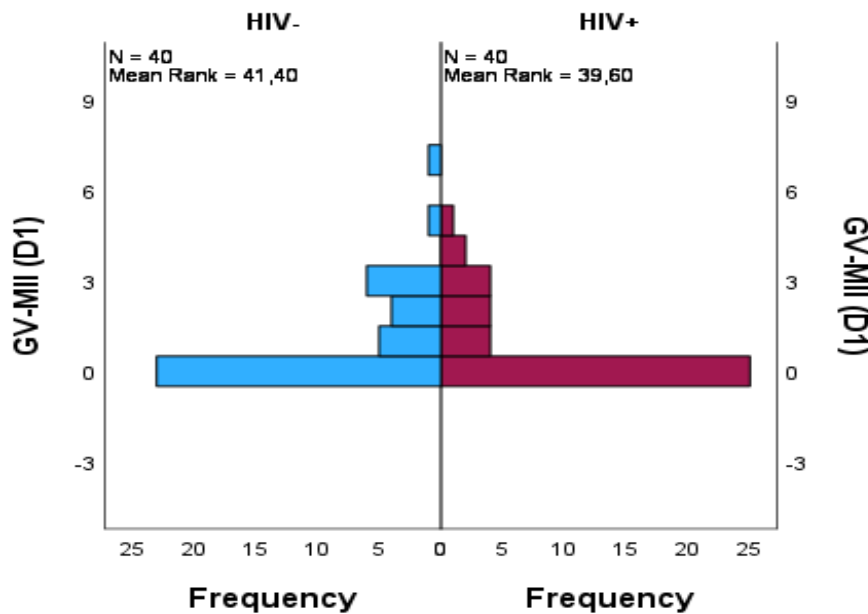


Figure 27: Independent-Samples Wilcoxon Mann-Whitney U Test analysis illustrating the frequency distribution patterns of the number of rescue-IVM oocytes that were initially immature (GV oocytes) on the day of oocyte retrieval, but matured following extended culture, between HIV-infected and HIV-uninfected women. P-value 0.695.

4.6.3 Fertilisation and Embryo Development

Fertilisation was assessed at +/- 17 hours after insemination, and the results are presented in Table 25; these were compared by HIV serostatus. Since the insemination procedure was ICSI, the fertilization rate was calculated by dividing the number of fertilized eggs by the number of total injected eggs- MII oocytes (Rao *et al.* 2022:3). An independent-Samples T-test analysis was done which analyzed the mean fertilization rates in each group and the results as shown in Table 25 show that there were no statistically significant differences (all p-values ≥ 0.05) in the mean numbers of fertilized oocytes between the HIV infected and HIV uninfected women.

These results exclude one patient whose egg was immature even after an extended 24-hour incubation period and all the oocytes that were cryopreserved.

Table 25: Fertilisation rates observed between HIV-infected and HIV uninfected women.

Group Statistics						p-value
	HIV STATUS	N	Mean	Std. Deviation	Std. Error Mean	
Ratio of fertilisation	HIV-	40	0.6315	0.33759	0.05338	0.175
	HIV+	40	0.5259	0.35201	0.05566	

Embryo quality evaluation and grading (Appendix K) was performed by a qualified embryologist (the principal researcher) on the chosen day of transfer or embryo

cryopreservation for surplus embryos or in freeze all cycles, and the best quality embryo/s were selected for transfer and/or cryopreservation. Embryos that were deemed not to be of good quality were discarded. Embryo transfer or cryopreservation was done on either day 3 (cleavage stage embryos) or day 5 (blastocysts). Selection of the day of embryo transfer was at the embryologist's/physician's discretion.

Embryo grading was based on the Gardner grading system developed by Professor D. Gardner (Gardner and Schoolcraft 1999:385) (Appendix K). Day 3 embryos- Cleavage stage embryos, were graded based on the quantity, size and homogeneity of the proliferating cells, and the extent of fragmentation observed; day 5 embryos- Blastocysts, were scored by assessing blastocoel enlargement, and the distinction and characteristics of the inner cell mass (ICM) and trophectoderm cells (TE) (Shukla *et al.* 2022:9-14).

The quantity of preimplantation embryos to transfer to the recipient female was selected by the receiving patient after counselling with the clinician, embryologist, and the support of the partner. Table 26 and Table 27 show the number and quality of the embryos observed on the day of embryo transfer to the recipient female between the HIV-infected and HIV-uninfected women.

Table 26: Distribution analysis showing the quality and the number of embryos transferred on the day of embryo transfer (Day 3/Day 5) in the population of women living with HIV.

Number+ embryo grade			
	Day of transfer		
	Day 3	Day 5	Total
1-8II	1	0	1
1-6I	1	0	1
2-3CB, 4AC	0	1	1
2-3BB, EB	0	1	1
2-Morula, 8II	0	1	1
2-4AA, 4AA	0	1	1
2-5AA, 3CC	0	1	1
2-5AB, 4AB	0	1	1
2-8II, 8II	1	0	1
2-9II, 7II	1	0	1
2-8II, 8II	1	0	1
2-3BB,4BB	0	1	1
2-Morula, 9II	1	0	1
2Morula, Morula	0	1	1
2-4BB, EB	0	1	1
2-3AB, 3AB	0	1	1
Total	n=6	n=10	n=16

Table 27: Distribution analysis showing the quality and the number of embryos transferred on the day of embryo transfer (Day 3/Day 5) in the HIV-negative women.

Number+ embryo grade			
	Day of transfer		
	Day 3	Day 5	Total
1-8II	1	0	1
1-8II	1	0	1
2-5AA, 5AB	0	1	1
2-5AB, EB	0	1	1
2-3BB,4BB	0	1	1
2-4AA,4AA	0	1	1
2-4AB, 4AC	0	1	1
2-4AA, 10II	0	1	1
2-12II, 10II	1	0	1
2-6II,7II	1	0	1
2-Morula, EB	0	1	1
2-Morula, EB	0	1	1
2-Morula, 8II	1	0	1
2-Morula, 10I	1	0	1
Total	n=6	n=8	n=14

Based on the data collected on all the embryos obtained during the investigation, using the frequency cross-tabulation and Pearson Chi-Square tests, an assessment was done to compare the quality of the embryos attained between the HIV-infected females and HIV uninfected women. The result of this analysis is presented in Table 28. a, 28. b and 28. c. These results were interpreted based on a study by Zilberberg *et al.* (2021:1084) who grouped and graded embryos based on the simplified Society for Assisted Reproductive Technology (SART) grading system. This system classified day 3 cleavage embryos as: “Grade I—embryos with even blastomeres and no cytoplasmic fragmentation, Grade II—embryos with ≤10% cytoplasmic fragmentation, Grade III—embryos with 10–25% cytoplasmic fragmentation, Grade IV—embryos with 25–50%% cytoplasmic fragmentation, Grade V—embryos with ≥50% cytoplasmic fragmentation; and blastocysts as ‘good’- grade AA or AB blastocysts, ‘fair’- grade BB, BC, BA and AC blastocysts and ‘poor’- grade CC or CB blastocysts”. Solely for this study, the embryos were combined- day 3 and day 5, and based on the SART classifications, embryos were grouped as ‘good’, ‘fair’ and ‘poor’.

When the entire study population's embryos were grouped based on the SART categories and then a comparison was made between the HIV-infected women and HIV-uninfected women, no statistically significant differences (all p-values ≥0.05) were found in the quality of embryos between the two types of women.

Table 28.a: SART grading 'Good' embryo frequency distributions between the HIV-infected and HIV-uninfected women.

		Category: 'Good'			Total	p-value
		0-2	3-5	≥6		
HIV Group	HIV-	21	6	2	29	0.284
	HIV+	26	2	2	30	
Total		47	8	4	59	

Table 28.b: SART grading 'Fair' embryo frequency distributions between the HIV-infected and HIV-uninfected women.

		Category: 'Fair'			Total	p-value
		0-2	3-5	≥6		
HIV Group	HIV-	21	5	3	29	0.558
	HIV+	23	6	1	30	
Total		44	11	4	59	

Table 28.c: SART grading 'Poor' embryo frequency distributions between the HIV-infected and HIV-uninfected women.

		Category: 'Poor'			Total	p-value
		0-2	3-5	≥6		
HIV Group	HIV-	14	11	4	29	0.468
	HIV+	12	10	8	30	
Total		26	21	12	59	

An analysis was conducted to show the patterns of gamete and/or embryo cryopreservation across the entire study population. These results are shown in Table 29. The results in this table suggest that there is no significant pattern in gamete and embryo cryopreservation between HIV positive and HIV negative participants, meaning that HIV serostatus does not influence the decision to cryopreserve samples. Therefore, cryopreservation of samples could be due to other factors.

The selection of embryo cryopreservation day is most frequently at the embryologist's and clinicians' discretion, and this is done on either day 3, day 4 or day 5 of embryo culture. This result section included patients who froze all their embryos (n=14 HIV positive participants and n=17 HIV negative participants) and had no embryo transfer at all. Some participants had an embryo transfer and froze excess embryos (n=13 HIV positive participants and n=11 HIV negative participants). In some cases, for various reasons such as certain diseases, some medical conditions requiring surgical interventions, patient-related factors, certain socio-economic factors and male factor-caused issues, patients will opt for fertility preservation (oocyte freezing). These types of cases in this study are presented in Table 29 under the day 0 row.

Table 29: Number of patients who froze surplus embryos and had freeze-all cycles where all embryos were frozen. Categorised according to HIV serostatus and day of cryopreservation.

		HIV STATUS		Total
		HIV-	HIV+	
DAY OF CRYOPRESERVATION	DAY 0	7	9	16
	DAY 3	5	4	9
	DAY 4	2	0	2
	DAY 5	23	23	46
Total		37	36	73

4.7 Conclusion

Since this research study comprised a population of women undergoing ART procedures, and outcomes were compared based on HIV serostatus, it was of interest to assess the group of women who did not have any known conditions that may alter their IVF outcomes and only compare them against each other based on their HIV status. This was an additional attempt to highlight any effect caused by the HIV disease itself. The supplementary statistics section assessed the original research study's aims and objectives only in women with the same infertility diagnosis, one that is known not to be an ovarian stimulation confounder, tubal factor caused infertility.

Based on the results presented in this section, this study was able to show that when the entire population was grouped by a single diagnosis (tubal factor infertility) and compared by serostatus, there were no statistically significant differences (all p-values > 0.05) in follicle growth, total oocytes retrieved, and the number of mature-MII oocytes observed.

Furthermore, because this research study investigated oocytes, which are merely the female gametes, it became of interest to extend the study into presenting results of the rescue-IVM process (this is routinely conducted at the ART facility where the study was conducted), fertilisation rates and embryo development findings of these oocytes obtained during the investigation. The study showed that the number of mature-MII oocytes acquired following rescue-IVM, the fertilisation rates and the quality of the preimplantation embryos observed between the two groups were similar.

Lastly, HIV serostatus did not influence the decision to cryopreserve samples as observed in Table 29.

CHAPTER 5

DISCUSSION

This research study's rationale was developed based on observations from IVF laboratory practice and results from a fraction of the existing Sub-Saharan African literature (Cassim and Mohamed 2018, Eldib and Tashani 2018, Udjo 2018, Ombelet and Onofre 2019, Dwyer-Lindgren *et al.* 2019, Lucas *et al.* 2021 and Nik-Hazlin *et al.* 2022) on infertility and HIV infection in women. While assisted reproduction opens the possibility for infertile couples to have biological children, some couples still face challenges with procreation even with assistance from ART (Santulli *et al.* 2016:1084). Infertility is a major health issue on its own, and many factors are implicated in female infertility, among these are BBVs such as HIV (Yahya *et al.* 2018:179). The complexity of the viral invasion and its multiple effects on normal bodily functioning make the HIV-infected community very fragile for consistent probing for research purposes. Furthermore, the need for immediate treatment following diagnosis (World Health Organization 2015:250) adds to the unfeasibility of assessing the direct viral invasion effect on fertility. Even though attempts have been made to shed some light on the association between infertility and HIV infection, developing countries such as South Africa still have a paucity of literature on HIV infection and female fertility potential (Botha, Shamley, and Dyer 2018:9, Ombelet and Onofre 2019:68, Eldib and Tashani 2018:38 and Yahya *et al.* 2018:178). The knowledge gap, particularly in developing regions, propels the need for more studies, such as the present study, to be conducted, to provide better treatment to the infertile community living with HIV if the need exists.

This study aimed to provide a comparative analysis of the mature and immature oocyte yields in oocyte cohorts obtained from HIV-infected females against HIV-uninfected women of reproductive age, post-controlled ovarian hyperstimulation (COH). Additionally, the study examined the potential influence of factors associated with HIV infection, infertility, and ART, such as age, BMI, viral load, CD4+ lymphocyte counts, antiretroviral medication intake, ovarian stimulation protocols and ovulation trigger injections on oocyte maturation outcomes. To achieve this, oocyte cohorts from a group of HIV seropositive women and HIV non-infected controls in their childbearing years and attending an ART clinic were evaluated and compared, and their COH-ICSI cycle outcomes were recorded. The aim was to observe if oocyte maturation, oocyte retrieval rates and follicular growth post-COH may be impacted by the HIV infection, HIV associated and patient-related factors, thus promoting infertility.

5.1 Oocyte Maturation Rates in HIV Infected and HIV Uninfected Women Attending ART

The number of harvested oocytes is directly proportional to the chance of achieving a live birth (Esteves *et al.* 2020:1), this makes the optimization of OH in ART one of the most important steps (Guan *et al.* 2021:2). In fresh cycles, the retrieval of at least 11 oocytes promotes live birth rates and cumulative live birth rates require a minimum retrieval of 20 oocytes (Magnusson *et al.* 2018:61).

Evaluating both the quality and quantity of the oocytes aspirated from a patient following COH is clinically relevant since both these factors play a role in live birth rates. Developmentally competent oocytes would have undergone the necessary procedures *in vivo* to attain complete developmental potential and thus will have the capability to fertilise-be activated by the spermatozoon, and could develop into embryos for implantation (Robker, Hannebold and Russell 2018:3216). This is achieved by the ovarian follicular microenvironment and hormonal signalling channels that start and mediate the process of oocyte development, maturation, and release from the follicles (Richani and Gilchrist 2018: 2).

Competent oocytes are mature MII oocytes. For an oocyte to reach full developmental competence, it must undergo multiple lengthy processes timeously and eventually achieve both nuclear and cytoplasmic maturation (Palhares *et al.* 2022:962). Nuclear maturation encompasses the process of meiosis that ensures correct chromosome arrangement, and cytoplasmic maturation describes the oocytes' ability to assemble the required mitochondria, enzymes and extracellular matrix components for fertilisation and subsequent embryo development (Jiang *et al.* 2023:5). This process occurs during the oocyte's development in the follicle and by the time ovulation occurs, the oocyte should by all definition be developmentally competent-mature.

While attempts are made to successfully reach the goal of OH through proper selection; adherence and timing, in some cases, oocyte cohorts will have approximately 10%-30% immature (MI+GV) oocytes (Braga *et al.* 2020:70). This further aggravates infertility, and in some facilities, the patients' immature oocytes would be provided with extended *in vitro* culture (rescue *in vitro* maturation-R-IVM), or an entirely new cycle would be done. An extended culture period of 24 hours *in vitro* in normal culture media allows for the conversion of the oocytes from the immature to the mature phase (Palhares *et al.* 2022:962 and Esbert *et al.* 2024: para. 1 line 9).

The rationale for this study is based on past studies that have found lowered fertility rates among people living with HIV. This observation has been seen in the natural setting and the setting of IVF. The lowered reproductive potential observation has been associated with menstrual cycle abnormalities (Scherzer *et al.* 2017, Zanni *et al.* 2020, King *et al.* 2020 and Saxena and Ghumman 2020), hormonal imbalances (Yahya *et al.* 2018, Kalem, Şimşir, and Var 2019 and Merlini *et al.* 2021) and ovarian dysfunction (Snider and Wood 2019 and Cassim and Mohamed 2018). The functional capacity of the ovarian reserve becomes important when hormonal and ovarian dysfunctions are mentioned, since the ovarian microenvironment governs and is mediated by a variety of hormones, especially sex hormones.

The ovarian reserve is described as the number and quality of oocytes a woman has, and hormones such as AMH, FSH and AFC measurements are widely used quantifiers of the ovarian reserve and help direct and predict OH outcomes (Sharma and Chakrabarti 2019:5-6).

Any impact on ovarian function is commonly seen when conceiving is desired, and past studies in ART investigated ovarian dysfunction by assessing the ovarian reserve through its biomarkers such as AMH, FSH and AFC. To date, none of the existing studies that have assessed the status and functioning of the ovarian reserve have reached a consensus

concerning the impact of HIV infection on female fertility. It is beneficial to assess the level of the ovarian reserve as it guides ART; however, also assessing the quality of the oocytes that develop from within the follicles and any factors that may alter oocyte quality is also important and beneficial for the success of ART.

Since oocytes need to undergo both nuclear and cytoplasmic maturation to obtain the ability to fertilise and thus provide sufficient embryos and increase the chance of achieving a live birth, it is not enough to only measure the level of the ovarian reserve, especially in the context of ART, where oocyte maturity is of importance. This is why this study focused on evaluating oocyte number and oocyte maturation- observing nuclear maturation in oocyte cohorts from women living with HIV against women free from the virus.

The aim was to add to the information currently available on this fragile community by not only providing education on the importance of the measurement of the number of oocytes a patient can have, but also shining some light on the impact of oocyte maturity status, thus helping to realise the realistic probability of ART success in this population. This will best serve patients during first or repeat counselling so that better plans that suit the patient are made and patient expectations are directed accordingly.

In this study, the distribution of oocyte cohorts retrieved from the HIV-infected versus HIV-uninfected women is depicted in Figure 17. In the HIV-infected women (Table 6), the means of MII, MI and GV stage oocytes between the two groups showed comparable rates. This means that within each group, oocyte maturation patterns were similar, despite the participants having different HIV sero-status. Furthermore, based on an analysis of the frequency distribution patterns observed in figures 18, 19, 20, 21 and 22 between the HIV-infected and HIV-uninfected women, this study showed that there are no statistically significant differences when comparing the mean number of follicles (p-value 0.794), total oocyte retrieval rates (p-value 0.686), MII oocyte numbers (p-value 0.429), immature- MI oocyte numbers (p-value 0.759) and immature- GV oocyte numbers (p-value 0.753) between the HIV-infected and HIV-uninfected women.

Additionally, independent-samples median test analyses' (Table 14, Table 15, and Table 16) revealed no statistically significant differences in the means obtained for mature- MII oocytes (p-value 0.264), immature- MI oocytes (p-value 0.352) and immature- GV oocytes (p-value 0.258) in oocyte cohorts aspirated from the HIV infected and HIV uninfected women following COH. The results found in this study, therefore, confidently show that the number of mature and immature oocytes in oocyte cohorts aspirated from reproductive-aged women is similar regardless of HIV serostatus. In essence, following COH, oocyte cohorts from females living with HIV do not have a higher incidence of immature oocytes than mature oocytes compared to oocyte cohorts from HIV-uninfected women. Oocyte maturation asynchrony, therefore, does not seem to be associated with HIV infection.

This research study aimed to address oocyte heterogeneity among females living with HIV and HIV uninfected women following ovarian hyperstimulation in ART. The results of this study have shown that women living with HIV do not suffer lower fertility rates due to producing an abnormally larger number of immature (MI+GV) oocytes following COH than women living without the virus undergoing the same IVF interventions.

This result, however, was anticipated and could be explained by the population's characteristics, being that this research study only included patients of reproductive age with

good ovarian reserves- AMH > 1.22ng/ml. These characteristics (excluding HIV serostatus) generally place a female at greater odds of obtaining a good number and quality of oocytes.

Interestingly, some patients were hyper-responsive (excessive total oocyte number and MII oocyte number) (Table 5) despite being advanced in age and living with HIV. This phenomenon has been observed in one previous study (Zhang *et al.* 2019:7) but could not be explained, such as in this study.

This study showed that oocyte retrieval rates and MII oocyte numbers in the HIV-infected women of reproductive age and HIV-uninfected controls were comparable. However, when the entire population was assessed, age was significantly associated with oocyte retrieval rates (p-value 0.054) and mature-MII oocyte rates (p-value 0.037). This result was expected since age is generally associated with a fecundity decline (Moghadam *et al.* 2022:105). This means that as a female grows older, her probability of conceiving without difficulties reduces. Moreover, a significant trend was observed wherein older women (>35 years of age) living with HIV had significantly fewer total oocyte numbers and MII oocyte numbers (Table 24. a, 24. b and 24. c). These results were surprising since they seemed to be a one-sided significant decline in fertility in women living with HIV. This observation could not be elucidated in this study. This suggests that this observation could be related to other HIV-associated factors or the HIV infection acting through a particular pathway that is yet to be investigated. As such, a study by Van Ommen *et al.* (2023:776) proposed that immune ageing may play a more significant role than we know, and this lower oocyte yield observed in this group may be justified by this phenomenon.

Importantly, the HIV-infected group in this study were all on antiretroviral therapy with negligible viral load values and had acceptable CD4+ lymphocyte counts. This made it difficult to investigate the impact of these variables on oocyte maturation. Furthermore, no association was found between BMI (Table 22. a, 22. b and 22. c), ovarian stimulation protocol type (Table 17), trigger injections used (Table 18) and oocyte maturation asynchrony between women living with HIV and women living free of the virus.

In conclusion, some studies have found lower fertility potential and lower ovarian reserves in females living with HIV attending ART compared to HIV-uninfected women (Marston *et al.* 2017, Mataro *et al.* 2017, Vankerkem *et al.* 2017, Cassim and Mohamed 2018, Snider and Wood 2019, Liu *et al.* 2020 and Vianna *et al.* 2021). In contrast, this present study found that following ovarian stimulation, HIV-infected women stimulated at similar rates to HIV-uninfected women, and comparable OH outcomes were found. The observation made in the older women living with HIV is confusing since the result is statistically significant within the single group; however, the result seems to be insignificant when the two groups are compared. This may be due to the use of an arithmetic mean for analysis of the entire population.

Overall, it could be that in 'healthy' (undetectable viral load, normal CD4+ lymphocyte counts with consistency and proper intake of ART medication) women living with HIV, age is the major independent factor that mediates ovarian reserve depletion or perhaps there are some other HIV-associated factors that play a role in ovarian ageing that could not be elucidated in this study and are visible over a certain period (Kotlyar and Sneifer 2021). While these results are statistically accurate, caution should be taken in using them for the entire population of women living with HIV due to the small sample size in this research study.

5.2 Relationship Between HIV Infection (Viral Load) and Female Fertility

HIV infection impacts the ovarian reserve more harshly than other chronic viral illnesses (Bourdon *et al.* 2021:1073), and approximately 20% of the infertile individuals in South Africa are living with HIV (Yahya *et al.* 2018:179).

Several publications exist that have investigated the impact of HIV infection on fertility potential in Africa and South Africa (Udjo 2018, Yahya *et al.* 2018, Johnson *et al.* 2020, Mbita *et al.* 2019, Cassim and Mohammed 2018 and Iyer *et al.* 2019).

In people living with HIV, the adherence to and correct use of antiretroviral treatments drastically reduces viral loads by halting the rapid viral replication that follows the invasion. With undetectable viral loads (the number of viral particles is below detectable levels in the blood) and the absence of genital infections, viral transmission to the next person is limited and nearly impossible (Baza *et al.* 2019:1558).

In this study, all patients (n=40) living with HIV were required by IVF standards to undergo blood tests to measure their viral load levels, and the result had to be undetectable before commencing IVF (Ethics Committee of the American Society for Reproductive Medicine 2021:861). Due to all patients having an undetectable viral load, this research study was unable to evaluate the direct relationship between varying degrees of viral loads and female fertility. However, this study managed to provide a comparative analysis of the total oocyte number and MII oocyte numbers retrieved from the HIV-infected females against the HIV negative controls. Tables 14, 15 and 16 showed comparable oocyte maturation rates between the women living with HIV and the HIV uninfected controls. Although the results are limited, this is the only way this study could show that living with HIV does not automatically predispose an individual to poorer ART outcomes compared to HIV-uninfected women of similar age.

Studies by Baza *et al.* (2019:1560) and Lucas *et al.* (2021:639) have studied the relationship between HIV and fertility rates, and none of these studies found significant differences in the probability of conception between women living with HIV and uninfected women of the same age group. The results obtained in the current study are like those of previous studies, proving that when the viral infection is well managed, fertility is not compromised. A scientific reason would be that, since infection with HIV disrupts normal immune function (Wadkar *et al.* 2022:1) and immunity plays a role in fertility potential (Ehsani *et al.* 2019:275), when PLWH are not immunologically compromised, their fertility potential is not negatively affected.

There have also been some studies that have studied the effect of the period of viral infection on female fertility (Marston *et al.* 2017). Unfortunately, this study could not add any information on the impact of the duration of HIV infection on female fertility because most patients did not really know when they were infected and could only remember the date of initial diagnosis. This could potentially have created biased statistics and not provided applicable information regarding the subject. Furthermore, no information was available on the number, if any, of participants who at any point in their lives progressed to the most advanced stage of HIV, AIDS.

The build-up of the virus and the fast decline in immune function increase susceptibility to other infections and diseases (Wadkar *et al.* 2022:1). According to Cohen *et al.* (2019:28)

and Davey *et al.* (2019:11), obstetric statistics show a higher prevalence of HIV infected women presenting with an STI. In this study, of the 40 HIV-infected women, only 10 patients mentioned remembering contracting an STI at some point in their lives; however, none of these patients mentioned contracting the infection when they were already diagnosed with the virus. In this study, no laboratory-measured prevalence data on STIs in the HIV-infected group were collected. Therefore, this study cannot add any valuable information on the prevalence of STIs in the HIV-infected population due to the limitations in the collected data. Moreover, since the data on the likelihood of contracting an STI in the HIV population was not derived from any blood test results but from patients themselves, it would have been futile to even attempt to study the small sample that mentioned contracting an STI as the result would have been unreliable, biased, and not a true reflection of the prevalence rate.

In conclusion, the limitation in this study's data (no variations in viral loads) made it difficult to conduct a comparative analysis to assess the direct impact of HIV infection (by viral load levels) on total oocyte number and oocyte maturation. Future investigations into the population of PLWH would be interesting if they evaluate oocyte number and oocyte maturation rates at various points of infection with HIV, such as when they are initially diagnosed, when they initiate ART, at any time if the ART regimen is changed, and at a minimum of 6-month intervals between taking the antiretroviral medication. In addition, the viral load and CD4+ lymphocyte count would also be measured at every set interval. This would provide a more in-depth and broad analysis of the pace at which fertility declines, if it does. At least then, earlier attempts at reversing the damage, if possible, would be made.

5.3 Relationship Between CD4⁺ Lymphocyte Counts and Female Fertility

The infiltration and rapid mutation of HIV in immune cells destabilise and weaken the body's natural defence mechanism, immunity (Brenna and McMichael 2022:38).

Measuring the CD4+ lymphocyte counts, a prerequisite for all HIV-infected individuals before initiating IVF treatments and interventions, allows for the assessment of the individual's CD4+ T-cell reservoir. A couple where one or both partners are living with HIV may be considered for ART provided, they are on antiretroviral therapy, have undetectable viral loads and an elevated CD4+ lymphocyte level (Ethics Opinion 2018:88). The WHO guidelines in 2013 had recommended that adults (> 19years old) diagnosed with HIV should start antiretroviral therapy immediately when the initial diagnosis is made and if the CD4+ lymphocyte cell count is ≤ 500 cells/mm³, with the amended guidelines in 2015 stating that all people living with HIV should start treatment when diagnosed, irrespective of their CD4+ lymphocyte cell counts at the time (World Health Organization 2015:25).

CD4+ lymphocyte cells have been observed to have an impact on fertility hormones, thus contributing to infertility (Amadi and Akanno 2023:1), and they have also been found to play a role in the ability to achieve a live birth, and the risk of repeated pregnancy loss (RPL) (Modzelewski, Kajdy and Rabijewski 2019:109-110).

All the HIV-infected patients (n=40) in this study had normal CD4+ lymphocyte counts (≥ 500 cells/ μ L). The mean CD4+ Lymphocyte count was 749.3 cells/ μ L. In this research study, there were no patients with low CD4+ lymphocyte counts; owing to this limitation (lack of variation), the impact or relationship between CD4+ lymphocyte counts and female fertility, unfortunately, could not be established in this study.

Embryo implantation essentially is a cell invasion or introduction of a foreign body into the human body, and like all other invasions, the body's defence system will attempt to fight off the invasion or provide a serene environment that allows the cell to proliferate and flourish. Therefore, it would be reasonable to allude that controlling the utilisation and regeneration of the CD4+ lymphocyte reservoir may potentially play a significant role in promoting environments suitable for embryo implantation, thereby increasing the probability of achieving a live birth (Modzelewski, Kajdy and Rabijewski 2019:109-110 and Ehsani *et al.* 2019:275). Furthermore, according to a publication by Akinbami *et al.* (2015:116), advancing age and pregnancy have been reported to cause a significant decline in the quantity of CD4+ cells. This, however, does not reduce the necessity of managing the CD4+ cells at an acceptable level, as this provides benefit to individuals' health and promotes fertility.

5.4 Relationship Between Antiretroviral Therapy and Female Fertility

Antiretroviral therapy is a set of drugs that were developed for the treatment and management of HIV and began with a single drug treatment, Zidovudine, around the year 1982 (Zuger 1996). In 1993, a non-nucleoside reverse transcriptase inhibitor (NNRTI) called Nevirapine was introduced. This drug stopped the spread of the virus and was suitable for both adults and children, and prevented mother-to-child transmission when taken at birth (Akorn 2021). Nevirapine exists in tablet and liquid form and is often used with other antiviral drugs (HIVinfo.NIG.GOV. 2024). Around the year 1994, dual nukes (dual therapy) were initiated, which meant that a combination of drugs could be taken at a certain time as 2 tablets or 1 (combined drugs), and these drug combinations were noted to be effective at combating the virus with minimal adverse effects (I-base 2022).

Highly Active Antiretroviral Therapy (HAART) and Protease Inhibitors (PI's) were developed around 1996; HAART is the use of more than 2 drugs simultaneously to prevent the virus from making copies of itself and causing worsening of the disease and works on the concept that if one drug is ineffective there are at least several other drugs executing the same action (Eggleton and Nagalli 2023), while PI's work through enzymes to change the physiology of the viral replicas thus making them completely ineffective once produced (HIVinfo.NIH.GOV 2021).

Around the year 2008, a TLE fixed-dose single tablet (combined tenofovir disoproxil fumarate-TDF, Lamivudine and Efavirenz) was introduced as a form of first-line treatment for HIV type-1 (Adiningsih, Widiyanti and Rokhmad 2023:158). This means that the 3 drugs were combined into a single tablet at fixed doses. Between the years 2010-2019, the development of Pre-exposure Prophylaxis (PrEP) was seen, and PrEP lowered the risk of

being infected with HIV when taken daily by HIV uninfected individuals (National Institute of Allergy and Infectious Disease 2024).

In 2018, TLD, a more potent, faster-acting, with fewer side effects version of TLE was developed, this is a combination of 2 Nucleoside reverse transcriptase inhibitors (NRTIs)- TDF and Lamivudine, and replaces the non-nucleoside reverse transcriptase (NNRTI)- Efavirenz with an Integrase Strand Transfer Inhibitor (INSTI)- Dolutegravir (DTG) (LINKAGES 2019: n.p). Recent and future developments and advancements include long-acting injectable PrEPs, dapivirine vaginal rings, dissolvable vaginal films, rectal douches and a product that incorporates PrEP into a contraceptive (National Institute of Allergy and Infectious Disease 2024).

This study investigated the impact of the affiliation between ART outcomes and a blood-borne virus, HIV. While the focus was to assess the effect of the virus on fertility to provide better assistance and care to this already fragile community, one could not neglect to at least attempt to observe the impact, or lack thereof, of the medication developed to counteract the negative effects of the infection. Investigating the treatments for a disease or infection, especially a chronic one, assists physicians in counterattacking possible short-term or long-term toxic effects of drug interactions.

More than half of the HIV-infected population is receiving antiretroviral treatment in Eastern and Southern Africa (Mbita *et al.* 2019:2). This study attempted to investigate the correlation between antiretroviral use and fertility. The aim was to assess the association between IVF success and antiretroviral treatment intake in women living with HIV using HIV-uninfected women as controls.

For an individual or couple living with HIV to be eligible to undertake any IVF procedures, they are required to be on ART for a minimum of 4-6 months and have undetectable viral loads before initiating IVF treatments and interventions (Ethics Committee of the American Society for Reproductive Medicine 2021:861). This is important because it prevents partner-to-partner and mother-to-child transmission of the virus and ensures the protection of the medical personnel who will handle the patient's blood samples from possible transmission. Based on guidelines from reputable committees, in this study, all the participants (n=40) living with HIV had been using ART for variable periods, and an undetectable viral load had been achieved at the time of starting IVF treatments, which provided the raw data. The lack of raw data relating to participants' health or fertility status before or after antiretroviral therapy resulted in the inability of this study to provide a comparative analysis of the role of antiretroviral therapy regimens and the length of taking antiretroviral medications on female fertility. Therefore, this study could not provide substantial evidence-based information in the current literature.

Current literature, however, has conflicting notions regarding female fertility potential and antiretroviral therapy use, and while some studies say fecundity improves significantly with the proper use of ART (Mbita *et al.* 2019:2), other studies have mentioned that no differences exist in reproductive outcomes whether a female is using ART or not (Johnson *et al.* 2020:1953). Furthermore, studies by Awodele *et al.* (2018) and Kitsou *et al.* (2020) have

investigated the relationship between different types of ART regimens and female fertility. These studies found that some regimens are toxic to the ovarian reserve (number and quality of oocytes) in females. This present study, however, cannot add any significant information regarding the impact of various ART regimens on female fertility potential because of a lack of raw data and the study's population size.

Limitations in this study prevented the assessment of the impact of the length of antiretroviral use on female fertility. One of the HIV positive participants, however, had only started antiretroviral medication just before commencing IVF. The other participants had been on antiretroviral medication for at least a year; one patient's result was similar to those who had been on antiretroviral medication longer, and HIV negative women. Future evidence-based studies could potentially investigate female fertility and the duration of antiretroviral therapy use.

In conclusion, studying the relationship between ART and fertility in humans is challenging due to health implications related to the impact of the viral invasion and the need for prompt action once diagnosed. The effects of antiretroviral treatments on female reproduction have been explored, as shown in studies by Awodele et al. (2018) and Kitsou et al. (2020); however, these studies were conducted on rodents that were not HIV-infected. Nevertheless, these studies have found a positive link between antiretroviral drug use and female infertility. This finding is significant because the medication intended to improve the quality of life for an HIV-infected woman may also pose a threat to her fertility potential.

5.5 Relationship Between Ovarian Stimulation, Ovulation Trigger Injection and Female Fertility

Ovarian stimulation is fundamental to the success of ART. Through OH, it is possible to obtain many oocytes with good quality, thus increasing the chance of successfully achieving a live birth (Tso *et al.* 2021:205). The optimisation of OH rests highly on the selection of the best type and dose of the exogenous gonadotropin suited to the patient (La Marca and Sunkara 2014:126).

According to the African Network and Registry for Assisted Reproductive Technology (ANARA), in the year 2021, 3574 stimulation cycles were initiated in South Africa (ANARA Africa 2021). Unfortunately, at the time of writing this dissertation, the researcher could not find information regarding the years 2022 and 2023, as the data is currently pending. The information would have been beneficial since the two-year unpublished data includes the period of this study. This study only included 80 stimulated cycles.

The types of exogenous gonadotropins for ovarian stimulation used in this study were Gonal-F®, Menopur®, Menopur® pre-mix, and Pergoveris®. Initial doses ranged between 150IU, 225IU, and 300IU. 52.5% of patients in this study used Gonal-F® for ovarian stimulation, 27.5% of patients used Menopur®, 16.25% used Menopur® pre-mix, and 3.75% used Pergoveris® as shown in Table 7.

When the study population was matched based on the type of exogenous gonadotropin used and compared by HIV serostatus, the independent-Samples t-test analysis yielded no significant differences (all p-values > 0.05) in outcomes such as follicle number, total oocyte number and MII oocyte number (Table 17).

To take it a step further, this study evaluated the endometrial growth between the two groups during OH. The endometrial lining is sensitive to hormones produced by the growing follicles and oocytes, therefore, assessing endometrial growth in conjunction with follicular growth can be used to assess response to ovarian stimulation and predict pregnancy outcome (Onogi *et al.* 2020:6). The results obtained in this study show comparable endometrial lining growth between the HIV infected and HIV uninfected groups at the time of triggering ovulation. These results are similar to those observed by Onogi *et al.* (2020 and are associated with a positive clinical pregnancy outcome. All the results presented in this study therefore support and show that the HIV-infected female responds comparably to the HIV-uninfected woman when they are clinically healthy.

According to Paul, Atilan and Tulay (2019:347), the number of stimulation cycles a woman undergoes does not impact oocyte quality and embryo development. Unfortunately, this study did not investigate this, and therefore, no significant evidence-based information can be provided on the association between repeated ovarian stimulation and oocyte retrieval or maturation rates. Furthermore, Vianna *et al.* (2017:7) observed that women living with HIV needed higher doses of gonadotropins and were stimulated longer than HIV-uninfected women. A limitation in this study (raw data not included in stats analysis) meant that this study could not provide any evidence to corroborate or dispute this observation in any way.

The importance of the ovulation trigger in promoting oocyte maturation following stimulation and growth in response to the exogenous gonadotropins meant that to complete this study, an analysis of the association between ovulation trigger injections and oocyte maturity had to be included. Two different types of trigger injections were used in this study, leuprolide acetate trigger- Lucrin® and hCG trigger- Ovitrelle®. Lucrin® was given at a dose of either 40IU or 80IU, and Ovitrelle® was standardly given at 250mcg. The type and dose of the ovulation trigger injection were selected based on follicle number, size, and anticipated response to stimulation by the clinician.

For ovulation triggering (Table 8), 70% of the total study population used Ovitrelle® at a dose of 250mcg, 18.75% of patients used Lucrin® at 40IU, and 11.25% of patients used Lucrin® at 80IU. Upon investigating the association between ovulation trigger use and oocyte retrieval rates and MII oocyte rates, results from patients matched by triggers Lucrin® at 40IU and Ovitrelle® at 250mcg yielded no statistically significant differences in outcomes (Table 18). However, in the category of patients who used Lucrin® 80IU as an ovulation trigger, no results could be obtained due to the sample size of the HIV-infected test group (only 1 patient fell under this category).

In conclusion, the results of this study on ovarian stimulation and ovulation trigger injections showed that when using the GnRH-antagonist protocol, the participants did not exhibit different ART outcomes (response to stimulation, follicular growth, total oocyte number and

MII oocyte numbers) because they were living with HIV. This means that the efficiency of the type and dose of the exogenous gonadotropins and ovulation trigger injections was not influenced by HIV infection. This result was expected since the type of ovarian stimulation protocol used in this study population is the most widely used and recommended for this type of female population.

5.6 Relationship Between Age and Female Fertility

Advancing maternal age is associated with lowered oocyte number and quality (Esbert *et al.* 2024: para. 1 line 1). The pace at which this depletion occurs for most women is governed naturally by age. At birth, a female will have approximately 1 million primordial follicles; this number is reduced to 300000 by puberty and continues to decrease with advancing age (Moghadam *et al.* 2022:105). Primordial follicles are oocyte-containing sacs. Therefore, unlike in males, female gametes cannot be regenerated every short period as women are born with a calculated gamete pool that, whether utilised or not to attain offspring, will gradually deplete over time.

The female reproductive system ages faster than any other organ system in the human body (Machlin *et al.* 2021:1). Both natural ageing and ovarian ageing play a role in female fertility. Infertility prevalence rises with advancing age, at 20-24 years infertility prevalence is 6%, at ages 25-29 years the prevalence increases to 9%, 15% between ages 30-34 years, 30% at 35-39 years and reaches a pinnacle of 40% at ages 40-44 years (Scheffer *et al.* 2018:215).

In some instances, however, some forces can speed up this depletion process, thus leading to the female suffering infertility. Interestingly, these forces or factors that cause a rapid decline in fertility may act independently of age, which is why there are some cases where a young woman will exhibit low ovarian reserve levels despite their age and will therefore seek ART assistance. In the absence of such factors, though, age seems to be the most constant driving force in ovarian depletion.

The ovarian reserve describes the number and quality of oocytes a woman has and can be measured using its biomarkers, AMH and/or AFC (Moolhuijsen and Visser 2020:3361). In ART, while the number of oocytes is important as it increases the chance of a live birth (Malchau *et al.* 2019:176), equally important is the quality of the oocytes obtained, as these oocytes need to be used to create good-quality preimplantation embryos.

Another objective of this study included the assessment of the relationship between age and female fertility potential. This research study investigated an age association with follicular growth, oocyte retrieval rates and oocyte maturation rates in HIV seropositive females and the HIV uninfected control group during their reproductive years (18-39 years).

Over half (52.5%) of the entire population were women ≤ 35 years old, and 48.75% were 36 years and older (Table 11). No significant differences (p-value 0.131) were found in the spread of patients from the total study population across different age ranges.

Table 19 shows that in the entire study population, there is no statistically significant difference (p-value 0.92) in the mean serum-AMH values between the HIV-infected and HIV-uninfected women. When further stratified by age, in the 26–30-year-old group (Table 20), a statistically significant difference (p-value 0.030) was found when comparing the mean

serum-AMH values between the women living with HIV and the HIV uninfected controls. However, the sample size was small (HIV infected: n=3 and HIV uninfected: n= 5) and therefore these results should be interpreted with caution. In the other age groups, in ages 31–35-year-old and 36–39-year-old, no statistically significant differences (p-value 0.374 and p-value 0.461, respectively) were found when comparing the mean for serum-AMH between the HIV-infected and HIV uninfected women. The results presented in Table 20 simply compare the serum-AMH levels between the HIV-infected women and HIV-uninfected women after matching by age, and did not investigate the age impact on serum-AMH, which may be impacted by age.

According to Hsieh *et al.* (2020:1), maternal advancing age mediates the production of AMH. A Pearson correlation coefficient test (Table 23) and a Fisher-Freeman-Halton test (Table 24. a) in this present study agree with Hsieh *et al.* (2020) as the statistical analysis shows that in the entire study population, age is significantly associated with the relationship between AMH-follicle number-oocyte number, oocyte reservoir level, and number of MII oocytes obtained on the day of oocyte retrieval.

Furthermore, in Table 21, when the entire study population was matched by age and compared by serostatus, no statistically significant differences (all p-values > 0.05) were found when evaluating follicular growth rates, oocyte retrieval rates and oocyte maturation rates. When taking into account the fact that serum-AMH levels describe the number and quality of oocytes within a woman, and the study's population characteristics (reproductive-aged women with AMH > 1.22ng/ml and similar serum-AMH mean values across the entire population when matched by age), this result is acceptable.

However, upon assessing the oocyte quantity and quality patterns in each group, women ≥36 years old living with HIV had fewer oocyte numbers and MII oocytes (Table 24. b and 24. c) compared to younger women living with HIV and age-matched HIV uninfected controls. This is an important observation because at least 6-15 oocytes are necessary to achieve a live birth with fewer oocytes associated with poor pregnancy outcomes (Magnusson *et al.* 2018:59) and between 7-10 MII oocytes have been observed to give rise to at least 1 chromosomally normal blastocyst in woman in their mid-thirties (Esteves *et al.* 2020:12). This phenomenon could not be explained unfortunately.

A study by Regan *et al.* (2018:219) revealed that older women present with hormonal imbalances, specifically FSH and inhibin, and this imbalance alters normal follicular growth. This manifests as follicular growth with smaller-sized follicles and fewer granulosa cells caused by receptor signalling faults, and this plays a role in infertility. They, however, did not mention the ages of the patients, and due to a limitation in this study of not testing FSH and inhibin-B, the researcher cannot justify the observation made in this study with the results found by Regan *et al.* (2018).

A study by Cassim and Mohamed (2018) concluded that apart from advancing maternal age, HIV infection negatively affected female fertility by increasing the rate at which the ovarian reserve depletes. They quantified the ovarian reserve based on serum AMH levels and therefore found lower serum AMH levels in women living with HIV. While this study's results contradict the results by Cassim and Mohamed (2018) on HIV causing lower fertility in HIV-infected reproductive-aged women compared to HIV-uninfected reproductive-aged women, the observation made in the older women living with HIV suggests there may be an

impact to some degree on the ovarian reserve by being infected with HIV, which may be governed by age. However, this cannot be elaborated on due to the small sample size and that this was beyond the scope of this current research study.

Even though the outcomes reached were different, this present study and the study by Cassim and Mohamed share similarities, being that both studies were conducted on HIV-infected females undergoing ART, the sample size was similar, and all patients were on antiretroviral therapy with negligible viral loads and acceptable CD4⁺ lymphocyte counts and were of reproductive age.

Furthermore, in the same age category (36-39 years), surprisingly, some patients did exceptionally well and were hyper-responsive (>20 oocytes), despite their age and HIV status. This phenomenon has been described by Zhang *et al.* (2019:7) as older women who have normal AMH results and respond to OS the way younger women would respond. In this study and the other study, this phenomenon could not be elucidated. Moreover, this event is an exception and does not occur in all women of advanced age.

In conclusion, this study did not directly compare the effect of age on AMH and oocyte retrieval and MII oocyte rates but rather provided an age-matched comparison of AMH levels, oocyte retrieval and MII oocyte rates between HIV-infected and HIV-uninfected women undergoing OH. The population type (reproductive-aged women with serum AMH $\geq$ 1.22 ng/ml) in this study promotes the outcome of normal oocyte rates and MII oocyte numbers at retrieval post-ovarian hyperstimulation. This is why it was acceptable to obtain a result of no differences in serum AMH values across the entire study population and comparable oocyte retrieval rates and MII oocyte numbers regardless of HIV serostatus. However, multiple studies (Cassim and Mohamed 2018, Esbert *et al.* 2024 and Moghadam *et al.* 2022) have associated advancing maternal age with reductions in the ovarian reserve, number and quality of oocytes. This begins in the early 30s and quickly progresses by the mid-30s (Lucas *et al.* 2021;639 and Sun *et al.* 2020:1). Therefore, based on these studies, it is acceptable to have a result wherein older women living with HIV presented with lower oocyte yields. However, one could be more inclined to expect such lower oocyte numbers from women in their early 40s and above, and not necessarily in their late 30s (Appendix N), and to not be one-sided, such as presenting only in women living with HIV.

It could very well be possible that there are other factors at play that this research study's investigation could not define and elaborate on. One plausible explanation would be from a study by Van Ommen *et al.* (2023:776) who found that immune ageing significantly impacts serum-AMH levels in women over the age of 35 years, which would then lead to lower oocyte yields. Furthermore, it is widely known that the immune system plays an active role in the ability of the reproductive system to produce an oocyte and support gestation (Shirasuna and Iwata 2017:1); this same immunity naturally declines with advancing age (Lawton 2020:44). Since HIV mainly attacks the immune system causing a rapid decline in function (Durandt *et al.* 2019: S41), it may be that the observation in older women stems from both the natural decline in immunity caused by advancing age, and the impact of HIV on an individual's immunity overtime. This ultimately presents as lower oocyte yields following ovarian hyperstimulation. This promotes the need for even more studies to be conducted in this population, to evaluate whether the observation made exists in a series of patients living with HIV or could have just been random in this study.

5.7 Relationship Between BMI and Female Fertility

An individual's BMI measures their body mass relative to their height (Wu and Wu 2023:1). Since an individual's BMI can contribute to infertility (World Health Organization 2023:3), and BMI has been investigated and associated with HIV and antiretroviral therapy use (Koethe *et al.* 2020:2), this research study investigated a relationship between BMI and fertility potential in HIV infected and HIV uninfected women undergoing IVF treatments. In some instances, at the clinician's discretion, a patient will be asked to consult a dietician to raise their chance of conceiving or maintaining gestation.

Table 12 describes the spread of the study population across all BMI categories, namely, underweight (BMI<18.5), normal weight (BMI 18.5-24.9), overweight (BMI 25.0-29.9), and obese (BMI ≥30). The frequency distribution of patients under each BMI category was similar (p-value 0.145) in the HIV-infected and HIV-uninfected females. It would be possible to assume there would be differences in the weight distributions in one group against the other since immunosuppressed individuals are often advised to eat healthier and control what they introduce into their bodies, while people in the general population often do not follow any diets unless provoked by infertility issues or curb unwanted weight gain. Furthermore, this study found no statistically significant differences (all p-values> 0.05) in outcome patterns (multi-follicular growth, oocyte retrieval rates and MII oocyte numbers) between the HIV-infected group and the HIV uninfected group after patients were matched by BMI category (Table 22. a, Table 22. b, Table 22. c). The results found in this study show that BMI is not associated with oocyte maturation rates.

The result found in this study is in accordance with a study by Donno, García-Martínez and Polyzos (2023:1), which found no relationship between BMI and oocyte maturation rates. However, both this present study's result and the result by Donno, Garcia-Martínez and Polyzos are not in line with earlier studies (Mirabi *et al.* 2017:162 and Bou Nemer *et al.* 2019:409) that found lower oocyte maturation rates to be associated with BMI changes, especially in BMI≥30. These studies, however, could not agree on the exact cause for this, but they hypothesised that it could be linked to changes in follicular fluid components' concentration levels. Their hypothesis may have been rooted in the fact that the follicular fluid contains materials that mediate follicular development, which houses the oocyte and influences its growth and can be influenced by weight changes or fat deposition changes (Piccinni *et al.* 2021: e722). Moreover, a study by Wu and Wu (2023:2-8) found lower oocyte retrieval rates, lower oocyte maturity rates and lower endometrial thickness, suggesting poor ovarian response, in patients with increased weight. No such evidence was found in this study.

In conclusion, while this study and others have found no association between BMI and oocyte maturation, it is still important for patients to be advised to care for their weight gain or weight loss for health reasons. It could be possible that certain food diets significantly impact female fertility through weight fluctuations, and therefore, it would be more beneficial to develop a study with the assistance of a dietician, which will investigate certain food and beverage choices that increase or decrease an individual's weight that may also impact female reproductive potential.

5.8 Relationship Between Serum AMH, Follicle number and Oocyte number

Ageing affects both the quantity and quality of a female's oocyte pool, and while the pace at which this impact occurs cannot be predicted accurately, the oocyte pool can be measured at various stages of life, allowing one to be proactive (Ulrich and Marsh 2019:228).

AMH is the most widely used biomarker of the ovarian reserve (Moolhuijsen and Visser 2020:3361), however, according to Cedars (2022:1512) the flaw in measuring AMH alone is that AMH only quantifies the ovarian reserve and does not describe the quality of the oocytes, which is why it can be used to predict OS outcome but not pregnancy rates.

Existing studies have found conflicting results between HIV and AMH levels (Swann and Murray 2022:255), lower AMH levels were found in HIV-infected women (Cassim and Mohamed 2018:1), and Van Ommen *et al.* 2023:771 found no association between high viremia levels and low CD4⁺ lymphocyte counts and altered AMH levels.

In this study, no significant differences (p-value 0.92) in mean serum-AMH values were found between the HIV-infected females and the HIV-uninfected females (Table 19). This may be due to the population's characteristics. This result agrees with a result by King, Swann, and Murray (2022:255), who also found no association between AMH and HIV infection. What could be confirmed in this study was the positive correlation in the relationship between AMH-follicle number-oocyte number and age in both the HIV-infected women and HIV-uninfected women (Table 23). AMH is produced by the antral follicles; these antral follicles are found in the ovaries, and they contain oocytes, a decline in the ovarian reserve (the antral follicles) can be measured by serum AMH levels (Shrikhande, Shrikhande, and Shrikhande 2020:338). It is widely known that the ovarian reserve depletes with advancing age; therefore, the result in this study, wherein there was a significant association between age and the relationship between AMH, follicle number and MII oocyte number, was expected and is justified.

In another facet, Van Ommen *et al.* (2023:776) found that AMH levels in women over the age of 35 years were more impacted by immune ageing than natural ageing. This could explain the observation made in this study wherein older women (36-39 years) living with HIV had the least number of total harvested oocytes and MII oocytes (Table 24. a, and Table 24. c). As such, another stronger way to investigate the impact of HIV infection on fertility would be to assess the relationship between immune ageing and ovarian ageing by using cellular markers of immune ageing. This could provide a better understanding of possible HIV or immune-associated pressures that perhaps accrue over time and play a role in ovarian ageing. Neither in this present study nor in the study by Van Ommen *et al.* (2023) was the relationship between immune ageing and AMH elucidated.

It is important to note that there are other factors, except HIV and age, that can affect serum AMH levels (King, Swann, and Murray 2022:255). Hence, to test the relationship between AMH and any other variable, confounders always need to be restricted.

In conclusion, the role of HIV infection in infertility is important and continuously warrants more investigation. This research study could not test the role of HIV on serum AMH as the entire test group had undetectable viremia levels and normal CD4⁺ lymphocyte counts, which would have made it impossible to accurately test the varying disease degree impact on AMH. However, it may be possible that disease progression and worsening immune

function influence the ovarian reserve, and this may not immediately be seen but can become apparent over a certain period, as observed in this study. Also, it could be alluded that the medication used to combat the virus may play a more distinct role in the negative impact seen on the ovarian reserve of older patients.

Essentially, it is undeniable that age plays a role in the relationship between AMH, follicle number and MII oocyte numbers, and based on what is already known about female fertility and advancing age, this is acceptable. Furthermore, it is important to know that in this present study, this association was observed among the entire population, regardless of HIV status. The result of this study further adds to the current literature (Kotlyar and Sneifer 2021) that claims that age is the most constant influential factor in infertility.

Lastly, more controlled, larger sample studies are still needed to accurately test the relationship between HIV and AMH or rather, the ovarian reserve. This should be done by controlling AMH confounders and HIV-associated factors that could affect the true relationship reflection.

5.9 Supplementary Statistics

5.9.1 Ovarian Hyperstimulation Outcomes When the Infertility Diagnosis is the Same

Patients with polycystic ovary syndrome (PCOS) generally have elevated serum-AMH levels (King, Swann and Murray 2022:248) and they tend to hyperstimulate (Asada 2018:274) while patients with endometriosis tend to hypo stimulate (Zhao *et al.* 2020:1). Furthermore, patients with PCOS suffer anovulation thus, PCOS plays a significant role in infertility (Tanbo *et al.* 2018:1161).

Infertility can be caused by many factors, one of which is the tubal factor. Tubal factor causing infertility occurs when there is an occlusion, scarring, obstruction or anatomical defect of one or both of a female's oviducts (Sun *et al.* 2020:1). A high incidence of menstrual abnormalities and tubal defects have been observed to be linked with HIV infection (Cito *et al.* 2019:856). Furthermore, according to Yahya *et al.* (2018:180), a study on black women in sub-Saharan Africa revealed a higher frequency of tubal factor-caused infertility in HIV-infected women; Lucas *et al.* (2021:641) found no such prevalence. Nevertheless, a substantial percentage of couples who seek ART commonly suffer from tubal infertility (Abebe, Afework and Abaynew 2020:2).

To investigate this study's aim using another angle. Due to some infertility diagnoses influencing OH outcomes, this additional investigation provided an analysis on the follicular growth rates, oocyte retrieval and MII oocyte rates when the infertility diagnosis was the same and known not to be a confounder to OH. In figures 23, 24 and 25, when all patients' infertility diagnosis was the same- tubal factor caused, no statistically significant difference was found in follicle growth (p-value 0.537), oocyte retrieval rates (p-value 0.745) and mature-MII oocyte distribution (p-value 0.580) across the entire study population.

The distinction was important since this study was essentially evaluating if a pattern of lower maturation rate exists in women of reproductive age living with HIV. Patients attend ART for various reasons and are diagnosed differently, and a diagnosis can have an impact on the outcome of ART (Asada 2018:274 and Zhao *et al.* 2020:1). Therefore, to be able to test a

hypothesis, if confounders are controlled, the outcome is more reliable and a better true reflection of the relationship between two variables can be observed. In conclusion, this study aimed to investigate oocyte asynchrony in oocyte cohorts aspirated from women living with HIV and HIV negative women undergoing ART and this supplementary result proved that even when the diagnosis is similar and does not modify ART outcome, the follicular growth, oocyte retrieval rates and mature-MII oocyte rates between HIV infected women and HIV uninfected women of reproductive age are comparable.

5.9.2 Outcomes in Rescue *in vitro* Matured (R-IVM) Oocytes

Rescue *in vitro* maturation (R-IVM) is a subset of *in vitro* maturation, which allows immature oocytes harvested from stimulated cycles to reach maturity by culturing the immature oocytes in the laboratory. This provides surplus preimplantation embryos for selection on the day of transfer to the recipient female, or cryopreservation for future use (Faramarzi *et al.* 2018:430). Within 24 hours, at least 70% of the previously immature oocytes would have matured *in vitro* when the environment is favourable (Escrich, Pellicer and Meseguer 2018:638). This method has not only provided more preimplantation embryos but has also been found to achieve live births (Qin *et al.* 2022:2). However, according to Yuan *et al.* (2021: e135), caution should be taken when using R-IVM-derived embryos because most have aneuploidy. They recommend genetic testing of all R-IVM embryos.

Nevertheless, rescue *in vitro* maturation is frequently used, although not routinely. The facility where this present study was conducted provides R-IVM to all patients. In the HIV-infected group, post-R-IVM, 36% of the previously immature-GV oocytes had matured, and in the HIV, HIV-uninfected group, 50% of the previously immature-GV oocytes had matured. Similarly, 54% of the previously immature-MI oocytes matured in the HIV-infected group, while 73% of the oocytes were mature post-R-IVM. A study by Esbert *et al.* (2024:8) found a 71.5% maturation rate in R-IVM oocytes (GV+MI) but reported that there are studies that have found 42.5% and 82.6% maturation rates. In this present study, in the HIV-infected group, following R-IVM of all immature oocytes, a 42.67% maturation rate was observed, and in the HIV-uninfected group, a maturation rate of 58.82% was observed. The methods in which R-IVM is conducted differently by different clinics and research studies, therefore, may account for the differences in maturation rates observed.

All the oocytes that had matured following R-IVM were inseminated using the ICSI technique and cultured for use. The results in Figure 26 (MI-MII: p-value 0.590) and Figure 27 (GV-MII p-value 0.695) show there were no significant differences (all p-values > 0.05) in the number of mature-MII oocytes achieved through R-IVM between the HIV infected and HIV uninfected women following ovarian stimulation. This present study, however, cannot comment or add any valuable information on the aneuploidy rate, clinical pregnancy, and live birth rates of these R-IVM-derived oocytes, as this was beyond the scope of this present study's investigation. The total fertilisation rates exhibited in Table 24 included the oocytes that were matured *in vitro*, and no significant differences (p-value 0.175) in fertilisation rates were found in this study.

5.9.3 Fertilisation and Embryo Development

From the moment the spermatozoon enters the egg cell, a cascade of events begins that eventually gives rise to a fertilised oocyte-zygote, which quickly develops into an embryo. The oocyte gains fertilizing and embryo development capabilities during growth in the ovary (Jiang *et al.* 2023:5). For an oocyte to have developmental competence whether *in vivo* or *in vitro*, it should undergo and achieve nuclear maturation-resumption and completion of meiotic division and cytoplasmic maturation- packed with mitochondrial material, cytoplasmic enzymes, and organelles (Braga *et al.* 2020:70). Essentially, numerous cellular and molecular mechanisms intrinsic to the oocyte are essential for fertilization and early embryo development, and these are acquired by the oocyte during development (Reader *et al.* 2017:1). Cell division occurs shortly after the process of fertilization, and the fertilized egg cell now becomes a new cell type, the embryo, and will carry correct combinations of maternal and paternal genetic material (Mohammadzadeh *et al.* 2018:117).

Since most of the oocyte's material is the material from which the embryo evolves, any factors that affect the oocyte's ability to attain full developmental competence could impact fertilisation and subsequent embryo development. One such factor is advancing maternal age, which is associated with a reduction in the quality and number of oocytes (Moghadam *et al.* 2022:105). Female age has also been linked with oocyte-inherent factors that alter fertilisation (Mohammadzadeh *et al.* 2018:119).

Furthermore, according to Wu *et al.* (2019:164) and Sauerbrun-Cutler *et al.* (2020:1665), the sperm mitochondrial DNA (mtDNA) remains active in the oocyte during fertilisation and until early embryo development (4-8 cell stage) after which the embryonic genome overtakes, thus sperm is actively involved in the successful fertilization of the oocyte and has an impact on embryo development. In addition to this, sperm has a 10-15% impact in achieving a live birth (Rao *et al.* 2022:2). While the ICSI technique is associated with higher fertilisation rates compared to the traditional IVF method, the fertilisation rates post-ICSI can be impacted by the damage to organelles caused by the way the embryologist does the technique (Sauerbrun-Cutler *et al.* 2020:1665).

Embryo development is equally important as the process of fertilisation, and, as in the case of fertilisation, some factors can affect embryo development. The inability to successfully achieve a clinical pregnancy has been found by Tomic, Kasum and Vucic (2020:1328) to be associated with embryo quality in at least 30% of cases. Moreover, paternal age is another factor that is known to affect embryo development, particularly during early embryo development (Van Opstel *et al.* 2021:111). With the knowledge that oocyte maturation plays an active role in fertilisation and embryo growth to a certain degree, it was of interest in this study to include the assessment of fertilisation and embryo development rates. This study aimed to assess whether the number of mature (MII) oocytes in oocyte cohorts from women living with HIV would be lower when compared to HIV-uninfected women, even after undergoing controlled ovarian stimulation. In the case where the number of mature-MII oocytes would be lower, then that would mean fertilisation rates and embryo development would follow the same pattern.

In this part of the study, the aim was to assess the fertilisation rates between the HIV-infected women and HIV-uninfected women following COH-ICSI, and the results (Table 4) revealed that the fertilisation rates were comparable (78.80% versus 76.67%) between the two

groups. This result is in discordance with a study by Vianna *et al.* (2017:7) who observed lower fertilisation rates in women living with HIV following assisted reproduction. It is important to note that 70-80% of fertilisation rates are associated with the use of the ICSI insemination method with normal semen parameters (UCSF Health 2024: n.p), and the results presented in this study include fertilisation rates in R-IVM oocytes, which could be the reason for the high rates- more oocytes.

Furthermore, the total cleavage rates were 76.72% in the HIV-infected group and 74.60% in the HIV-uninfected group. The blastocyst formation rate, calculated as the number of blastocysts by the number of fertilised oocytes (2PN), was 45.42% and 44.44% in the HIV-infected group and HIV uninfected controls, respectively. According to Speyer *et al.* (2019:652), a 77.3% blastocyst rate is associated with ICSI inseminated oocytes. At face value, the blastulation rates obtained in this study within each group fall outside of the levels presented by the study by Speyer *et al.* (2019), but when compared with each other, there were no statistically significant differences (p-value 0.175) in blastocyst formation rates (Table 26). Sauerbrun-Cutler *et al.* (2020:1665) did state that blastulation may be affected by sperm quality and rescue *in vitro* matured oocytes. R-IVM oocytes have higher chromosomal abnormalities (Yuan *et al.* 2021: e.135). This study included partners with normal sperm parameters and R-IVM oocytes. This may justify why in this present study in Table 4, there are high fertilisation rates, but the cleavage rates are lower, and blastocyst rates are even lower. Additionally, there could be some laboratory factors that may be the cause of this.

It should be noted that there were more blastocyst-stage embryo transfers (HIV+: n=10 vs HIV-: n=9) and blastocyst stage cryopreserved embryo cohorts (HIV+: n=23 vs HIV-: n=23) than cleavage-stage embryo transfers (HIV+: n=6 vs HIV-: n=6) and cleavage stage embryo cryopreservation cohorts (HIV+: n=4 vs HIV-: n=5). A plausible explanation is that the transfer of blastocysts has been associated with superior live birth rates owing to better endometrium-embryo synchronisation and embryo natural selection (Kontopoulos *et al.* 2019:294).

Fundamentally, oocyte fertilisation and embryo development rely on the correct development and maturation of the oocyte and sperm, the proper interaction between the sperm and egg which activates the processes within the egg cell to promote genetic material exchange and for fertilization to occur and complete, and finally differentiation of the oocyte into the preimplantation embryo required for pregnancy to occur. The quality of the oocyte, spermatozoon and embryos are all important for successful procreation. Furthermore, it is indisputable that there are factors that threaten the quality of the oocyte, spermatozoon and embryo, which arise from either the female or the male, and these factors impact these cells at various levels of development and various degrees. Finally, in this study, no evidence supports the assumption that women living with HIV have poorer fertilisation and embryo development rates when undergoing COH-ICSI cycles.

At the time of embryo transfer to the recipient female, only one or a maximum of two embryos are transferred at a single time. The choice is at the patient's discretion with advice from the clinician. The excess embryos that are of good quality are then cryopreserved for use at a later stage. Moreover, in some instances, a patient will hyperstimulate and produce many oocytes (>20 oocytes) in a single cycle, or the patient will be booked to undergo a certain theatre procedure that would hinder implantation; in these cases, all the resultant

preimplantation embryos are frozen (freeze-all cycle). In the HIV-infected group, a total of 9 patients had an embryo transfer to the recipient female and froze excess embryos on day 5, 4 patients had a day 3 embryo transfer and froze excess embryos, and 14 patients underwent freeze-all embryo cycles. In the HIV uninfected group, a total of 8 patients had day 5 transfers and had excess embryos frozen, 3 patients had day 3 transfers and had excess embryos frozen, and 17 patients had freeze-all embryo cycles (Table 27). Only 2 Patients had transferable embryos and never had any left-over embryos to freeze in the HIV-infected group, and 3 patients only had transferable embryos and no left-over embryos to freeze in the HIV-uninfected control group.

5.10 Study Limitations

AMPATH Laboratories recommends (Appendix F) that ultrasonography visualisation (AFC) and hormonal profiling (AMH, FSH, Estradiol, inhibin B) be used together when deciding on a patient plan for ovarian induction (Ampath Laboratories, n.d.). While ultrasound scans were conducted in all patients at the first consultation, this study did not accurately note the measurements of the antral follicle count before ovarian stimulation induction. Furthermore, this study only used serum AMH levels to quantify the ovarian reserve and provide an indication of a patient's response to stimulation, it did not account for the baseline measurements of hormones FSH, inhibin-B, estradiol in conjunction with AMH, this could have been useful in providing in-depth assessment of the ovarian microenvironment, hypothalamic-pituitary axis and provided better direction for ovarian stimulation and expected response. However, the justification for only using AMH as an ovarian reserve biomarker over other hormones is that there are studies (King, Swann and Murray 2022:248) that have stated that AMH is a more reliable indicator than the other hormones.

Another limitation was that there was no note of the number of previous cycles, and the study was also not stringent in analysing the possible effect of the timing between ovulation trigger administration and the time the oocyte retrieval procedure took place. Although, as per the facility protocol, the time between ovulation trigger administration and oocyte pick-up is standardly 36 hours, and the patients are required to confirm the time they administered the injection before the procedure begins, none of the patients in this study had a note of late ovulation trigger injection administration.

One other important limitation of this study was that the entire study was done using traditional methods by a single embryologist, who is the principal researcher and therefore, no double witnessing of outcomes was provided. This means that there might be biased judgment by human error since traditional methods are limiting. The use of specialised microscopes and materials to assist with evaluating the oocyte's cytoplasmic maturity and the use of an AI time-lapse monitoring system would have provided a better evaluation of the oocyte's complete maturation outcome, fertilisation, and embryo development. This would have allowed the use of an AI-automated machine to choose an embryo based not only on its physical outlook but also its developmental journey, as there are some internal issues not visible to the naked eye that may render an embryo unsuitable for implantation.

Finally, this study did not present data on the clinical pregnancy rates and live birth rates of all patients studied. This is because this was a prospective study, and at the time of writing up this dissertation, not all of the participants had had an embryo transfer and from the participants who had an embryo transfer, some had not given birth; therefore, there was a raw data limitation.

5.11 Study strengths and weaknesses

One of the most prominent strengths of this study is that most past studies that have investigated the connection between HIV infection and fertility or infertility in women have mostly focused on quantifying the ovarian reserve through its most widely used biomarkers, AMH and AFC, while in this study, the focus was oocyte nuclear maturity and its relationship to HIV. Shared similarities with other studies include the women being of reproductive age and undergoing ART. Moreover, studies mentioned by Mataró *et al.* (2017:6) that investigated oocyte quality are from European countries, and according to the extensive literature search conducted through various search engines (Google Scholar, PubMed, Research Gate, Science Direct, Medline, Cochrane library, EPUB Reader and internet sources such as google), in South Africa, no research investigation of oocyte maturation rates in oocyte cohorts from women living with HIV against HIV negative women has been attempted before this present study.

Another distinct strength of this study is that it was a prospective research study, conducted in one of the countries (South Africa) and provinces (Gauteng) with the highest prevalence of HIV infection and in women of reproductive age in whom HIV infection claims the highest prevalence.

One of the identifiable weaknesses of this research study is the small population size and that it was a single-centre study, which could have increased biased results. Nevertheless, it is a unique research study, the first of its kind in South Africa, and adds important evidence-based value to the population of women living with HIV seeking assisted reproduction.

CHAPTER 6

CONCLUSION

This research study's rationale was developed based on observations from IVF laboratory practice and results observed from a fraction of existing Sub-Saharan African literature (Cassim and Mohamed 2018, Eldib and Tashani 2018, Udjo 2018, Ombelet and Onofre 2019, Dwyer-Lindgren *et al.* 2019, Lucas *et al.* 2021 and Nik-Hazlin *et al.* 2022) on infertility and HIV infection in women. While assisted reproduction opens the possibility for infertile couples to have biological children, some couples still face challenges with procreation even with assistance from ART (Santulli *et al.* 2016;1084). Infertility is a major health issue on its own, and many factors are implicated in female infertility, among these are BBVs such as HIV (Yahya *et al.* 2018:179). The complexity of the viral invasion and its multiple effects on normal bodily functioning make the HIV-infected community very fragile for consistent probing for research purposes. Furthermore, the need for immediate treatment following diagnosis (World Health Organization 2015:250) adds to the unfeasibility of assessing the direct viral invasion effect on fertility. Even though attempts have been made to shed some light on the association between infertility and HIV infection, developing countries such as South Africa still have a paucity of literature on HIV infection and female fertility potential (Botha, Shamley, and Dyer 2018:9, Ombelet and Onofre 2019:68, Eldib and Tashani 2018:38 and Yahya *et al.* 2018:178). The gap in knowledge, particularly in developing nations, propels the need for more studies, such as the present study, to be conducted to provide better treatment to the infertile community living with HIV if the need exists.

This study aimed to provide a comparative analysis of the mature and immature oocyte yields in oocyte cohorts obtained from HIV-infected females against HIV-uninfected women of reproductive age, post-controlled ovarian hyperstimulation (COH). Additionally, the study examined the potential influence of factors associated with HIV infection, infertility, and ART, such as age, BMI, viral load, CD4+ lymphocyte counts, antiretroviral medication intake, ovarian stimulation protocols and ovulation trigger injections on oocyte maturation outcomes. To achieve this, oocyte cohorts from a group of HIV seropositive women and HIV non-infected controls in their childbearing years and attending an ART clinic were evaluated and compared, and their COH-ICSI cycle outcomes were recorded and statistically analysed.

The evidence provided by this study does not support the notion that HIV infection promotes poorer IVF outcomes in females of reproductive age undergoing ART. There were no statistically significant differences in the number of total oocytes and Metaphase 2 (MII) oocytes in oocyte cohorts aspirated between HIV-infected women and HIV-uninfected women following ovarian hyperstimulation. The number of mature and immature oocytes was observed to be similar when outcomes were compared between women living with HIV and those who were HIV-uninfected. This means that being infected with HIV does not necessarily place an individual at a higher risk of having more immature than mature oocytes following ovarian hyperstimulation, nor do they present with drastically lowered ovarian reserve levels when compared to HIV-uninfected women of the same age category.

Oocyte maturation outcomes under factors such as the type of stimulation protocol, ovulation trigger injection and BMI were not affected by being infected with HIV, as found by

this study. In this study, an age-oocyte number and maturity status association was particularly observed in older women living with HIV. This was in accordance with one previous study that observed older women living with HIV having fewer oocyte yields; the study linked this reduction to immune ageing rather than natural ageing. Furthermore, a positive correlation was also found in the relationship between AMH, follicle number and MII oocyte numbers with age. With an increase in the number of older women aged 36-44 seeking ART (Moghadam *et al.* 2022:105), this observation of older women presenting with fewer mature oocytes should be investigated further. This study did not conduct an analysis on which age group seeks ART the most.

It is important to note, however, that there are a multitude of factors apart from the ones presented in this study that can affect the number of oocytes retrieved post COH and ART success. These include smoking, alcohol consumption, some diseases, other infections, and issues within the ovarian microenvironment.

While patient individualisation and current ART recommendations of specific population types may provide better ART success, the present study does provide a blueprint into the future of IVF treatments in people living with BBVs such as HIV. Furthermore, even though the results supplied by this study show benefit to PLWH, caution should be taken with using this study for the entire HIV population group due to its sample size. Therefore, results of this study should only be used as a counselling and advisory tool for patients living with HIV seeking ART assistance. Additionally, because none of the existing studies have reached a consensus regarding the role of HIV infection on female fertility, most studies having limited human-based evidence, small population samples and the effect of population demographics and geographic locations (Deshpande and Gupta 2019:287 and Eldib and Tashani 2018:43), more randomized controlled studies are still very much needed.

This is the first South African evidence-based study that has successfully investigated and provided evidence that women living with HIV stimulate comparably to women who are HIV negative. This evidence further reinforces the use of ART to all individuals who need and can afford it.

Recommendations

1. More randomised controlled multi-centre studies for an in-depth assessment of oocyte maturation rates in patient oocyte cohorts from women living with HIV. This will assist with observing any distinct patterns or confirm if oocyte maturation is not impacted by the HIV infection or any HIV associated factors.
2. Investigating immune ageing further to identify possible associations.
3. The continuous use of the dual trigger injection technique for ovulation induction. This is when patients administer two types of triggers for ovulation triggering. This method is associated with the harvest of more mature oocytes on the transvaginal oocyte aspiration procedure day and better embryo quality (Dong *et al.* 2022:6).
4. Use of the Progestin-Primed Ovarian Stimulation (PPOS) protocol for ovarian stimulation. Developed in the year 2015 by Dr. Yanping Kuang in China, the protocol employs the use of a progestin rather than an antagonist to inhibit a premature LH surge in conjunction with the commonly used exogenous gonadotropins (Guan *et al.* 2021:1).

CHAPTER 7

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APPENDICES

APPENDIX A: Permission request letter.

08 AUGUST 2022

Dr Jackson Ndhlovu
BSc, MBChB (MEDUNSA), MMED " O&G" (UP), FCOG (SA),
Dip HIV Man (SA)
PGA (Warwick), Certificate in Reproductive Medicine (SA).
Certificate in Endoscopic Surgery level 2 (SA)
PR number: 0270237, MP: 0528587
TEL: 0152965156 Cell: 0680869477

Request for Permission to Conduct Research

Dear Doctor Ndhlovu and FemiCare Fertility Management

My name is Nombuso Felicia Ndlela, a Master of Health: Clinical Technology student at the Durban University of Technology. As part of obtaining a master's degree qualification, I am required to conduct research in my field and submit a completed research dissertation at the end of the study term. The research I wish to conduct for my master's Dissertation is based on evaluating the ratio of mature oocytes to immature oocytes in oocyte cohorts aspirated from HIV sero-positive and HIV sero-negative females post controlled ovarian hyperstimulation.

I am hereby seeking your consent to use your ART practice as my research site. I will gather data from the patient's files relating to results from routine IVF patient assessment and other patient health information that is linked to and will enable me to complete my research study. For the proposed study, no other procedures apart from the general IVF procedures that the patient would have agreed to will be conducted.

I have provided you with a copy of my research proposal, which includes copies of the data collection tools and consent forms to be used in the research process, as well as a copy of the approval letter which I received from the Institutional Research Ethics Committee (IREC).

If you require any further information, please do not hesitate to contact me on my Cellphone: 0604156342 or Email: nombusofndlela@gmail.com. Thank you for your time and consideration in this matter.

Yours sincerely,

Nombuso Felicia Ndlela
Durban University of Technology

Signature (Gatekeeper)

Date

Time

APPENDIX B: Confirmation letters from the clinician and the research study's facility management granting access to proceed with the research.

Dr Jackson Ndhlovu
BSc, MBChB (MEDUNSA), MMED " O&G" (UP), FCOG (SA),
Dip HIV Man (SA)
PGA (Warwick), Certificate in Reproductive Medicine (SA).
Certificate in Endoscopic Surgery level 2 (SA)
PR number: 0270237, MP: 0528587
TEL: 0152965156 Cell: 0680869477

To whom it may concern

This letter serves the purpose to acknowledge receipt of the researchers (Miss N.F NDLELA, IREC 138/22) request to utilize our facility to conduct research, and we have accepted the request. All relevant forms (letter of information, consent form, data collection tool, short questionnaire), how the research is to be conducted and its outcome and benefit have been deliberated and accepted. Thank you.

Kind regards

Dr. Jackson Ndhlovu

16/9/2022.
Date





Femicare

Fertility and Advanced Endoscopic Surgery Centre

Suite 1, Femina Woman's Hospital, 460 Belvedere Street, Arcadia Pretoria
Tel: +27 12 326 8213, Fax: +27 86 578 2287, Email: reception@femicarefertility.co.za

Dr Jack Biko

Gynaecologist and Fertility Specialist

MB, BCh (Wits), MMed O&G (Pretoria), FCOG (SA), PGA
Assisted Reproduction (Warwick, UK), Msc Human Reprod
(Valencia, Spain), Cert Advanced Endo Surg (Kiel, Germany)

Dr Thato Maoto

Obstetrician and Gynaecologist

MBChB (UCT), FCOG (SA), MMed (UP)

Pr No: 0204013

2023/08/04

This serves to confirm:

Ms Felicia N. Ndlela (ID# 9507050214084), serving in the position of Embryologist at our clinic, has permission to conduct a clinical study at Femicare Fertility Centre.

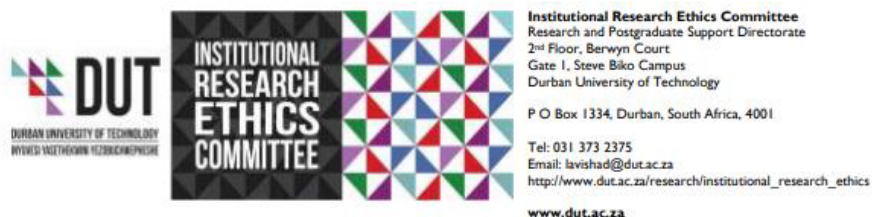
Thank you for your consideration.

Dr Thato Maoto

Gynaecologist & Reproductive Medicine Specialist



APPENDIX C: Ethical Clearance Letter.



21 September 2022

Ms N F Ndlela
P.O Box 2414
Margate
4275

Dear Ms Ndlela

Evaluating the ratio of mature oocytes to immature oocytes in oocyte cohorts aspirated from HIV sero-positive and HIV sero-negative females post controlled ovarian stimulation

Ethical Clearance number IREC 138/22

The DUT-Institutional Research Ethics Committee acknowledges receipt of your gatekeeper permission letter.

Please note that FULL APPROVAL is granted to your research proposal. You may proceed with data collection.

Any adverse events [serious or minor] which occur in connection with this study and/or which may alter its ethical consideration must be reported to the DUT-IREC according to the DUT-IREC Standard Operating Procedures (SOP's).

Please note that any deviations from the approved proposal require the approval of the DUT-IREC as outlined in the DUT-IREC SOP's.

Yours Sincerely

Prof J K Adam
Chairperson: DUT-IREC

APPENDIX D: Letter of information.



LETTER OF INFORMATION

Title of the Research Study: Evaluating the ratio of mature oocytes to immature oocytes in oocyte cohorts aspirated from HIV seropositive and HIV seronegative females post-controlled ovarian stimulation.

Principal Investigator/s/researcher: Nombuso Felicia Ndlela. BTech: Clinical Technology

Co-Investigator/s/supervisor/s: 1. Dr. D.R Prakashchandra. PhD

2. Mr. M.E Gabriel. BSc, BSc (Hons), MSc

Dear Participant

I am Miss N.F Ndlela, currently registered at the Durban University of Technology for a Master of Health Sciences in Clinical Technology. As part of the basic requirements to obtain a master's degree, I am required to conduct a study of my choosing in my field of study. The study I have chosen to conduct is based on assisted reproduction, where I will be evaluating the ratio of mature oocytes to immature oocytes in oocyte cohorts aspirated from HIV seropositive and HIV seronegative females post-controlled ovarian stimulation. I am therefore humbly pleading for your approval to be a participant in the abovementioned study.

In this study, as a participant, you will only do tests and procedures that you are booked for as a patient of *IVF*, the results of those tests will then be used for the study; you will not be required to have any additional tests or procedures outside of the ones already recommended by your physician. Should you agree to participate in this study, you will be asked only to complete two forms, a consent form to validate your verbal consent in giving the researcher permission to use your procedure and test results for the study; and a short questionnaire to assist with gathering important information that will not be found from the tests and procedures you will undertake, but will greatly assist in interpreting data. To ensure that your identity will remain hidden, the researcher will give you a specific code that you will be called from the start to the end of the study.

You are allowed to ask as much about the study as you like from the researcher and address any issues you may have. It is important that before providing written consent, you are fully aware and understand what the study is about and what is expected from you; for this purpose, a copy of the letter of information document will be provided to you to take home. You are also allowed to discuss the study with your partner, family, friends, and your attending doctor. It is also important that you know you are under no obligation to participate in the study nor respond to the researcher's request on the same date of the request.

This study like others, is expected to provide more information about the HIV-infected population to help provide better clinical management to this population in vitro.

Brief Introduction and Purpose of the Study:

In the clinical setting, the researcher has noticed that there are quite a few HIV-infected females who attend an ART facility and are stimulated the same way as HIV-negative females with the same diagnosis, but the HIV-positive female will on the day of taking out the eggs (TVOA), have more immature eggs than mature eggs. The problem with this outcome is that immature oocytes cannot be used until they reach maturity, which may occur after an additional 24 hours of culture, or the oocytes may never mature at all. In conventional IVF, the cumulus cells surrounding the oocyte are not removed therefore we never know which are mature to use and which are not, and the unfertilized oocytes will be discarded the next day, this decreases the number of embryos for the patient; in intracytoplasmic sperm injection (ICSI) however, the cumulus cells surrounding the oocytes are removed, and the egg is assessed to see if it is mature and can be used if the oocyte is immature, the oocyte is given another day to reach maturity then used.

The purpose then for this study is to see whether or not being HIV positive places you at a risk of having more than expected immature oocytes than that of an individual who is HIV negative.

Outline of the Procedures:

The following is the outline of the procedure you would routinely go for as part of your treatment at the clinic:

- On day 3 of the patient's menstrual cycle, they are given a medication timetable and will start medication to initiate multiple follicular growth. Subsequently, the patient will come back to the doctor once or twice to assess the progress of follicular growth.
- Thirty-six (36) hours before the transvaginal oocyte aspiration (TVOA) procedure, the patient will receive the HcG trigger which will initiate the completion of oocyte maturation. On the same day, the patient will be tested for Covid-19, as per hospital rules and regulations since they will be admitted into the hospital ward for post-op monitoring.
- The procedure is done in the morning under conscious sedation.
- The patient arrives at the procedure room, empties their bladder, and is attended to by the attending anaesthetist who will administer the anaesthesia.
- Once the patient has been sedated, the specialist doctor will start by cleaning the vaginal area with sterile saline water, thereafter, he will administer a local anaesthetic on the cervix. The doctor will then, assisted by the nurse, conduct the transvaginal oocyte aspiration procedure under ultrasound guidance.
- The aim is to use an aspiration needle attached to a probe (which is inserted into the vagina) to aspirate follicular fluid from the expanded follicles in the ovaries while taking great care to bypass the uterus and other major blood vessels.
- The aspirated follicular fluid is then handed over to the embryologist to evaluate for the presence of any oocytes, regardless of their maturation status.

- Once all the oocytes have been aspirated, the patient is awoken and taken to the hospital resting ward for monitoring, until the anaesthesia wears off.
- Pain medication is prescribed when the patient gets discharged.

Risk or Discomforts to the Participants: Minimal pain can be expected following the oocyte aspiration procedure.

Benefits: No benefit to the participant.

For the researcher, the benefit expected is to obtain a qualification.

Reason/s why the Participant May Be Withdrawn from the Study: The participant may, at any stage, without prejudice, withdraw their consent and participation in the study.

Remuneration: None

Costs of the study: None

Confidentiality: Every participant will be allocated a file number (code). The participant at all times will be referred to as the file number (code) allocated and no personal identification information will be shared with anyone.

Research-related Injury: No research-related injury or adverse reaction is expected as the research is based on normal laboratory techniques and treatment options that the patients have already consented to.

Persons to Contact in the Event of Any Problems or Queries:

Please contact the principal researcher Miss N.F Ndlela (Tel no. :060-415-6342), and my supervisor Dr. D.R Prakashchandra. (Tel no. 083-446-7735) or the Institutional Research Ethics Administrator on 031-373-2375.

APPENDIX E: Consent form.



CONSENT

Statement of Agreement to Participate in the Research Study: Evaluating the ratio of mature oocytes to immature oocytes in oocyte cohorts aspirated from HIV seropositive and HIV seronegative females post-controlled ovarian stimulation.

- I hereby confirm that I have been informed by the researcher, _____
(Name of researcher), about the nature, conduct, benefits and risks of this study - Research Ethics Clearance Number: _____
- I have also received, read and understood the above-written information (Participant Letter of Information) regarding the study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- Given the requirements of research, I agree that the data collected during this study can be processed in a computerized system by the researcher.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.
- I understand that significant new findings developed during this research which may relate to my participation will be made available to me.

_____	_____	_____	_____
Full Name of Participant	Date	Time	Signature/Right Thumbprint

I, _____ (name of the researcher) herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

_____	_____	_____
Full Name of Researcher	Date	Signature

_____	_____	_____
Full Name of Witness (If applicable)	Date	Signature

_____	_____	_____
Full Name of Legal Guardian (If applicable)	Date	Signature

APPENDIX F: Serum AMH reference ranges.

From:	Ampath laboratory		
Priority:	Urgent		
Status:	Order results completed		
Sample:	2016/06/21 10:37		
Analysis:	Anti-Mullerian Hormone	0.27	(ng/ml)
Analysis:	Anti-Mullerian Hormone	1.9	(pmol/l)

Serum AMH levels may be used as a predictor of ovarian response following ovarian stimulation during IVF treatment and should be used in combination with clinical and ultrasound findings (antral follicular count).

Suggested cut off values:

ng/ml	pmol/L	Response to ovarian stimulation treatment
<0.17	<1.2	Negligible/non-responders
0.17-1.21	1.2-8.6	Reduced/poor response (=2 eggs at oocyte retrieval)
1.22-2.30	8.7-16.4	Normal response (3-20 eggs at oocyte retrieval)
2.30	16.4	High/excessive response (>20 eggs at oocyte retrieval)

Beckman Access method

Analysis: Full text report See text

[Less..](#)

*** DRS DU BUISSON, BRUNETTE & KRAMER Ing/Inc ***
PRAC.NO.:052 000 5200431

24 Hour Contact No: 012 678 0700

2023/10/16 printed by Felicia Ndlela, Miss

Serum AMH reference values (AMPATH Laboratories)

APPENDIX G: WHO 2010/2021 semen parameters reference ranges.

	WHO 2010	WHO 2021
Semen volume (mL)	1.5 (1.4–1.7)	1.4 (1.3–1.5)
Sperm density (10 ⁶ per ejaculate)	15-250	16-250
Total motility (%)	40 (38–42)	42 (40–43)
Progressive motility (%)	32 (31–34)	30 (29–31)
Non-progressive motility (%)	1	1 (1–1)
Immotile sperm (%)	22	20 (19–20)
Vitality (%)	58 (55–63)	54 (50–56)
Normal forms (%)	4 (3–4)	4 (3.9–4)

WHO 2010 (5th Edition) and WHO 2021 (6th Edition) lower fifth percentile (with 95% confidence interval) of semen parameters from men in couples starting a pregnancy within one year of unprotected sexual intercourse leading to a natural conception (Boitrelle *et al.* 2021:6).

WFS: _____
 Diagnosis: _____
 Stimulation: _____
 Antagonist: _____
 WVI: _____
 Semen analysis: _____
 Procedure: _____
 TLL (TV): _____

Day	8	9	10	11	12	13	14
Date	03/11/2023	04/11	05/11	06/11	07/11	08/11	09/11
Lucrin/Cetrotide	0.25	0.25	0.25	0.25			
Menopur/Gonalif	300	300	300				
Estradiol							
Trigger							
Aspiration							
Endometrium							
7							
Left ovary							
10mm x8							
Right ovary							
12mm x10							

ICSI / IVF

How many follicles $\geq 14\text{mm}$:

How many follicles measured $\geq 14\text{mm}$:

How many follicles aspirated:

How many ampules MMG:

How many oocytes aspirated:

Embryo transfer: Cook/Tomcat/Total Embryo's: _____

/ Grading of embryo's:

Easy or Difficult:

Date: BHCg:

APPENDIX I: Data capture sheet.



DATA CAPTURE SHEET

Research topic: Evaluating the ratio of mature oocytes to immature oocytes in oocyte cohorts aspirated from HIV seropositive and HIV seronegative females post-controlled ovarian stimulation.

Study Number

Participant Code:

Participant Age:

Age range:

18-25 years	26-30 years	31-35 years	36-39 years
-------------	-------------	-------------	-------------

AMH value	
Viral load (Detectable/undetectable)	
CD4 ⁺ Lymphocyte count (cells/ μ l)	
BMI value	
Antiretroviral therapy (Yes/No)	
Duration of Antiretroviral therapy intake	
Stimulation Protocol	
Exogenous gonadotropin	
Trigger type	
Number total follicles	
Number total oocytes	
Number of MII oocytes (D0)	
Number of MI oocytes	
Number of GV oocytes (D0)	
Number of MII oocytes (D1)	
Endometrial thickness @TVOA	
Diagnosis	
Semen sample type	
Number fertilised	
Number total embryos	
Number and day of embryo transfer	
Quality of transferred embryo(s)	
Total oocytes cryopreserved	
Total embryos cryopreserved	

APPENDIX J: Data capture sheet questionnaire.



DATA CAPTURE SHEET QUESTIONNAIRE

Study number:	
Research topic: Evaluating the ratio of mature oocytes to immature oocytes in oocyte cohorts aspirated from HIV seropositive and HIV seronegative females post-controlled ovarian stimulation.	

AGE

DURATION OF INFECTION

PREVIOUS HISTORY OF STI/STD YES NO

ANTIRETROVIRAL THERAPY YES NO

➤ If yes, how long before attending a fertility clinic have you been on treatment?

APPENDIX K: Cleavage stage and blastocyst stage embryo scoring techniques.

DAY 3 Cleavage Stage Embryos

- Grade I— Cleavage stage embryos with similar cell (blastomere) sizes with no visible cytoplasmic fragmentation.
- Grade II— Non-linear blastomere size with at least 10% visible cytoplasmic fragmentation.
- Grade III— approximately 10–25% cytoplasmic fragmentation found in the embryo.
- Grade IV— 25–50% visible cytoplasmic fragmentation.
- Grade V— More than half of the embryo are cytoplasmic fragments.

Cleavage stage embryo grading system (Zilberberg *et al.* 2021:1084).

Blastocoel cavity expansion stages	Inner Cell Mass (ICM) quality	Trophectoderm (TE) quality
1- Early blastocyst: blastocoel is half the size of the embryo.	A- Clearly visible, has many cells compacted together.	A- Many cells forming a cohesive epithelium.
2- Blastocyst: blastocoel > half of the embryo size.	B- Several cells that are loosely grouped.	B- Adequate number of cells forming a loose epithelium.
3- Full blastocyst: blastocoel occupies the entire embryo space.	C- Contains very few cells.	C- A loose epithelium formed by a few larger cells.
4- Expanded blastocyst: fully expanded blastocoel, and the zona pellucida is thinner than in the initial stages of embryo growth.	D- No visible cells or the presence of degenerated cells.	D- Sparse or degenerating cells surrounding the inner cell mass.
5- Hatching blastocyst: trophectoderm begins to herniate		

through the zona pellucida.		
6- Hatched blastocyst: the blastocyst has completely broken free from the zona.		

Human blastocysts scoring technique. (Adapted from Gardner and Schoolcraft (1999:385) and The Working Group on the update of the ESHRE/ALPHA Istanbul Consensus *et al.* (2025:1019).

APPENDIX L: Hospital COVID-19 testing sheet and assessment.

PATHOLOGISTS		Switchboard Numbers:		LAB NO.		LAB NO.	
LANCET LABORATORIES Key to Diagnostic Excellence		JHB	0027 (0) 11 358 0800	→		Rev: 015	
		PTA	0027 (0) 12 483 0100	→		→	
		KZN	0027 (0) 31 308 6500	→		→	
		CPT	0027 (0) 21 673 1700	→		→	
		POLOKWANE	0027 (0) 15 294 0400	→		→	
		RUSTENBURG	0027 (0) 14 597 8500	→		→	
		NELSPRUIT	0027 (0) 13 745 9000	→		→	

Referring Doctor	Copy Doctor	Copy Doctor
	Femi NP	

Patient Details		Person Responsible for Payment of Account	
Patient I.D. No.		I.D. No.	
Patient Surname		Surname & Initials	
Patient Initials & First Name		First Name	Title
Patient Date of Birth		Postal Address	
Hospital / Folio Number			
Patient Cell No.		Telephone No. (C)	(H)
Patient Email		Email	
Patient Guardian Signatures: My signature indicates my understanding of, and my agreement to: comply with the terms of the legal declaration, provide consent for the processing of personal information and the releasing of test results as documented on the back of this form. I give consent for tests and guarantee payment of any amounts. I consent that ICD10 codes may be provided to my medical aid as per statutory requirements on my account.		Employer	(W)
Hospital Patient	Y N Fasting Random Routine Urgent	Medical Aid Name	
Telephone / Fax Number		Med. Aid #	
Collection Date		Cash Receipt #	
Venesectionist		Authorisation #	Dependent Code
Submitted		Patient Membership Card Verified?	Account No.
Specimen (Taken & Received) (Refer to the back of this form for the key guide)		Patient MRI#	

SARS-CoV-2 (COVID-19) Request			
C290	☐	PCR SARS-CoV-2	
Sample Type: Sputum () Nasopharyngeal Swab () Throat Swab () Nasal Swab () Tracheal Aspirate () Broncho Alveolar Lavage ()			
Travel History			
Country	Date of Departure	Date of Return	
Clinical Presentation			
Date of onset of symptoms			
Symptoms (tick all that apply):			
Fever (≥38°C)	☐	Shortness of breath	☐
Cough	☐	Nausea / Vomiting	☐
Chills	☐	Diarrhoea	☐
Sore Throat	☐	Other	☐



health

Department:
Health
REPUBLIC OF SOUTH AFRICA



List of persons you have been in close contact with

Please list all the people you have been in close contact with over the last 7 days
(close contact refers to being within 2 meters of someone).

	Name of person in close contact with	Mobile number of person in contact with
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		

COVID-19 testing and contact evaluation forms. (Netcare Femina Women's Hospital, 2020).

APPENDIX M: ESHRE recommendations on starting ART post-COVID-19.

Procedure for patients

- All patients planning to start treatment should have a triage questionnaire (paper, email or phone) two weeks before commencing treatment.
- A preliminary triage of both partners should be performed two weeks before starting the ART treatment.
- A further triage of both partners should be performed during ovarian stimulation.
- Triage should be performed according to the same procedures used for staff members. Both partners should undergo triage. Patients, suspected of infection after triage should get regular SARS-CoV-2 IgM/IgG testing or equivalent tests. Additional testing can be considered in line with national recommendations and/or availability of tests.
- All patients with a previous confirmed COVID-19 infection should present medical evidence of clearance in order to be eligible for treatment. If patients have been on respiratory support during the COVID-19 infection episode, they should additionally provide evidence of assessment and a medical specialist report.

Scenarios for patients

Scenario I [include]:

- Both patients are triaged as low risk (negative clinical history, lifestyle compatible with low/minimal risk of contact with potentially infected individuals)
- Both patients are asymptomatic

3

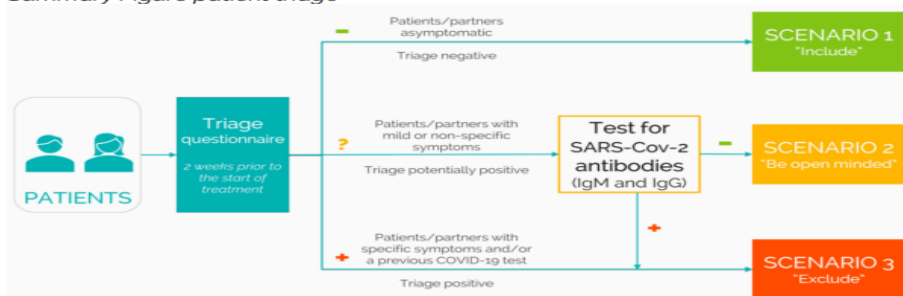
Scenario II [be open minded]

- Patients who have recovered from a previous COVID-19 infection, proven by certified medical evidence of clearance, should have SARS-CoV-2 IgM/IgG testing prior to starting treatment.
- (IIa) Presence of non-specific symptoms in one of the partners before starting ovarian stimulation:
 - ⇒ Repeat the triage at the beginning of ovarian stimulation
 - If negative: Continue the treatment
 - If symptoms persist: Perform SARS-CoV-2 IgM/IgG testing to decide
 - If IgM/IgG negative: Continue the treatment
 - If IgM/IgG positive: Postpone the treatment and refer for further testing.
- (IIb) Non-specific symptoms arising during ovarian stimulation
 - ⇒ Perform SARS-CoV-2 IgM/IgG testing
 - If IgM/IgG negative: Continue the treatment
 - If IgM/IgG positive: Postpone the treatment and refer for further testing.

Scenario III [exclude]

- If patients and/or partners are symptomatic or COVID-19 positive, postpone the treatment and refer for further testing and follow-up.

Summary Figure patient triage



Appendix 1 - ART Triage Questionnaire

1. Have you been sick in the last two weeks?
2. Do you have fever (over 37,5°C)?
3. Are you coughing at present?
4. Do you have a sore throat?
5. Have you lost your sense of smell or taste?
6. Have you been in contact with somebody who has any of these symptoms?
7. Have you travelled to an area at high risk for COVID-19, nationally or internationally?
8. Do you work in a hospital/nursing home or healthcare facility?
9. Have you been in contact with somebody who has COVID-19?
10. Have you been you diagnosed with COVID-19?
11. Do you live in a household with somebody who has been diagnosed with COVID-19 infection or has COVID-19 symptoms (fever, cough, loss of smell)?
12. If you have been COVID-19 positive and recovered, do you have certified medical evidence of clearance ?
13. Do you have a severe medical condition like diabetes, respiratory disease, chronic kidney disease, etc.? *(this question can be skipped when using the ART triage questionnaire for staff)*

ESHRE recommendations on starting ART post-COVID-19 (The ESHRE COVID-19 Working Group *et al.* 2021:277)

APPENDIX N: Expected serum AMH values by age.

Age (y)	Age expected AMH (ng/ml)
39	1.73
38	1.95
37	2.16
36	2.37
35	2.58
34	2.78
33	3.02
32	3.25
31	3.47
30	3.70
29	3.82
28	3.94
27	3.99
26	4.02
25	4.05
24	4.07
	Total

Expected serum AMH values by age (Cassim and Mohamed 2018:3)

APPENDIX O: Biostatistician letter.

13 Apsley Court
443 Musgrave Road
Essenwood
Durban

Department: Biomedical and Clinical Technology.
Faculty: Health Sciences

11 November 2023

To whom it may concern

Re: Statistical Assistance for Ms Nombuso Felicia Ndlela

This serves to confirm that I assisted Ms Ndlela with the statistical analysis for her Masters of Health Science in Clinical Technology.

Title: Evaluating the ratio of mature oocytes to immature oocytes in oocyte cohorts aspirated from HIV sero-positive and HIV sero-negative females post controlled ovarian stimulation.

Yours sincerely

GB Matthews

Glenda Matthews