NAMIBIA UNIVERSITY
OF SCIENCE AND TECHNOLOGY

FERTILITY AND PREGNANCY OUTCOME AMONG WOMEN UNDERGOING ASSISTED REPRODUCTIVE TECHNOLOGY TREATMENT IN WINDHOEK, NAMIBIA.

By

Mr Adão Francisco Lucas

200990330

"Thesis presented in fulfilment of the requirements for the degree of Master of Health Sciences"

in the Faculty of Health and Applied Sciences

Supervisor: Dr. Yapo G. Aboua

Co-supervisor: Professor Stefan S. Du Plessis

April 2020

DECLARATION

I, Adão Francisco Lucas hereby declare that the work contained in the thesis entitled "Fertility and pregnancy outcome among women undergoing assisted reproductive technology treatment in Windhoek, Namibia." is my own original work and that I have not previously in its entirety or in part submitted it at any university or other higher education institution for the award of a degree.

Signature:  Date: 10th February 2020

Supervisor Signature:  Date: 10th February 2020

Co-supervisor Signature: ..... Date: .....

Retention and Use of Thesis

I, Adão Francisco Lucas being a candidate for the degree of Master of Health Sciences accept the requirements of the Namibia University of Science and Technology relating to the retention and use of theses deposited in the Library and Information Services.

In terms of these conditions, I agree that the original of my thesis deposited in the Library and Information Services will be accessible for purposes of study and research, in accordance with the normal conditions established by the Librarian for the care, loan or reproduction of theses.

Signature:  Date: 10th February 2020

ACKNOWLEDGEMENTS

I wish to thank:

- God, who has been my rock and fortress throughout this journey from beginning to end, to Him be all the glory.
- My wife, Justine Lucas, for all the support and cheering me on when I barely could make time for her and other family commitments.
- Cape Windhoek Fertility Clinic and its patients for making this study possible. A special thank you to Drs Axel Vandendael and Fred Kigozi.
- My supervisors, Dr. Yapo Aboua and Prof. Stefan Du Plessis, for their constant support and guidance.
- The statistician, Dr. Dibaba Gemechu, for assisting with the statistical analysis of the data collected during the study.
- My former employer, High Care Laboratory, for affording me flexible working hours so that I could be able to attend to my studies when the need arose.
- My friends and relatives who have occasionally spoken a word of encouragement when the going was tough.

DEDICATION

This thesis is dedicated to my parents, Francisco Sebastião Lucas and Margarida Comboio Mateus, who despite all odds have prioritized our education at all costs throughout the years. They are among my unsung heroes.

ABSTRACT

BACKGROUND: Infertility is a worldwide burden that requires attention, and yet has been largely unappreciated and understudied, particularly in sub-Sahara Africa where there is a high prevalence. The stigma of infertility among African women is a serious socio-economic concern that needs to be tackled and alleviated. Infertility has been defined as a couple's failure to conceive after continuous and unprotected coitus for one year or six months, depending on the age of the female counterpart. Although infertility can be caused by both male and female factors, the female is often to blame and bear the consequences, particularly in cultures that have placed a high premium on children such as those found in Africa. This study therefore, explored the effectiveness of assisted reproductive technology (ART) treatment on pregnancy outcomes and assessed possible risk factors that lead to infertility among Namibian women.

OBJECTIVE: The primary aim of this study was to determine the prevalence of successful pregnancy outcomes among infertile women undergoing ART treatment at the Cape Windhoek Fertility Clinic in Windhoek, Namibia.

METHODOLOGY: This was a prospective and descriptive cross-sectional case reference study encompassing 178 infertile women visiting the Cape Windhoek Fertility Clinic for ART treatment. The study was approved by the Ministry of Health and Social Services (MoHSS) ethics committee and the Namibia University of Science and Technology (NUST) higher degree committee. Written informed consent was acquired before the commencement of the study from all participants. Venous blood collected from participants were analysed for Human Immunodeficiency Virus (HIV), Syphilis, Hepatitis B surface antigen (HBsAg), Hepatitis C virus (HCV), Thyroid-Stimulating Hormone (TSH), Follicle-Stimulating Hormone (FSH), Anti-Mullerian Hormone (AMH), and Prolactin (PRL) levels before the commencement of treatment. Human Chorionic Gonadotropin (hCG) levels were measured after treatment was administered to determine the effectiveness of the treatment and the pregnancy outcome. Statistical analysis was done using SPSS version 25.0 and the Chi-square test was used. $P<0.05$ were considered to be statistically significant.

RESULTS: The women enrolled in the study ranged from 25-50 years of age and most 97 (54.5%) were in the age group of 30-39 years. The great majority 145 (81.5%) of the participants were married women. There was a high prevalence of infertility 67 (69.1%) in the age group of 30-39 years. From the 178 infertile women, 96 (53.9%) suffered from primary infertility and 82 (46.1%) women suffered from secondary infertility. The predominant cause of complications for infertility among the women under study was defective ovulation 51 (28.7%) and the most common ART treatment administered was IVF/ICSI 93 (52.2%). Of the 178 women who received ART treatment only 59 (33.1%) eventually fell pregnant, while the vast majority 119 (66.9%) did not fall pregnant.

CONCLUSION: The high prevalence of infertile women (66.9%) in this study, calls for immediate remedial measures and interventions both at the national and continental level. However, access to affordable ART treatment remains a challenge in low-resource settings (i.e. Africa) seen that for the most part they are only offered by privately owned hospitals and/or clinics at costly fees.

Keywords: Infertility; Human chorionic gonadotropin; Anti-mullerian hormone; Pregnancy outcome; Female age; Ovarian reserve; Hormonal imbalance; Assisted reproductive technology; Involuntary childlessness; Ovulation.

DEFINITION OF KEY CONCEPTS

These are definitions of some key concepts used in the study as defined by Bishop, Fody, and Schoeff (2010); Grynnerup, Lindhard, and Sørensen (2012); "Fertilitypedia" (n.d.); and Zegers-Hochschild et al., (2017):

Amenorrhea	The absence of a menstrual period in women of reproductive age
Anovulation	Failure of the ovaries to release an egg over a period of time generally >3 months
Assisted reproductive technology	All interventions that include the in vitro handling of both human oocytes and sperm or of embryos for the purpose of reproduction
Anti-Mullerian Hormone	Is a dimeric glycoprotein of the transforming growth factor-β super family that is involved in growth and differentiation. In males it is expressed by the sertoli cells and in females it is expressed in the granulosa cells
Azoospermia	The absence of spermatozoa in the ejaculate
Biochemical pregnancy	A pregnancy diagnosed only by the detection of β-hCG in serum or urine
Childlessness	A condition in which a person, voluntarily or involuntarily, is not a legal or societally-recognized parent to a child, or has had all children die
Clinical pregnancy	A pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs or definitive clinical signs of pregnancy. In addition to intra-uterine pregnancy, it includes a clinically documented ectopic pregnancy
Coitus	Sexual intercourse
Oocyte/Egg donation	The use of oocytes from an egg donor for reproductive purposes or research
Electrochemiluminescence	An analytical method whereby quantification of an analyte is based on emission of light resulting from a chemical reaction
Frozen-thawed embryo transfer cycle	An ART procedure in which cycle monitoring is carried out with the intention of transferring to a woman, frozen/thawed embryo (s)
Follicle-stimulating hormone	A hormone secreted by the anterior pituitary gland. It regulates the development, growth, pubertal maturation and reproductive functions of the body
Gonadotropin-releasing hormone	A releasing hormone secreted from the hypothalamus and is responsible for the release of FSH and LH from the anterior pituitary

Hormone	Are chemical messengers that control the actions of specific cells or organs
Human Chorionic Gonadotropin	A hormone that supports the normal development of an egg in a woman's ovary, and stimulates the release of the egg during ovulation
Hypothalamus	A region of the forebrain that regulates body temperature, some metabolic processes and governs the autonomic nervous system
Immunoassay	An analytical method that involves the binding of antibody to antigen for the specific and sensitive detection of an analyte
Intracytoplasmic Sperm Injection	A procedure in which a single spermatozoon is injected into the oocyte cytoplasm
Intra-uterine insemination	A procedure in which laboratory processed sperm are placed in the uterus to attempt a pregnancy
In Vitro Fertilization	A sequence of procedures that involves extracorporeal fertilization of gametes. It includes conventional in vitro insemination and ICSI
Luteinizing hormone	A hormone that stimulates ovulation and the development of the corpus luteum in females, and the production of androgens in males
Menstrual cycle	Is a repetitive sequence of events that occurs in fertile women roughly every 28 days
Oestradiol	A steroid and oestrogen sex hormone predominantly produced in the ovaries of females, but small amounts are produced by the adrenals and testis
Oestrogen	The primary female sex hormone responsible for the development and regulation of the female reproductive system and secondary sex characteristics
Oligomenorrhea	Generally refers to infrequent menstruation. More strictly, it is menstrual periods occurring at intervals of greater than 35 days, with only four to nine periods in a year
Pituitary gland	An endocrine gland, about the size of a pea, whose secretions control the other endocrine glands and influence growth, metabolism, and maturation
Polycystic ovary	An ovary with at least 12 follicles measuring 2–9 mm in diameter in at least one ovary (Rotterdam criteria). PCO may be present in women with PCOS, but also in women with normal ovulatory function and normal fertility
Prevalence	The extent to which a population is affected by a particular condition (e.g. disease) at a given time period

Progesterone	A steroid hormone, secreted by the ovaries, whose function is to prepare the uterus for the implantation of a fertilized ovum and to maintain pregnancy
Prolactin	A peptide gonadotrophic hormone secreted by the pituitary gland that stimulates growth of the mammary glands and lactation in females
Survey	An examination of opinions, behaviour, etc., performed by asking people questions
Thyroid gland	One of the largest endocrine glands in the body, controls the rate of use of energy sources, protein synthesis, and body's sensitivity to other hormones
Thyroid hormones	Tyrosine-based hormones produced by thyroid gland and that regulate metabolism, heat production, protein synthesis, and many other body functions. These hormones include T3, T4, and calcitonin
Thyroid-stimulating hormone	A hormone that stimulates the thyroid gland to produce thyroxine, and then triiodothyronine, which stimulates the metabolism of tissue in the body
Thyrotropin-releasing hormone:	A hormone produced by the hypothalamus that stimulates the release of thyrotropin (TSH) and prolactin from the anterior pituitary

LIST OF ABBREVIATIONS

AFC	Antral Follicle Count
AI	Artificial Insemination
Ag/Ab	Antigen/Antibody
AMH	Anti-Müllerian Hormone
Anti-HBs	Hepatitis B Surface Antibody
ART	Assisted Reproductive Technology
CDC	Centers for Disease Control and Prevention
CMIA	Chemiluminescent Microparticle Immunoassay
ECL	Electrochemiluminescence
ED	Egg Donation
ET	Embryo Transfer
FET	Frozen-thawed Embryo Transfer
FSH	Follicle-Stimulating Hormone
GIFT	Gamete Intrafallopian Transfer
GnRH	Gonadotropin-Releasing Hormone
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
hCG	Human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HSG	Hysterosalpingography
ICSI	Intracytoplasmic Sperm Injection
IUI	Intrauterine Insemination
IVF	In Vitro Fertilization
LH	Luteinizing Hormone

MoHSS	Ministry of Health and Social Services
PID	Pelvic Inflammatory Disease
PM	Photomultiplier
PRL	Prolactin
RLU	Relative Light Unit
S/CO	Signal to Cut-off
STIs	Sexually Transmitted Infections
TP	Treponema Pallidum
TPA	Tripropylamine
TRH	Thyrotropin-Releasing Hormone
TSH	Thyroid-Stimulating Hormone
WHO	World Health Organization
ZIFT	Zygote Intrafallopian Transfer

LIST OF TABLES

TABLE OF CONTENTS

CHAPTER ONE:

INTRODUCTION

Infertility is defined as the failure of a couple to conceive after continuous unprotected coitus for one year in women under 35 years of age or 6 months in women above 35 years of age (Agenor & Bhattacharya, 2015; Benksim, Elkhoudri, Ait Addi, Baali, & Cherkaoui, 2018; Emokpae, Osadolor, & Ohonsi, 2011; Tabong & Adongo, 2013) and is classified into primary and secondary infertility (Agenor & Bhattacharya, 2015; Mascarenhas, Flaxman, Boerma, Vanderpoel, & Stevens, 2012; Tabong & Adongo, 2013). The probability of women conceiving varies and is dependent on the length of sexual exposure, frequency of coitus, and age (Anwar & Anwar, 2016; Eniola, Adetola, & Abayomi, 2012).

Primary infertility occurs when a couple fails to conceive and give birth to a first live child after one year of unprotected coitus, while secondary infertility occurs when a couple has previously conceived and given birth to a first live child but is now failing to obtain a second live child. However, it is necessary to distinguish infertility from sterility. Sterility is defined as the absolute inability to conceive (Bernardi, Cohen, & Stephenson, 2013; Pfeifer et al., 2015).

Infertility is a global burden and requires attention. According to the World Health Organization (WHO), 25% of the couples in developing countries are affected by infertility (World Health Organization, 2012). A study seeking to establish global trends of infertility conducted by Mascarenhas et al. (2012), listed Sub-Saharan Africa and South Asia among the regions with the highest prevalence rates of infertility. Moreover, that study also noted that the prevalence of primary infertility was higher in Central sub-Saharan Africa compared to Eastern and Southern Africa where it was lower. Studies have further shown that the prevalence of couples worldwide affected by infertility was between 10% to 15%, while 20 – 60% of couples are affected in Africa alone (Eniola et al., 2012; Pedro & Andipatin, 2014).

In the 1990s, motives for parenthood were categorized as happiness, well-being, fatherhood/motherhood, identity, continuity, and social status (van Balen & Trimbos-Kemper, 1995). These motives led to fewer social consequences in developed countries as opposed to developing countries as a result of childlessness due to infertility (Afolabi, 2018; Dyer, 2007; Ibisomi & Mudege, 2014). In most of the developing world, particularly Africa, having children is highly prized as evidenced by the motives for parenthood among African couples such as carrying the family name, to provide assistance with household chores, to provide for and look after the elderly, as well as, to obey a religious command to reproduce, for joy and companionship, for respect and status in society.

Therefore, infertility is not taken lightly in some cultures and may have negative implications on the psychological life, marital relationship, sexual relationship, and quality of life (Afolabi, 2018; Ibisomi & Mudege, 2014; Tabong & Adongo, 2013).

Although infertility can result from both the male and/or the female, often the female partner is the one that faces the most pressure since it is believed that the failure to conceive could be due to the inability to fulfil one's role as a woman and this echoes the Chadian proverb that says: "A woman without children is like a tree without leaves." (Dyer, 2007; Tabong & Adongo, 2013). The implications of involuntary childlessness due to infertility in the African context to name but a few range from polygamous relationship and therefore risk of Human immunodeficiency virus (HIV) acquisition, divorce, domestic abuse or maltreatment, loss of social security, lack of domestic support in the home, poverty or high dependency among elderly people, lack of respect and status in society, social isolation and humiliation (Afolabi, 2018; Fledderjohann, 2012; Hess, Ross, & Gililland, 2018; Ibisomi & Mudege, 2014; Luk & Loke, 2015).

Could assisted reproductive technology be the answer to the plight of infertile women who have not been able to conceive naturally? This is to our knowledge, the first study to assess fertility and pregnancy outcome in infertile women undergoing assisted reproductive (ART) technology treatment in Windhoek, Namibia.

1.1 Problem statement

In the developing world, fertility care is among the most unattended to and underestimated health care concern (Ombelet, 2011). According to the WHO data, more than 180 million couples in developing countries suffer from primary or secondary infertility. The social stigma of childlessness still leads to isolation and abandonment in many developing countries (Ombelet & Onofre, 2019).

Although the negative consequences of childlessness are much more pronounced in developing countries when compared to developed countries, interest of the international community and local health care providers to boldly create awareness and bring solutions to the affected individuals and families is still lacking (Ombelet, 2014; Ombelet, Cooke, Dyer, Serour, & Devroey, 2008).

The fundamental human right to basic medical care such as assistance to fertility care concerning socio-cultural, ethical and political differences is often neglected in many developing countries (Ombelet, 2011). Although ART has given hope to many families across the globe, little is done to give support to men, women, and families affected by infertility in sub-Sahara Africa.

Therefore, the purpose of this study was to determine the effectiveness of ART treatment on pregnancy outcomes and assess possible risk factors that lead to infertility in order to address the challenge of involuntary childlessness that is so common in developing countries such as Namibia.

1.2 Justification of the study

Infertility is a global burden and requires attention. According to the WHO, 25% of the couples in developing countries are affected by infertility (World Health Organization, 2012). The stigma of infertility among women in Africa is a serious socio-economic concern that needs to be addressed and alleviated (Dyer & Patel, 2012).

Fertility treatment is costly and is only currently performed at a few privately owned hospitals and/or clinics in Namibia. This study will serve to raise awareness about infertility prevention and management since this subject has been understudied in Namibia as very few published studies done in Namibia were found during the undertaking of this research project.

1.3 Aim and objectives of the study

The primary aim of this study was to determine the prevalence of successful pregnancy outcomes among infertile women undergoing ART treatment at the Cape Windhoek Fertility Clinic in Windhoek, Namibia.

The study purposed to achieve the following objectives:

- To determine the cause of infertility among women seeking and/or undergoing ART treatment.
- To assess the impact of sexually transmitted infections (STIs) in infertile women.
- To determine suitable ART treatment methods for successful pregnancy outcomes among women undergoing ART treatment at the Cape Windhoek Fertility Clinic.

1.4 Research Questions

This study sought to answer the following research questions:

- What were the causes of infertility among women seeking and/or undergoing ART treatment?
- What were the impacts of STIs such as HIV, Syphilis, HBsAg, HCV, among women seeking and/or undergoing ART treatment?
- What was the most suitable ART treatment method (s) for successful pregnancy outcomes among women undergoing ART treatment at the Cape Windhoek Fertility Clinic?

1.5 Significance of the study

This study will add knowledge to the medical field in terms of reproductive biology within the Namibian context since little research has been conducted. It will further help stakeholders such as the MoHSS to develop intervention measures that would prevent infertility and/or increase the prospects of positive pregnancy outcome for those undergoing treatment such as:

- Educating the public about the significance of age in family planning thus avoiding delayed parenthood since fertility starts to decline as one gets older.
- Educating the public about the impact lifestyle choices (i.e. smoking, drinking, exercise, body weight, eating a balanced diet, etc) have on either boosting or decreasing fertility.
- Encouraging individuals to go for annual screening, diagnosis, and treatment of other conditions (e.g. diabetes) that may, in turn, affect their fertility prospects.
- Educating the public about the impact of HIV and other STIs on fertility thereby encouraging abstinence or the practice of protected coitus.
- To create awareness about the various infertility treatment options available to couples who fail to conceive, thus removing the stigma that comes with involuntary childlessness in certain cultures.
- Moreover, the outcome of this study may help couples seeking ART treatment to have realistic expectations given the screening results before initiation of treatment.
- The study may also further serve as a guideline to assist couples who desperately want to conceive but are not succeeding, to consider other alternatives such as adoption.
- Finally, the study may help in the development of educational material and tools for infertility.

CHAPTER TWO:

LITERATURE REVIEW

This section presents the literature review that is relevant to the topic under investigation with regards to existing information.

Educated women spent more time pursuing fertility care than uneducated women, and women with secondary infertility spent less time pursuing fertility care than women who had primary infertility (Wu, Elliott, Katz, & Smith, 2013). The overall cost is one of the major reasons as to why patients choose not to seek ART treatment and/or discontinue treatment before falling pregnant (Indongo & Pazvakawambwa, 2012; Lande, Seidman, Maman, Baum, & Hourvitz, 2015; Palamuleni, 2017). Furthermore, patients that spend more time pursuing fertility care tend to have more care-related stress than those who spent less time seeking solutions. Psychological disorders such as depression and anxiety are predominant among infertile women (Unuane, Tournaye, Velkeniers, & Poppe, 2011a; Wu et al., 2013; Yusuf, 2016).

2.1 The function of the reproductive system

The reproductive system ensures the continued existence of the human species by producing, storing, nourishing, and transporting functional male and female reproductive cells called gametes. During coitus, semen from the male is deposited into the vagina, the sperm then travels upward in the female reproductive tract. The process of fertilization begins upon the sperm cell encountering an oocyte in the fallopian tubes. The uterus will then enclose and support the developing embryo as it grows into a foetus and prepares for birth (Martini, Nath, & Bartholomew, 2018).

The reproductive system consists of primary (i.e. testes and ovaries) and secondary sex organs (i.e. in the male: ducts, glands, penis, and in the female: uterine tubes, uterus, cervix, and vagina), which are necessary for reproduction (Saladin, 2010; Shier, Butler, Lewis, Day, & Pilcher, 2016).

2.1.1 The female reproductive system

The female reproductive system exerts two major functions: exocrine (secretion has extracellular effects) and endocrine (secretion has intracellular effects).

The organs of the female reproductive system are specialized to produce and maintain the female sex cells (exocrine function); transport these cells to the site of fertilization; provide a favourable

environment for the developing offspring; move the offspring to the outside, and produce female sex hormones (endocrine function). The primary sex organs (gonads) of this system are the two ovaries, which produce the female sex cells (oocytes) and sex hormones (progesterone and oestrogens). The accessory sex organs (secondary sex organs) of the female reproductive system are the internal (uterine tubes, uterus, cervix, and vagina) and external (mons pubis, clitoris, labia minora, labia majora, vaginal orifice, accessory glands, and erectile tissues) reproductive organs (Saladin, 2010; Shier et al., 2016).

2.1.2 The female reproductive cycle (Sexual cycle)

Shier et al. (2016) described the reproductive lives of women as consisting of cycles such as the reproductive cycle (sequence of events from fertilization to giving birth) and the sexual cycle (events that occur every month when pregnancy does not occur). The sexual cycle further consists of two cycles controlled by hormone secretion fluctuations in the female body: the ovarian cycle (events in the ovaries) and the menstrual cycle (parallel changes that take place in the uterus):

The ovarian cycle: Follicular phase→ Ovulation→ Luteal phase

The menstrual cycle: Proliferative phase→Secretory phase→Premenstrual phase→Menstrual phase

On average, the sexual cycle may last 28 days but it differs from individual to individual and from month to month. Saladin (2010) and Shier et al. (2016) outlined the sequence of events that takes place during a female's sexual cycle in the following order:

- The Gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates the pituitary gland to produce FSH and LH.
- The anterior pituitary gland secretes FSH and LH.
- FSH stimulates maturation of a dominant follicle.
- Granulosa cells of the follicle produce and secrete oestrogens, which maintains secondary sex traits and causes the endometrium to thicken.
- The anterior pituitary gland releases a surge of LH leading to ovulation.
- Follicular and thecal cells become luteal cells, which secrete oestrogens and progesterone. Oestrogen continues to stimulate uterine wall development; progesterone stimulates the endometrium to become more glandular and vascular; oestrogens together with progesterone inhibit secretion of FSH and LH from the anterior pituitary gland.

- If the secondary oocyte is not fertilized, the corpus luteum degenerates and no longer secretes oestrogens and progesterone.
- As the concentration of luteal hormones decline, blood vessels in the endometrium constrict.
- The uterine lining disintegrates and sloughs off, producing a menstrual flow.
- The anterior pituitary gland is no longer inhibited and again secretes FSH and LH.
- The reproductive cycle repeats.

2.2 The endocrine system and reproductive functions

The human reproductive cycles are controlled by the interaction between the hormones from the hypothalamus and anterior pituitary with the hormones from reproductive tissues and organs. The hypothalamus is responsible for monitoring and stimulating the release of FSH and LH from the pituitary gland. At puberty, the adrenals release the hormones that must be present for GnRH to be produced (Saladin, 2010; Shier et al., 2016).

Although FSH and LH are named after their functions in female reproduction, they are produced in both genders and play important roles in controlling reproduction. In females, FSH assists with the growth of ovarian follicles and secretion of oestrogen, while in males, it assists with sperm production. LH, on the other hand, assists with ovulation and maintenance of corpus luteum in females, while it assists with testosterone secretion in males. Other hormones, such as PRL serve more specified functions in the male and female reproductive systems. In both males and females, androstenedione serves as a precursor to testosterone and oestrogens, while activin stimulates the production and release of FSH and assists in menstrual cycle regulation (Molnar & Gair, 2012).

The thyroid gland is the body's largest single organ responsible for the production of thyroid hormones and calcitonin. It is located in the lower anterior neck and posterior to it are the parathyroid glands. It is composed of two lobes that rest on each side of the trachea, connected by a band tissue called the isthmus, which gives it its butterfly-like shape (Gabriel, 2008; Gardner, Shoback, & Greenspan, 2011).

Iodine is a key structural component of thyroid hormones; therefore its metabolism is vital in determining thyroid function. This micronutrient can be found in seafood, iodized salt, dairy products, iodine-enriched bread, and vitamins (Bishop et al., 2010). The WHO daily recommendation for iodine intake are 150µg for adults, 200µg for pregnant and lactating women, and 50-250µg for children (Gardner et al., 2011; World Health Organization, 2015). The failure to take adequate amounts of

iodine renders the thyroid gland unable to manufacture adequate amounts of thyroid hormones leading to thyroid deficiency disorders such as hypothyroidism.

The prevalence of iodine deficiency (42%) in Africa remains high despite efforts to eliminate it. Iodine deficiency is the predominant cause of thyroid dysfunction, particularly in Africa. The common iodine deficiency disorders encountered are hypothyroidism, goitre, cretinism, and congenital anomalies. Areas well supplied with iodine are predominantly faced with autoimmune thyroid disorders such as Grave's disease and Hashimoto's thyroiditis (Ogbera & Okosieme, 2014; Sidibé, 2007).

The two main thyroid hormones are Thyroxine (T4) and Triiodothyronine (T3), which are made up of glycoproteins and iodine. The earlier consisting of 4 iodine molecules while the latter of 3 iodine molecules. T3 is more metabolically active compared to T4 and is often considered to be the active form of thyroid hormone, while T4 is regarded as the prohormone (Bishop et al., 2010; Evers, 2012; Welsh & Soldin, 2016).

The production of thyroid hormones is regulated by TSH while the secretion of TSH is regulated by the amount of circulating T4 and T3 in the blood through a mechanism called negative feedback that is trigged by Thyrotropin-releasing hormone (TRH). Sufficient concentrations of T4 and T3 in the blood inhibits the secretion of TSH while a decrease in their concentrations triggers the secretion of TSH (Gabriel, 2008; Gardner et al., 2011).

Hypothyroidism is characterized by a decrease in the concentration levels of thyroid hormones in the blood circulation. The growth and function of the thyroid gland are regulated by the hypothalamus-pituitary-thyroid axis. Hypothyroidism resulting from a disorder of the thyroid gland is referred to as primary hypothyroidism, whilst the disorder from another organ (e.g. pituitary and/or hypothalamus) is referred to as secondary hypothyroidism (Dave et al., 2015; Franklyn, 2013; Gaitonde, Rowley, & Sweeney, 2012).

Overt hypothyroidism is identified by low concentration levels of T3 and T4 with high concentration levels of TSH, whereas subclinical hypothyroidism is identified by normal concentration levels of T3 and T4 with high concentration levels of TSH (Khandelwal & Tandon, 2012; Nazarpour, Tehrani, Simbar, & Azizi, 2015; Teng, Shan, Patil-Sisodia, & Cooper, 2013).

The symptoms of hypothyroidism vary from non-specific to more specific ones, which may include: cold intolerance, fatigue, weight gain, weakness, constipation, depression, hair loss, memory impairment, dry skin, slow heart rate, menorrhagia or secondary amenorrhea (Gabriel, 2008; Gaitonde et al., 2012). The reliance on laboratory measurements of TSH, T4, and T3 levels in serum is indispensable for the diagnosis of hypothyroidism. TSH has been established as the first-line test in

the diagnosis of thyroid disorder in cases of a stable hypothalamus-pituitary axis, whereas T4 as the second-line test in cases where the TSH proves not reliable. T3, on the other hand, provides little information that aids in the diagnosis when TSH is high (Hiraoka et al., 2016; Warren, 2014).

Hypothyroidism is more predominant in females than males and there a relationship between its prevalence and age (Evers, 2012; Welsh & Soldin, 2016). According to previous studies, the prevalence of hypothyroidism among women of child-bearing age varies between 2%-4% and is most commonly caused by thyroid autoimmunity (Eniola et al., 2012; Ogbera & Okosieme, 2014; Poppe, Velkeniers, & Glinoer, 2007; Saran et al., 2016). The problem of hypothyroidism can be corrected by early interventions with thyroxine therapy.

Hypothyroidism is among the most common endocrine disorders that lead to infertility (Dunn & Turner, 2016; Saran et al., 2016), therefore a proper and functional thyroid gland is vital for uncompromised reproductive health. In hypothyroidism, women tend to present with decreased levels of progesterone, oestradiol, FSH, and LH. Moreover, studies have also shown a correlation between high PRL concentration and hypothyroidism in infertile women (Cho, 2015; Evers, 2012; Murto, Bjuresten, Landgren, & Stavreus-Evers, 2013). Hyperprolactinemia causes an increase in the production of TRH, altering the secretion of GnRH which triggers the delay of LH response and inadequate corpus luteum (Binita, Suprava, Mainak, Koner, & Alpana, 2009; Fupare, Jambhulkar, & Tale, 2016; Nupur, Andaleeb, Premlata, Nisha, & Swati, 2015).

In hypothyroidism women of child-bearing age present with irregular menstruation cycles and decreased ovarian reserve (anovulation) which in turn leads to infertility (Ahmad, Priya, & Akhtar, 2015; Evers, 2012; Nupur et al., 2015). Women suffering of hypothyroidism who successfully conceive have higher risk of pregnancy-related adverse effects such as spontaneous pregnancy loss, preterm birth, low birth weight, pregnancy-induced hypertension, placental abruption, and postpartum haemorrhage (Hiraoka et al., 2016; Krassas, Poppe, & Glinoer, 2010; Medenica et al., 2015; Nazarpour et al., 2015).

2.2.1 Hormonal control of female reproductive function and secondary sex characteristics

The control of reproduction in females is more intricate. As with the male, the GnRH causes the release of FSH and LH from the anterior pituitary. Besides that, oestrogens and progesterone are released from the developing follicles. In females, oestrogen is responsible for endometrial re-growth, ovulation, and calcium absorption. Moreover, oestrogen is also responsible for the secondary sexual characteristics of females such as the development of breasts, widening of the hips, no facial hair, and

faster bone maturation. Progesterone, on the other hand, is responsible for endometrial re-growth, inhibition of FSH, and release of LH (Molnar & Gair, 2012).

In females, FSH stimulates the development of ova, which develop in structures called follicles. These follicles in turn release inhibin which is responsible for the inhibition of FSH production. LH also plays a role in the development of ova, induction of ovulation, and stimulation of oestradiol and progesterone production by the ovaries. Oestradiol and progesterone prepare the body for pregnancy and are also involved in the regulation of the menstrual cycle (Martini et al., 2018; Molnar & Gair, 2012; Shier et al., 2016).

2.3 Global trends and prevalence of infertility

Infertility is a global health issue and is characterized by the failure to establish a clinical pregnancy after 12 months of regular and unprotected sexual intercourse (Vander Borght & Wyns, 2018). An awareness and thorough knowledge of the prevalence and distribution of infertility around the world serves as a stepping-stone towards the development of measures and policies to aid in the reduction of the burden caused by infertility (Mascarenhas et al., 2012).

On average, between 8% and 12% of reproductive-aged couples worldwide are affected by infertility (Vander Borght & Wyns, 2018). Males are said to be responsible for 20–30% of infertility cases while female are responsible for about 37% of all infertility cases among couples. Secondary infertility is the most common form of female infertility around the globe, often due to reproductive tract infections (Unuane, Tournaye, Velkeniers, & Poppe, 2011b; Vander Borght & Wyns, 2018).

Sadly, areas of the world with the highest prevalence of infertility (20-60%) are often those with poor access to ART such as Africa (Inhorn & Patrizio, 2014a; Pedro & Andipatin, 2014). The differences in the occurrence of infertility in the developed world as compared to the developing world is owing to variations in access to reproductive health care (i.e. IVF centres) and different socio-cultural values as it pertains to procreation and childlessness (Ombelet, 2011).

A study seeking to establish global trends of infertility conducted by Mascarenhas et al. (2012), listed sub-Saharan Africa, and South Asia among the regions with the highest prevalence rates of infertility. Furthermore, East Asia and the Pacific, Central and Eastern Europe and Central Asia, are also known to have high a prevalence of infertility (Inhorn & Patrizio, 2014a).

2.4 Causes of infertility

Fertility depends on the timing of sexual intercourse coupled with the interaction of other factors such as hormones produced by the glands as well as the female and male reproductive organs. Like most of the body's systems, the endocrine system regulates the complex processes involved in male and female fertility through the release of hormones. Of the hormones responsible for regulating the reproductive system and cycles in both genders, the three key hormones are GnRH, LH, and FSH.

For ovulation and sperm production to take place, these hormones must be produced in the correct amounts, correct sequence and correct time by the body. If that fails to take place, there is a reduced probability of achieving conception. Ovulation is indispensable for a successful pregnancy outcome. Over the years researchers have been able to categorize the etiological factors of infertility among women into several classes: ovarian; tubal; luteal; cervical; endometriosis; uterine; male factor and unexplained factor (Jose-Miller, Boyden, & Frey, 2007; Poppe et al., 2007; Unuane et al., 2011b).

The Centers for Disease Control and Prevention (CDC) categorized the causes of female infertility into 3 broader categories, namely: defective ovulation, defective transport, and defective implantation (Anwar & Anwar, 2016):

- Defective ovulation occurs because of the following causes:
  - ✓ Endocrine disorders: ovulation may be hampered due to hormonal imbalances as a result of a dysfunctional endocrine system, which then leads to infertility.
  - ✓ Physical disorders: Certain physical disorders such as obesity, anorexia nervosa, and excessive exercise may lead to overweight or malnutrition, and later the menstrual cycle, thus making the couple infertile.
  - ✓ Ovarian disorders: Polycystic ovarian disease (PCO) can lead to infertility because of an increased amount of testosterone and LH and decrease uptake of glucose by muscle, fat and liver cells resulting in the production of large amounts of insulin by the pancreas. Low FSH levels also hinder the production of eggs from the ovarian follicles and lead to form fluid-filled ovarian cysts that eventually cover the whole ovaries and prevent conception.
  - ✓ Endometriosis: Occurs when sections of the uterine lining implant in the vagina, ovaries, fallopian tubes or pelvis. These implants grow with each passing menstrual cycle and eventually turn into scars that block the passage of the egg and delay pregnancy.

- Defective transport occurs because of the following causes:
  - ✓ Ovum: Tubal obstruction may occur resulting in the egg not being releasing or trapped thus delaying conception. This is owing to pelvic inflammatory disease (PID), gonorrhoea, peritonitis, previous tubal surgery, and fimbrial adhesions.
  - ✓ Scar tissue after abdominal surgery: After abdominal surgeries, the presence of scar tissue may alter the movement of the ovaries, fallopian tubes, and uterus, resulting in infertility.
  - ✓ Sperm: The presence of psychosexual problems such as vaginismus, or dyspareunia may hinder fertilization and make the couple infertile.
  - ✓ Cervix: Delay in pregnancy may occur as a result of trauma, surgery, infection, anti-sperm antibodies in the cervical mucus.

- Defective implantation can occur because of the following causes:
  - ✓ Congenital anomaly and fibroids: Congenital uterine anomaly such as bicornuate uterus and uterine fibroids near the fallopian tubes or cervix may alter implantation of the zygote and cause infertility.

Ovarian reserve is a good determining factor in assessing a female's fecundity since it reveals the quality and quantity of the ovarian follicle pool (Grynnerup et al., 2012). The incidence of infertility increases steadily with the age of the female partner as a result of decreasing and ageing ovarian reserve. Therefore, with increasing age (i.e. after 31 years of age) the percentage of women with infertility steadily increases because natural fertility begins to decrease, which is subsequent and notable after 37 years of age. Women older than 40 years were 71% less likely to achieve pregnancy compared with women less than 35 years (Balasch, 2010; Deatsman, Vasilopoulos, & Rhoton-Vlasak, 2016; Goodwin, Montoro, Muderspach, Paulson, & Roy, 2010; Shier et al., 2016; Smith et al., 2011; Somigliana et al., 2016).

In the developed world, hormonal dysregulation is often the cause of female infertility compared to the developing where the predominant causes of infertility include infections, unsafe abortions, post partum complications and tuberculosis (Ombelet, 2014).

2.4.1 Infertility and causes of infertility in sub-Saharan Africa

Despite infertility being a global problem, the highest prevalence is in low resource countries, where infection-related tubal damage is the commonest cause (Inhorn & Patrizio, 2014a; Sharma, Mittal, & Aggarwal, 2009). The prevalence of primary infertility is low when compared to that of secondary infertility in sub-Saharan Africa (Polis, Cox, Tunçalp, McLain, & Thoma, 2017). It has been observed

that the causes of infertility are related to geographical differences. While age was observed to be the most common risk factor of infertility in western countries, it was noted that in Africa, infections (i.e. STIs) was the most common cause (Afolabi, 2018; Gimenes et al., 2014; Ombelet & Onofre, 2019; Pedro & Andipatin, 2014).

Genital tract infections play a role in human infertility. STIs are caused by several pathogens, including bacteria, viruses and protozoa, and can induce male infertility through multiple pathophysiological mechanisms. Additionally, horizontal transmission of STI pathogens to sexual partners or vertical transmission to foetuses and neonates is possible. Chronic or inadequately treated infections seem to be more relevant to infertility than acute infections are, although in many cases the exact aetiological agents remain unknown (Gimenes et al., 2014).

In sub-Saharan Africa, STIs are responsible for more than 70% of cases of pelvic infections, with most being caused by Chlamydia and N. gonorrhoea. Of these two organisms, N. gonorrhoea is the causative agent of acute infection of the fallopian tubes thus requiring immediate treatment, even hospitalisation, and making diagnosis easier. Infertility due to tubal damage in women and altered spermatogenesis in men can be found in HIV infected individuals. These effects happen both directly and through increased susceptibility to other STIs. Other infections associated with infertility in developing countries include Lepromatous leprosy, schistosomiasis and malaria (Gimenes et al., 2014; Kjetland, Leutscher, & Ndhlovu, 2012; Sharma et al., 2009).

The causes of infertility can be further divided into two groups. The first group includes anatomical, genetical, hormonal and immunological problems. These have been described as the 'main causes' of infertility. The second group includes preventable cases which vary widely in the world. The preventable causes mostly include iatrogenic and infection-related causes. In Africa, about 85% of women had a diagnosis of infertility caused by infection, a figure which is more than double that of the rest of the world. The type and mode of infection vary from country to country depending on the social factors, health infrastructure, healthcare practices and environmental factors. Iatrogenic causes of infertility constitute approximately 5% of all causes in Western Europe compared to 15.5% in Africa (Ombelet et al., 2008; Sharma et al., 2009).

2.5. Investigations for the diagnosis of infertility

Fertility is not only determined by adequate functioning of the pituitary and the reproductive tract and its associated glands. Other endocrine organs such as the thyroid, the adrenal gland, the liver and total body composition also contribute to a normal body condition that allows conception therefore a correct diagnosis is a prerequisite to successful treatment (Smitz, 2019).

A female's fertility can also be affected by a myriad of factors such as: intoxication with alcohol which may harm the developing foetus; smoking which may reduce chances of conceiving; dietary supplementation with folic acid to avoid having babies with neural tube defects; obesity which may delay conception; low body weight and irregular menstruation which reduces chance of conception; environmental hazards due to the nature of occupation; interference by drugs; and frequency and timing of sexual intercourse. These factors should also be taken into account when investigations to determine cause of infertility are underway (Fields, Chard, James, & Treasure, 2013).

In females, a wide range of diagnostic tools are available to the infertility specialist to determine the cause of infertility (Smitz, 2019). Evaluation for infertility should be done earlier in patients with risk factors for infertility or in women older than 35 years. Detailed history should be taken and physical examination should be assessed as it helps to direct the course of action during evaluation (Anwar & Anwar, 2016; Lindsay & Vitrikas, 2015).

A thorough history of all obstetric and gynaecologic-related concerns should be taken alongside physical examination which may include among many others: the patient's height and weight, palpitation of the thyroid, breast exam, signs of endometriosis, and cervical abnormalities (Anwar & Anwar, 2016; Goodwin et al., 2010; Lindsay & Vitrikas, 2015).

Effective treatment of infertility depends largely on the correct diagnosis. Below is a list of investigations employed to aid with diagnosis of infertility:

<u>Investigation of suspected ovarian disorders</u>

- Ovarian reserve

  A woman's fertility is related to her ovarian reserve, which influences her chances of conceiving. Ovarian reserve declines with age, therefore age serves as a good indicator of a female's ovarian reserve status. Tests widely used to estimate a women's ovarian reserve

include measurement of AMH, FSH and transvaginal ultrasound measurement of total antral follicle count (AFC) (Goodwin et al., 2010; NCCWCH, 2013).

- The regularity of the menstrual cycle

Regular menstrual cycles within 26 to 36 days are usually indicative of ovulation. Ovulation can be confirmed by measurement of serum progesterone in the mid-luteal phase. For women with prolonged irregular cycles, this test may need to be performed later in the cycle. Moreover, serum gonadotrophins (FSH) should be measured in women with irregular cycles (Goodwin et al., 2010; NCCWCH, 2013).

Anovulation and oligo-ovulation are ovulatory disorders. The WHO categorizes ovulation disorders into three groups: Group I, II, and III.

Group I ovulation disorders are caused by hypothalamic-pituitary failure. Conditions included in this category are hypothalamic amenorrhea and hypogonadotropic hypogonadism. Women that present with amenorrhoea usually have low levels of gonadotrophins and oestrogen deficiency. It is found in approximately 10% of women (Jankowska, 2017; Mikhael, Punjala-Patel, & Gavrilova-Jordan, 2019; NCCWCH, 2013).

Group II ovulation disorders are caused by dysfunctions of the hypothalamic-pituitary-ovarian axis. Conditions included in this category are polycystic ovary syndrome and hyperprolactinaemic amenorrhoea. It is found in approximately 85% of women (Jankowska, 2017; Mikhael et al., 2019; NCCWCH, 2013).

Group III ovulation disorders are caused by ovarian failure. It is found in approximately 5% of women (Jankowska, 2017; Mikhael et al.,, 2019; NCCWCH, 2013).

Investigation of suspected tubal and uterine abnormalities

- Assessing tubal damage

Tubal blockage involves the proximal part (which is closest to the uterus), the mid part, or the distal part (which is furthest from the uterus). Causes of tubal disease may include tubal obstruction and pelvic adhesions due to infection, endometriosis and previous surgery (NCCWCH, 2013; Sadow & Sahni, 2014).

HSG should be offered to women known to not have comorbidities such as PID, previous ectopic pregnancy, and endometriosis since it is a reliable test for ruling out tubal occlusion, less invasive and most cost-effective in comparison to laparoscopy. On the other hand, hysterosalpingo-contrast-ultrasonography should be offered to women known to have comorbidities (Briceag et al., 2015; Goodwin et al., 2010; NCCWCH, 2013; Sadow & Sahni, 2014).

- Assessing uterine abnormalities

Hysteroscopy is regarded as the 'gold standard' test for identifying uterine abnormalities such as adhesions, polyps, submucous leiomyomas and septae, as it allows direct visualisation of the uterine cavity. Uterine leiomyoma is associated with a reduced chance of clinical pregnancy in women undergoing ART (Briceag et al., 2015; Goodwin et al., 2010; NCCWCH, 2013).

Transvaginal ultrasound is more accurate and reliable for the identification of pelvic anatomy. Ultrasound can be used in the evaluation of pelvic pathologies, such as endometriosis, endometrioma, cysts, polyp, leiomyoma, adnexal and ovarian abnormality, where such abnormalities are present (Briceag et al., 2015; NCCWCH, 2013; Sadow & Sahni, 2014).

Investigations for viral infection, cancer and Chlamydia

- Viral transmission

An important area of work for fertility specialists has been assisting couples where one has a sexually transmissible viral infection, such as HIV, to become pregnant while minimising the risk of viral transfer using ART treatments. The virus in question determines the approach used to minimise the risks of transmission (Goodwin et al., 2010; NCCWCH, 2013).

For HBV, transmission rates are minimised by the use of pre-exposure vaccination. HCV has a low transfer rate via sexual intercourse, but sperm washing has been used to reduce this risk of transmission. For HIV the standard approach for the female to male transmission is the use of ART, such as intrauterine insemination (IUI) or in vitro fertilization (IVF). For the male to female transmission, the standard approach has been sperm washing. Sperm washing serves to reduce the viral load to a very low or undetectable level in prepared sperm. The washed

sperm preparation can then be transferred to the women using IUI or used to fertilise eggs in IVF or intracytoplasmic sperm injection (ICSI) (NCCWCH, 2013; Zafer et al., 2017).

- Cervical cancer screening

  Abnormal cervical cytology that is overlooked may lead to an increased delay in fertility treatment because treatment of cervical intraepithelial neoplasia is more complicated during pregnancy (Goodwin et al., 2010; NCCWCH, 2013).

- Screening for Chlamydia trachomatis

  The 2016 global prevalence estimates of Chlamydia in women were 3.8% (Rowley et al., 2019). It is a major cause of PID, leading to chronic abdominal pain, ectopic pregnancy and tubal factor infertility. Asymptomatic chlamydial infection may go unrecognised and untreated (Goodwin et al., 2010; NCCWCH, 2013).

2.6 ART treatment modalities of infertility

Up until the discovery of ART, people had little control over their fertility and couples that could not get a child had no other choice but to accept the fact. In contrast, although today infertility is a relatively common problem that touches deeply the soul of couples affected, medical science has increased the chances of giving solutions to the problem with ART treatment (Inhorn & Patrizio, 2014a; Roupa et al., 2009)

In the 1960s, hormones (oestrogen derivatives) and gonadotrophins became available to stimulate ovulation (Buxton & Herrmann, 1960; Smitz, 2019). This was followed by the development of ART in the seventies, and in 1978 the first successful fertilization of human eggs in the laboratory took place, and the world's first "test-tube" baby, Louise Brown, was born (Steptoe & Edwards, 1978). This "test-tube baby" was a real milestone because it gave hope to the infertile couples as they now could achieve a successful pregnancy through ART. Furthermore, in the USA, the first successful childbirth in 1981 through ART led to rapidly increasing application of this method and the creation of specialized centres (Roupa et al., 2009). ART has since become widely implemented around the world and constant developments in every aspect of these technologies have led to their high level of efficacy in treating infertile couples (Smitz, 2019).

Over the past 6 decades, the therapeutic arsenal to treat infertility has substantially increased. The discovery of medication to regulate fertility has had a strong impact on society - the major

consequence being that couples can decide to postpone childbirth. On-hand availability of a complete panel of hormones and infectious disease parameters is of primary importance, especially for infertility doctors practising ART (Smitz, 2019).

ART involves all interventions that include the in vitro handling of both human oocytes and sperm or of embryos for reproduction. This includes but is not limited to, IVF and embryo transfer (ET), ICSI, embryo biopsy, preimplantation genetic testing (PGT), assisted hatching, gamete intrafallopian transfer (GIFT), gamete and embryo cryopreservation, semen, egg and embryo donation, and gestational carrier cycles (Zegers-Hochschild et al., 2017).

Treatment cycles play a role in the successful outcome for those seeking ART treatment (Chambers et al., 2014). Those failing IVF after two cycles may want to consider other treatment strategies, such as sperm donation, egg donation, or further modifications in the IVF or ICSI protocol because additional cycles seem less likely to increase reproductive success as much as earlier cycles (Gameiro, Verhaak, Kremer, & Boivin, 2013; Gnoth et al., 2011; Smith et al., 2011).

The female patient's basal endocrine profile, age, follicular reserve and expected response to gonadotrophin treatment should be considered when seeking to establish a diagnosis and propose a specific treatment program to be employed (Goodwin et al., 2010; Smitz, 2019).

Different treatment methods are available today from which women may choose from based on their infertility history, diagnosis and likelihood of success. Below is a list of ART treatment:

IUI

In IUI, sperm is inserted into the uterine cavity around the time of ovulation. IUI can be performed during a natural cycle without stimulation through the use of drugs (NCCWCH, 2013). However, stimulation may also be induced through the use of oral anti-oestrogens (course of tablets for 5 days) or gonadotrophins (fertility injections for 7-10 days). The ovarian response is monitored and assessed, once follicles of suitable size are identified, an hCG injection is given to induce ovulation. Insemination of prepared semen is carried out within 24-36 hours. IUI is carried out in cases of unexplained infertility, mild endometriosis, mild male factor infertility, disability (physical or psychological) preventing vaginal sexual intercourse, HIV positive (after sperm wash), and sperm donation (Goodwin et al., 2010; NCCWCH, 2013; Zegers-Hochschild et al., 2017).

IVF

In IVF, eggs are fertilized with sperm outside the body in a laboratory. In general, it is used as an alternative when other treatments fail to produce a successful pregnancy. An IVF treatment cycle can comprise the following seven sequential stages: pre-treatment, down-regulation, controlled ovarian stimulation, ovulation trigger, oocyte and sperm retrieval, embryo replacement, and luteal phase support (NCCWCH, 2013).

IVF may be carried out in cases of unsuccessful conception following: a period of expectant management in people with unexplained infertility, ovulation induction therapy, treatment for endometriosis. Further indications for use of IVF include: severe tubal disease, severe male factor infertility (IVF with ICSI may be the preferred option), failure of spermatogenesis following cancer treatment where cryopreserved semen has been unsuccessful at achieving conception with IUI, ovarian failure caused by cancer treatment where eggs or embryos have been cryopreserved, where egg donation is being used (Armstrong & Akande, 2013; Goodwin et al., 2010; NCCWCH, 2013).

ICSI

ICSI is an extension to conventional IVF treatment and can be used in cases where there is low sperm number, motility or morphology, or a combination of these parameters. ICSI can also be used in cases where sperm have been retrieved surgically from the epididymis or testicular tissue, couples with previously failed IVF cycles and non-male subfertility (Goodwin et al., 2010; NCCWCH, 2013; Tan, Lau, Loh, & Tan, 2014).

Oocyte donation

In oocyte donation, a fertile woman allows several of her oocytes to be aspirated, usually following ovarian stimulation, and used to enable another woman, who is infertile due to ovarian failure (WHO Group III), to conceive with IVF. The major indication for the use of donor oocytes is premature ovarian failure. It can further be used to reduce the risk of transmission of a genetic disorder in cases in which the carrier status of both partners is known (NCCWCH, 2013).

Women with markedly diminished ovarian reserve should be counselled on their low chances of conception using their gametes, even with ART, and should be offered the options of donor oocytes. Egg donation is the most effective treatment option for achieving pregnancy in perimenopausal women (Goodwin et al., 2010; NCCWCH, 2013).

Sperm donation

Sperm donation is used in situations where the male partner is infertile. In sperm donation, fertile male donates sperm at a clinic which is then stored for future use. Sperm donation may be used in ICSI to treat cases of azoospermia or severe abnormalities of semen quality. It can further be used to prevent transmission of an inheritable genetic condition and infection such as HIV (Goodwin et al., 2010; NCCWCH, 2013).

ZIFT

Although Zygote intrafallopian transfer (ZIFT) is not widely used, it has been developed alongside IVF using much of the same technology. When transcervical embryo transfer is impossible, laparoscopic transfer of embryos to the fallopian tube after fertilisation in vitro offers an alternative route (Goodwin et al., 2010; NCCWCH, 2013; Zegers-Hochschild et al., 2017).

GIFT

GIFT has been developed alongside IVF using much of the same technology, but where eggs, once collected, are transferred laparoscopically to the fallopian tube with prepared motile sperm to allow fertilisation to occur in vivo. It is not widely used due to its need for laparoscopy. It has been most commonly used in the management of people with unexplained male factor fertility problems, and where transcervical embryo transfer is impossible (Goodwin et al., 2010; NCCWCH, 2013; Zegers-Hochschild et al., 2017).

Gestational surrogacy

Where women don't have a uterus or functional uterus, the couple can decide to have a gestational carrier, who carries the couple’s embryo in the uterus. This is also used for women in whom pregnancy can endanger their health (Goodwin et al., 2010; NCCWCH, 2013; Zegers-Hochschild et al., 2017).

On the other hand prevention of infertility remains the most cost-effective treatment strategy particularly in countries with a high prevalence of pregnancy-related infections and STIs. Preventive measures could include education and creating public awareness, encouraging protected coitus, establishing healthy lifestyle habits (i.e. regular exercise, less alcohol consumption, and smoking, etc), establishing patient support networks, implementing accessible fertility services, and implementing low-cost ART treatment (Ombelet, 2011).

CHAPTER THREE:

METHODOLOGY

The following chapter focuses on the methodology used in this study. The research methodology can be defined as a systematic way of solving the identified research problem. It presents the research approach, design and study area, sampling method, materials used, data collection, data analysis, validity, reliability, and ethical considerations.

3.1 Research Design

This was a prospective and descriptive cross-sectional case reference study encompassing infertile women visiting the Cape Windhoek Fertility Clinic.

This was both a qualitative and quantitative study by design as we sought to measure specific variables to determine its effect on another variable, such as the impact of STIs on female infertility and the significance of screening infertile women for ART treatment on pregnancy outcome.

The study was approved by the MoHSS ethics committee and the NUST higher degree committee. Written informed consent was acquired before the commencement of the study from all participants. All information generated from the study was kept strictly confidential and there was no way to trace the identity of patients since they were all given unique codes as identifiers during the study.

3.2 Population and setting of the study

The population of the study was infertile women visiting the Cape Windhoek Fertility Clinic during the period from February to August 2019. This study was conducted in Windhoek, Namibia. The fertility clinic is situated at Klein Windhoek and provides ART treatment such as IVF, ICSI, sperm donation, egg donation, and surrogacy. The clinic attends to 360 patients per year, giving us an average of 30 patients per month.

3.3 Size of sample and sampling procedure

The sample size was determined using an acceptable margin of error of 7%, 95% confidence level and a single population proportion formula considering a prevalence of 35%, several studies in sub-Saharan Africa reported a prevalence between 10 – 50% (Masoumi et al., 2015; Okunola, Ajenifuja, Loto, Salawu, & Omitinde, 2017; Ombelet & Onofre, 2019; Pedro & Andipatin, 2014), while in Namibia, Inhorn reported a prevalence of 32% in some ethnic groups (Inhorn, 2003).

The total required sample size was calculated using Cochran's formula below:

$$n_0 = \frac{Z^2 pq}{e^2}$$

where n_0 is the sample size, z is the selected critical value of desired confidence level, p is the estimated proportion of an attribute that is present in the population, q = 1 - p and e is the desired level of precision (Sarmah & Hazarika, 2012).

A total of 178 infertile women visiting the Cape Windhoek Fertility Clinic were therefore selected to formulate the sample population for this study, where:

$$Z = 1.96,\ p = 0.35,\ e = 0.07$$

The population for this study was sampled using a purposive non-random sampling procedure. All women were given the opportunity to participate in this study after they were briefed about the research. When they voluntarily agreed to participate, all information gathered was handled with the highest level of confidentiality.

All participants were required to sign informed consent forms and return them prior to the commencement of the study.

Venous blood samples were collected by a qualified phlebotomist using serum gel tubes from each participant at the medical laboratory premises for hormone levels analysis and STIs screening. The samples were then centrifuged at 1500G for 10 minutes to separate the serum.

The serum samples were analysed for HIV, Syphilis, HBsAg, HCV, TSH, FSH, AMH, and PRL levels using an Architect and Cobas immunoassay analyser before the commencement of treatment.

After treatment, another blood sample was sent to the laboratory for analysis of serum hCG hormone level to determine whether or not the pregnancy was successful via the aid of the Architect immunoassay analyser.

3.4 Principle of immunoassay module of the analyser: Architect *c*8200*i*

The Architect *c*8200*i* immunoassay module (*i*2000SR*)* of the analyser uses the CHEMIFLEX method for analysing its samples. In chemiluminescence reactions, part of the chemical energy generated produces excited intermediates that decay to a ground state with the emission of photons. The emitted radiation is measured with a PM tube, and the signal is related to the analyte concentration. Most important, chemiluminescence reactions are oxidation reactions of luminol, acridinium esters, and dioxetanes characterized by a rapid increase in the intensity of emitted light followed by gradual decay. Usually, the signal is taken as the integral of the entire peak (Bishop et al., 2010; Cox, 2011).

3.4.1 Follicle-stimulating hormone

According to the manufacturer, the biological principle of the Architect FSH assay is a two-step immunoassay to determine the presence of FSH in serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex (Abbott Laboratories, 2016a):

- Sample and anti-β FSH coated paramagnetic microparticles are combined. The FSH present in the sample binds to the anti-β FSH coated microparticles.
- After washing, anti-α FSH acridinium-labelled conjugate is added to create a reaction mixture.
- Following another wash cycle, Pre-trigger and Trigger solutions are added to the reaction mixture.
- The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of FSH in the sample and the RLUs detected by the Architect *i*System optics.

Reference values:

	Follicular (Day 7)	Ovulation (Day 14)	Luteal (Day21)
Menstruating females	3.35 – 21.63 IU/L	4.97 – 20.82 IU/L	1.11 – 13.99 IU/L
Post-menopausal females	2.58 – 150.53 IU/L		

3.4.2 Prolactin

According to the manufacturer, the PRL assay is a two-step immunoassay used to determine the presence of PRL in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex (Abbott Laboratories, 2016b):

- Sample and anti-PRL (mouse, monoclonal) coated paramagnetic microparticles are combined. The PRL present in the sample binds to the anti-PRL (mouse, monoclonal) coated microparticles.
- After washing, anti-PRL (mouse, monoclonal) acridinium-labelled conjugate is added to create a reaction mixture.
- Following another wash cycle, Pre-trigger and Trigger solutions are added to the reaction mixture.
- The resulting chemiluminescent reaction is measured as RLUs. There is a direct relationship between the amount of PRL in the sample and the RLUs detected by the Architect *i*System optics.

Reference values: 25.2 – 628.5 mIU/L

3.4.3 Thyroid-stimulating hormone

As instructed by the manufacturer, the TSH assay is a two-step immunoassay use to determine the presence of TSH in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex (Abbott Laboratories, 2015):

- Sample, anti-β TSH antibody-coated paramagnetic microparticles and TSH assay diluents are combined. TSH present in the sample binds to the anti-TSH antibody-coated microparticles.
- After washing, anti-α TSH acridinium-labelled conjugate is added to create a reaction mixture.
- Following another wash cycle, Pre-trigger and Trigger solutions are added to the reaction mixture.
- The resulting chemiluminescent reaction is measured as RLUs. There is a direct relationship between the amount of TSH in the sample and the RLUs detected by the Architect *i*System optics.

Reference values: 0.35 – 4.94 mIU/L

3.4.4 Human immunodeficiency virus

The Architect HIV Ag/Ab combo assay is a two-step immunoassay to determine the presence of HIV p24 antigen and antibodies to HIV-1 (Group M and Group O) and HIV-2 in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex. According to the manufacturer, below are the biological principles of the procedure employed for testing for HIV (Abbott Laboratories, 2014a):

- In the first step, sample, Architect wash buffer, assay diluent, and paramagnetic microparticles are combined. HIV p24 antigen and HIV-1/HIV-2 antibodies present in the sample bind to the HIV-1/HIV-2 antigen and HIV p24 monoclonal (mouse) antibody-coated microparticles.
- After washing, the HIV p24 antigen and HIV-1/HIV-2 antibodies bind to the acridinium-labelled conjugates (HIV-1/HIV-2 antigens [recombinant], synthetic peptides, and HIV p24 antibody [mouse, monoclonal]).
- Following another wash cycle, Pre-trigger and Trigger solutions are added to the reaction mixture.
- The resulting chemiluminescent reaction is measured as RLUs. A direct relationship exists between the amount of HIV antigen and antibody in the sample and the RLUs detected by the Architect *i*System optics.
- The presence or absence of HIV p24 antigen or HIV-1/HIV-2 antibodies in the specimen is determined by comparing the chemiluminescent signal in the reaction to the cut-off signal determined from an Architect HIV Ag/Ab combo calibration. Specimens with a signal to cut-off (S/CO) values greater than or equal to 1.00 are considered reactive for HIV p24 antigen or HIV-1/HIV-2 antibodies.
- Specimens that are initially reactive in the Architect HIV Ag/Ab combo assay should be retested in duplicate. Repeat reactivity is highly predictive of the presence of HIV p24 antigen and HIV-1/HIV-2 antibodies.

Reference values:

Specimens with S/CO values <1.00 are considered non-reactive (Negative)

Specimens with S/CO ≥1.00 are considered reactive (Positive)

3.4.5 Hepatitis B surface antigen

The Architect HBsAg qualitative assay is a one-step immunoassay for the qualitative detection of HBsAg in human serum and plasma using CMIA technology, with flexible assay protocols, referred to as Chemiflex. (Note: The ancillary wash buffer is added in a second incubation step, so the assay file performs a two-step assay protocol). According to the reagent package insert, below are the biological principles of the procedure employed for testing for HBsAg (Abbott Laboratories, 2013):

- In the Architect HBsAg qualitative assay, sample, anti-HBS coated paramagnetic microparticles and anti-HBS acridinium-labelled conjugate are combined to create a reaction mixture. HBsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labelled conjugate.
- After washing, the ancillary wash buffer is added to the reaction mixture.
- Following another wash cycle, Pre-trigger and Trigger solutions are added to the reaction mixture.
- The resulting chemiluminescent reaction is measured as RLUs. A direct relationship exists between the amount of HBsAg in the sample and the RLUs detected by the Architect *i*System optics.
- The presence or absence of HBsAg in the sample is determined by comparing the chemiluminescent signal in the reaction to the cut-off signal determined from an active calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cut-off signal, the sample is considered reactive for HBsAg.

Reference values:

Specimens with S/CO values <1.00 are considered non-reactive (Negative)

Specimens with S/CO ≥1.00 are considered reactive (Positive)

3.4.6 Hepatitis C virus

As per the manufacturer, the Anti-HCV assay is a two-step immunoassay, using CMIA technology for the qualitative detection of anti-HCV in human serum and plasma (Abbott Laboratories, 2014b):

- Sample, recombinant HCV antigen-coated paramagnetic microparticles and assay diluent are combined. The anti-HCV present in the sample binds to the HCV coated microparticles.

- After washing, the anti-human acridinium-labelled conjugate is added to create a reaction mixture.
- Following another wash cycle, Pre-trigger and Trigger solutions are added to the reaction mixture.
- The resulting chemiluminescent reaction is measured as RLUs. A direct relationship exists between the amount of anti-HCV in the sample and the RLUs detected by the Architect *i*System optics.
- The presence or absence of anti-HCV in the sample is determined by comparing the chemiluminescent signal in the reaction to the cut-off signal determined from an active calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cut-off signal, the specimen is considered reactive for anti-HCV.

Reference values:

Specimens with S/CO values <1.00 are considered non-reactive (Negative)

Specimens with S/CO ≥1.00 are considered reactive (Positive)

3.4.7 Syphilis

The Syphilis Treponema Pallidum (TP) assay is a two-step immunoassay for the qualitative detection of antibody to TP in human serum or plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex (Abbott Laboratories, 2017):

- Sample, microparticles coated with recombinant TP antigens (TpN15, TpN17, and TpN47) and assay diluent are combined. Anti-TP antibodies present in the sample bind to the TP coated microparticles.
- After washing, acridinium-labelled anti-human IgG and IgM conjugate is added to create a reaction mixture.
- Following another wash cycle, Pre-trigger and Trigger solutions are added to the reaction mixture.
- The resulting chemiluminescent reaction is measured as RLUs. There is a direct relationship between the amount of anti-TP antibodies in the sample and the RLUs detected by the Architect *i*System optics.
- The presence or absence of anti-TP antibodies in the specimen is determined by comparing the chemiluminescent signal in the reaction to the cut-off signal determined from a previous

Architect Syphilis TP calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cut-off signal, the specimen is considered reactive for anti-TP.

Reference values:

Specimens with S/CO values <1.00 are considered non-reactive (Negative)

Specimens with S/CO ≥1.00 are considered reactive (Positive)

3.4.8 hCG

The Architect total β-hCG assay is a two-step immunoassay to determine the presence of β-hCG in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex. According to the reagent package insert, below are the biological principles of the procedure employed for testing for hCG (Abbott Laboratories, 2014c):

- In the first step, sample, and anti- β-hCG coated paramagnetic microparticles are combined. β-hCG present in the sample binds to the anti- β-hCG coated microparticles.
- After washing, the anti- β-hCG acridinium-labelled conjugate is added in the second step.
- Pre-trigger and Trigger solutions are then added to the reaction mixture.
- The resulting chemiluminescent reaction is measured as RLUs. A direct relationship exists between the amount of β-hCG in the sample and the RLUs detected by the Architect *i* optical system.

Reference values:

Positive	≥25.00 mIU/mL
Negative	≤5.00 mIU/mL

3.4.9 Principle of immunoassay module of the analyser: Cobas 6000

The Cobas 6000 immunoassay module (e601) of the analyzer uses Elecsys technology ECL (Electrochemiluminescence) for immunoassay detection. The development of ECL immunoassays is based on the use of a ruthenium-complex and tripropylamine (TPA). The chemiluminescence reaction for the detection of the reaction complex is initiated by applying a voltage to the sample solution resulting in a precisely controlled reaction (Bishop et al., 2010; Cox, 2011).

3.4.9.1 AMH

According to the reagent package insert (Roche diagnostics, 2019) below are the principles of the procedure employed for testing for AMH:

- 1st incubation: 50µL of sample, a biotinylated monoclonal AMH-specific antibody, and a monoclonal AMH-specific antibody labelled with a ruthenium complex) form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

Reference values:

	AFC	AMH (ng/mL)
Negligible response	3 – 6	<0.15
Reduced response	5 – 11	0.15 – 1.14
Safe/Normal response	9 – 16	1.15 – 2.56
Excessive response	13 – 26	>2.56

3.5 Data collection methods

All women were given the opportunity to participate in this study after they were briefed about the research. When they voluntarily agreed to participate, all information gathered was handled with the highest level of confidentiality.

All participants were required to sign informed consent forms and return them prior to the commencement of study.

This was accomplished by carrying out a survey through the administration of a questionnaire to all participating women. The questionnaire was administered by the consulting gynaecologist as part of taking patient history. The questionnaire served to gather first-hand information (i.e. age; Last menstrual period (LMP); race; work industry; marital status; lifestyle habits, etc.) from each participant.

3.6 Variables that were collected

This study entailed both qualitative and quantitative variables such as:

- Qualitative: Work industry, Race, Marital status, and Lifestyle habits.
- Quantitative: Age, Number of children, and Hormone levels.

3.7 Methods of data analysis

All raw data collected was initially sorted and grouped via the aid of Microsoft Excel software. To analyse our data we employed the analytics software, SPSS (Statistical Package for the Social Sciences version 25.0, SPSS Inc., Chicago, IL, USA) and the Chi-square test was used. $P<0.05$ were considered to be statistically significant.

Descriptive statistics (minimum, maximum, mean, and standard deviation) were applied for continuous variables and simple percentages for categorical variables.

3.8 Ethical considerations of the research

Ethical clearance was sought and obtained from the MoHSS ethics committee and the NUST higher degree committee to carry out the aforementioned study.

The participation in the study was entirely voluntary with the option of participant's withdrawal from the study at any point and time they wished to withdraw.

All participants read and signed informed consent prior to the commencement of study.

All data and information gathered from the study were kept confidential and secure. Moreover, there was no way to link the data to participants as they were identified by codes instead of their names.

There was no conflict of interest or any financial gain from the researcher’s side as a result of this study.

CHAPTER FOUR:

RESULTS

This chapter presents the results of this study. It describes the outcome and findings of the investigations.

The study consisted of 178 patients classified as ''infertile women'' visiting the Cape Windhoek Fertility Clinic for ART treatment. Of these 178 women, 96 were infertile, while the remainder 82 were sub-fertile.

The enrolled women in the study ranged from 25-50 years of age, with the youngest being 25 years old and the oldest being 50 years old. The great majority 145 (81.5%) of the participants were married women. Most infertile women 67 (69.1%) were in the age group of 30-39 years and the majority of women 97 (54.5%) were also in the same age group (30-39 years) as shown in Table 4.1.

From the 178 infertile women, 96 cases (53.9%) suffered from primary infertility and 82 (46.1%) women suffered from secondary infertility as shown in Table 4.2. Of those who presented with secondary infertility, most 40 (22.5%) of them had 2 children.

Most of the participants were Black women 144 (80.9%) and a large number of participants (82 representing 46.1%) indicated that they work in the Business/Finance/Admin industries.

In this study the predominant cause of complications for infertility among the 178 women was defective ovulation 51 (28.7%) and the most common ART treatment administered was split IVF/ICSI 93 (52.2%).

Most patients tested negative when screened for STIs as seen in Table 4.4. All 178 patients tested negative for HCV. The great majority indicated to be non-smokers 177 (99.4%) and they did not consume alcohol 143 (80.3%).

Of the 178 women who received ART treatment, 59 (33.1%) eventually fell pregnant compared to 119 (66.9%) who did not fall pregnant. Most women 130 (73.1%) received one ART treatment cycle.

4.1 Population demographics

4.1.1 Age group

From the 178 infertile women who participated in the study, majority 97 (54.5%) were in the age group 30-39 years old, followed by 69 (38.8%) falling in the age group of 40-49 years old, and the little minority 9 (5.1%) indicated to be in the age group 20-29 years old, with only 3 (1.7%) in the age group >49 years old as shown in Table 4.1 below. Out of the 97 patients, ages 30-39 years old, 67 (69.1%) were found not to be pregnant while 30 (30.9%) were proven to be pregnant. From the 69 patients, ages 40-49 years old, 47 (68.1%) were without pregnancy as compared to 22 (31.9%) pregnant. From the 9 patients, ages 20-29 years old, 5 (55.6%) were not pregnant while 4 (44.4%) fell pregnant. Surprisingly, all 3 (100%) patients above the age of 49 fell pregnant. From these results, one can observe that the possibility of falling pregnant among the women who underwent ART treatment in Windhoek, was not age-related (Chi-square; p-value=0.077).

4.1.2 Marital status

Of the 178 participants, 145 (81.5%) women were married and 33 (18.5%) were single as shown in Table 4.1. From the 145 married patients, 102 (70.3%) pregnancy outcomes were unsuccessful and 43 (29.7%) women fell pregnant. From the 33 single patients, 17 (51.5%) were found without pregnancy as compared to 16 (48.5%) pregnant after ART treatment. There is also a correlation between the outcome in pregnancy and the marital status among the women who underwent ART treatment (p=0.038).

4.1.3 Race

The great majority 144 (80.9%) of women in the study were black, followed by those of mixed race 23 (12.9%), with the white women being the minority 11 (6.2%). From the 144 Black women, 98 (68.1%) pregnancy outcomes were unsuccessful and 46 (31.9%) fell pregnant. From the 23 Mixed ancestry patients, 15 (65.2%) were found without pregnancy as compared to 8 (34.8%) pregnant. From the 11 White patients, 6 (54.5%) failed to be pregnant as compared to 5 (45.5%) pregnant. No significant difference was found in the outcome of pregnancy according to race among the women who underwent ART treatment (p=0.646) at the Cape Windhoek Clinic.

4.1.4 Work Industry

Among the 178 women who underwent ART treatment at the Cape Windhoek Fertility Clinic, 82 (46.1%) were from the Business/Finance/Admin industries followed by Education 35 (19.7%),

Law/Police/Military 24 (13.5%), 14 (7.9%) Home-maker, 14 (7.9%) Medical/Health, then 8 (4.5%) Construction/Engineering and 1 (0.6%) unemployed as shown in Table 4.1. The employment types and status did not have an impact on the pregnancy outcome (p= 0.132).

4.1.5 Gravida-para status

From the 178 infertile women who participated in the study, the majority 96 (53.9%) had no children, followed by 40 (22.5%) women who had two children, and 22 (12.4%) indicated to have more than two children, with only 20 (11.2%) as having one child as shown in Table 4.1 below. Although there is no correlation between the Gravida-para status and the pregnancy outcome (p=0.621), the study shows a decreasing trend of pregnancy outcome from women who had more two children (40.9%), to women with one child (40.0%), two children (35 %), then to a woman without child (29.2%).

Table 4.1: Population demographics (N = 178)

Variables	**Categories**	Human Chorionic Gonadotropin categories						
		Pregnancy Negative		Pregnancy Positive		Total		Chi-square (p-value)
		Count	%	Count	%	Count	%	
Age Group (Years)	20-29	5	55.6	4	44.4	9	5.1	0.077
	30-39	67	69.1	30	30.9	97	54.5	
	40-49	47	68.1	22	31.9	69	38.8	
	>49	0	0.0	3	100.0	3	1.7	
Marital Status	Single	17	51.5	16	48.5	33	18.5	**0.038***
	Married	102	70.3	43	29.7	145	81.5	
Race	Black women	98	68.1	46	31.9	144	80.9	0.646
	White women	6	54.5	5	45.5	11	6.2	
	Mixed ancestry women	15	65.2	8	34.8	23	12.9	
Work Industry	Business/Finance/Admin	51	62.2	31	37.8	82	46.1	0.132
	Construction/Engineering	8	100.0	0	0.0	8	4.5	
	Education	23	65.7	12	34.3	35	19.7	
	Home-Maker	12	85.7	2	14.3	14	7.9	
	Law/Police/Military	17	70.8	7	29.2	24	13.5	
	Medical/Health	8	57.1	6	42.9	14	7.9	
	Unemployed	0	0.0	1	100.0	1	0.6	
Gravida-para status	0	68	70.8	28	29.2	96	53.9	0.621
	1	12	60.0	8	40.0	20	11.2	
	2	26	65.0	14	35.0	40	22.5	
	>2	13	59.1	9	40.9	22	12.4	

*. The Chi-square statistic is significant at the .05 level.

4.2 Type of infertility and Lifestyle habits

4.2.1 Type of infertility

Of the 178 participating women, 96 (53.9%) presented with primary infertility while 82 (46.1%) presented with secondary infertility as shown in Table 4.2. Although there is no correlation between the type of infertility and the pregnancy outcome (p=0.222), the study shows that there is a higher rate of successful pregnancy outcome in women who previously had children (37.8%) compared to those who never had any child (29.2%). On the other hand, women who presented with primary infertility had more (70.8%) unsuccessful pregnancy outcome compared to those women with secondary infertility (62.2%).

4.2.2 Lifestyle habits: Smoking and Alcohol drinking

Of the 178 participants in this study, 177 (99.4%) indicated to be non-smokers while only 1 (0.6%) is a smoker (Table 4.2). On the other hand, 142 (80.3%) indicated to be non-alcohol consumers while 35 (19.7%) reported consuming normal amounts of alcohol (2 glass of wine or 2 bottles of Beer a day). There is no correlation between the pregnancy outcome versus smoking and drinking, (p=0.480) and (p=0.810) respectively.

Table 4.2: Type of infertility and Lifestyle habits

Variables	**Categories**	Human Chorionic Gonadotropin categories						Chi-square (p-value)
		Pregnancy Negative		Pregnancy Positive		Total		
		Count	%	Count	%	Count	%	
Type of infertility	Primary infertility	68	70.8	28	29.2	96	53.9	0.222
	Secondary infertility	51	62.2	31	37.8	82	46.1	
Smoking	No	118	66.7	59	33.3	177	99.4	0.480
	Yes	1	100.0	0	0.0	1	0.6	
Alcohol drinking	No	95	66.4	48	33.6	143	80.3	0.810
	Yes	24	68.6	11	31.4	35	19.7	

4.3 Cause of complications

The results of the present study as indicated by the gynaecologist showed that the most common cause of complication in female infertility was defective ovulation in 51 (28.7%) women, while the second most common cause was infertility due to unspecified/unknown cause in 41 (23%) of the women, the third most common cause was defective implantation in 33 (18.5%) of the cases, followed

by infertility due to defective ovulation and implantation in 22 (12.4%) of the cases. Lastly, 16 (9%) cases were indicated to be due to defective transport and implantation, 10 (5.6%) due to defective transport, 3 (1.7%) due to defective ovulation, transport and implantation and 2 (1.1%) cases due to defective ovulation and transport, as shown in Table 4.3.

Out of the 51 patients with infertility due to defective ovulation, 37 (72.5%) were found not to be pregnant while 14 (27.5%) fell pregnant. From the 41 patients with unspecified/unknown cause of infertility, 23 (56.1%) were without pregnancy as compared to 18 (43.9%) pregnant. From the 33 patients with infertility due to defective implantation, 21 (63.6%) were not pregnant while 12 (36.4%) fell pregnant. From the 22 patients with infertility due to defective ovulation and implantation, 16 (72.7%) were found not to be pregnant while 6 (23.7.0%) fell pregnant. From the 16 patients with infertility due to defective transport and implantation, 9 (56.3%) were found not to be pregnant while 7 (43.8%) fell pregnant. From the 10 patients with infertility due to defective transport, 8 (80.0%) were without pregnancy as compared to 2 (20.0%) pregnant. From the 3 patients with infertility due to defective ovulation, transport and implantation, 3 (100%) did not fall pregnant. Lastly, out of the 2 patients with infertility due to defective ovulation and transport, 2 (100%) were without pregnancy. From these results, one can observe that there was no correlation between the pregnancy outcome and cause of complications that led to infertility among the women who underwent ART treatment (Chi-square; p-value=0.383).

Table 4.3: Cause of complications

Variables	**Categories**	Human Chorionic Gonadotropin categories						
		Pregnancy Negative		Pregnancy Positive		Total		Chi-square (p-value)
		Count	%	Count	%	Count	%	
Cause of Complications	Defective Implantation	21	63.6	12	36.4	33	18.5	0.383
	Defective Ovulation	37	72.5	14	27.5	51	28.7	
	Defective Ovulation and Implantation	16	72.7	6	27.3	22	12.4	
	Defective Ovulation and Transport	2	100.0	0	0.0	2	1.1	
	Defective Ovulation, Transport and Implantation	3	100.0	0	0.0	3	1.7	
	Defective Transport	8	80.0	2	20.0	10	5.6	
	Defective Transport and Implantation	9	56.3	7	43.8	16	9.0	
	Unspecified/Unknown cause	23	56.1	18	43.9	41	23.0	

4.4 HIV, HBsAg, HCV, and Syphilis

Among the infections screened were HIV, HBsAg, HCV, and Syphilis as shown in Table 4.4. Of the participant women, the majority tested negative when screened for those infections: HIV 160 (89.9%), HBsAg 165 (92.7%), HCV 178 (100%), and Syphilis 176 (98.9%), whereas few tested positive: HIV 18 (10.1%), HBsAg 13 (7.3%), and Syphilis 2 (1.1%). Out of the 18 HIV positive patients, 12 (66.7%) were found not to be pregnant while 6 (33.3%) fell pregnant. From the 2 Syphilis positive patients, 1 (50.0%) was without pregnancy as compared to 1 (50.0%) pregnant. From these results, one can observe that there is no correlation between the pregnancy outcome and the HIV and Syphilis status, (p=0.986) and (p=0.611) respectively. Moreover, out of the 13 HBsAg positive patients, 5 (38.5%) were found not to be pregnant while 8 (61.5%) fell pregnancy. There is also a correlation between the outcome in pregnancy and the HBsAg status among the women who underwent ART treatment (p=0.024).

Table 4.4: HIV, HBsAg, HCV, and Syphilis

Variables	Categories	Human Chorionic Gonadotropin categories						Chi-square (p-value)
		Pregnancy Negative		Pregnancy Positive		Total		
		Count	%	Count	%	Count	%	
HIV	Negative	107	66.9	53	33.1	160	89.9	0.986
	Positive	12	66.7	6	33.3	18	10.1	
HBsAg	Negative	114	69.1	51	30.9	165	92.7	**0.024***
	Positive	5	38.5	8	61.5	13	7.3	
HCV	Negative	119	66.9	59	33.1	178	100.0	.a
	Positive	0	0.0	0	0.0	0	0.0	
Syphilis	Negative	118	67.0	58	33.0	176	98.9	0.611
	Positive	1	50.0	1	50.0	2	1.1	

*. The Chi-square statistic is significant at the .05 level.

a. The Chi-square test is not performed for this sub-table because row and column variables are identical.

4.5. ART treatment

The results of this study showed that the most common ART treatment administered to the participating women was IVF/ICSI in 93 (52.2%) of the cases, while the second most common treatment administered was IVF/ED in 54 (30.3%) of the cases, the third most common treatment administered was FET in 16 (9%) of the cases, then IVF/ET 8 (4.5%) and IUI 7 (3.9%) for treatment as shown in Table 4.5. Out of the 93 patients that received IVF/ICSI as treatment, 72 (77.4%) were found not to be pregnant while 21 (22.6%) fell pregnant. From the 54 patients that received IVF/ED as treatment, 30 (55.6%) were without pregnancy as compared to 24 (44.4%) pregnant. From the 16

patients that received FET as treatment, 13 (81.3%) were not pregnant while 3 (18.8%) fell pregnant. From the 8 patients that received IVF/ET as treatment, 2 (25.0%) were found not to be pregnant while 6 (75.0%) fell pregnant. Lastly, out of the 7 patients that received IUI as treatment, 2 (28.6%) were without pregnancy as compared to 5 (71.4%) pregnant. One can observe from these results that the possibility of falling pregnant among the women who underwent ART treatment in Windhoek, was related to the type of ART treatment received (Chi-square; p-value=<0.001).

4.5.1 ART treatment cycle

Of the 178 women who agreed to participate in this study, the majority 130 (73.1%) received one ART treatment cycle, followed by 38 (21.3%) receiving two ART treatment cycles, with only a little minority 10 (5.6%) receiving more than two ART treatment cycles as shown in Table 4.5. Although there is no correlation between ART treatment cycle and the pregnancy outcome (p=0.315), the study shows that there is a higher rate of successful pregnancy outcome in women that went for 2 ART treatment cycles (42.1%) compared to those who only had 1 treatment cycle (31.5%) and >2 treatment cycles (20.0%). Surprisingly, those who had more than >2 ART treatment cycles had a higher rate of unsuccessful pregnancy outcome (80.0%) compared to those who only had 1 treatment cycle (68.5%) and 2 treatment cycles (57.9%).

Table 4.5: ART treatment and Treatment cycle

Variables	**Categories**	Human Chorionic Gonadotropin categories						
		Pregnancy Negative		Pregnancy Positive		Total		Chi-square (p-value)
		Count	%	Count	%	Count	%	
ART treatment	FET	13	81.3	3	18.8	16	9.0	**<0.001***
	IUI	2	28.6	5	71.4	7	3.9	
	IVF/ED (donor eggs)	30	55.6	24	44.4	54	30.3	
	IVF/ET (own eggs)	2	25.0	6	75.0	8	4.5	
	IVF/ICSI (split)	72	77.4	21	22.6	93	52.2	
Treatment cycle	1	89	68.5	41	31.5	130	73.0	0.315
	2	22	57.9	16	42.1	38	21.3	
	More than twice (> 2)	8	80.0	2	20.0	10	5.6	

*. The Chi-square statistic is significant at the .05 level.

4.6 Biochemical pregnancy confirmation (hCG)

Of the 178 women who received ART treatment, 119 (66.9%) had a negative hCG (not pregnant) result while 59 (33.1%) had a positive hCG (pregnant) result, as shown in Table 4.6.

Table 4.6: Biochemical pregnancy confirmation (hCG)

Variables	Categories	Human Chorionic Gonadotropin categories						Chi-square (p-value)
		Pregnancy Negative		Pregnancy Positive		Total		
		Count	%	Count	%	Count	%	
hCG	Pregnancy unlikely (Negative)	119	100.0	0	0.0	119	66.9	.a
	Pregnancy likely (Positive)	0	0.0	59	100.0	59	33.1	
	Total	119		59		178	100.0	

a. The Chi-square test is not performed for this sub-table because row and column variables are identical.

4.7 Hormonal screening

4.7.1 TSH

From the 178 infertile women who participated in the study, the vast majority 177 (99.4%) had a normal TSH result, and 1 (0.6%) had a low TSH result as shown in Table 4.7 below. From the 177 patients with normal TSH result, 118 (66.7%) pregnancy outcomes were unsuccessful and 59 (33.3%) women were pregnant. From the 1 patient with low TSH result, there was 100% unsuccessful pregnancy outcome. There is no correlation between the outcome in pregnancy and the TSH result among the women who underwent ART treatment (p=0.480).

4.7.2 PRL

Of the 178 participants, majority 158 (88.8%) had a normal PRL result, followed by 11 (6.2%) and 9 (5.1%) that had high and low PRL results, respectively. Out of the 158 patients that had normal PRL results, 105 (66.5%) were found not to be pregnant while 53 (33.5%) fell pregnant. From the 11 patients that had high PRL results, 8 (72.7%) were without pregnancy as compared to 3 (27.3%)

pregnant. From the 9 patients that had low PRL results, 6 (66.7%) were not pregnant while 3 (33.3%) fell pregnant. One can observe from these results that there is no correlation between the pregnancy outcome and the PRL results among the women who underwent ART treatment at the Cape Windhoek Fertility Clinic (Chi-square; p-value=0.913).

4.7.3 AMH

From the 178 infertile women tested for AMH, the majority 59 (33.1%) fell in the category of safe/normal response, followed by 54 (30.3%) falling in category of reduced response, and the little minority 45 (25.3%) and 20 (11.2%) falling in the categories excessive response and negligible response, respectively. Out of the 59 patients that had a normal ovarian response, 34 (57.6%) were found not to be pregnant while 25 (42.4%) fell pregnant. From the 54 patients that had a reduced ovarian response, 41 (75.9%) were without pregnancy as compared to 13 (24.1%) pregnant. From the 45 patients that had an excessive ovarian response, 29 (64.4%) were not pregnant while 16 (35.6%) fell pregnant. From the 20 patients that had a negligible ovarian response, 15 (75.0%) were found not to be pregnant while 5 (25.0%) fell pregnant. One can observe from these results that the possibility of falling pregnant among the women who underwent ART treatment in Windhoek, was not related to the ovarian response as measured by AMH (Chi-square; p-value=0.173).

Table 4.7: Hormonal screening

Variables	**Categories**	Human Chorionic Gonadotropin categories						Chi-square (p-value)
		Pregnancy Negative		Pregnancy Positive		Total		
		Count	%	Count	%	Count	%	
TSH	Low	1	100.0	0	0.0	1	0.6	0.480
	Normal	118	66.7	59	33.3	177	99.4	
PRL	Low	6	66.7	3	33.3	9	5.1	0.913
	Normal	105	66.5	53	33.5	158	88.8	
	High	8	72.7	3	27.3	11	6.2	
AMH	Negligible response	15	75.0	5	25.0	20	11.2	0.173
	Reduced response	41	75.9	13	24.1	54	30.3	
	Safe/Normal Response	34	57.6	25	42.4	59	33.1	
	Excessive response	29	64.4	16	35.6	45	25.3	

Table 4.8: Summary of Mean and SD of Hormones

Hormones (Units)	Mean	95.0% Lower CL for Mean	95.0% Upper CL for Mean	Minimum	Maximum	Range	Standard Deviation
FSH (IU/L)	13.96	10.40	17.52	0.60	200.00	199.40	24.07
TSH (mIU/L)	1.72	1.59	1.85	0.33	4.38	4.05	0.89
PRL (mIU/L)	301.48	264.24	338.71	12.10	2531.60	2519.50	251.72
AMH (ng/mL)	1.88	1.62	2.14	0.03	9.27	9.24	1.74
hCG (mIU/mL)	3225	359	6092	<5	203215	203210	19378

CHAPTER FIVE:
DISCUSSION OF RESULTS AND CONCLUSION

This chapter will discuss and highlight the results of this study in relationship to what other researchers have done and concluded.

5.1 Discussion

ART remains inaccessible in many parts of the world, particularly in sub-Saharan Africa, where IVF clinics are still absent in most countries (Inhorn & Patrizio, 2014a). While infertility was found to have fewer and/or diminishing consequences in the developed world, the opposite is true for the developing world, where it often results in severe consequences due to the high premium placed on children in those cultures and the motivation for parenthood (Ombelet, 2011). Therefore, this study sought to determine the prevalence of successful pregnancy outcomes among infertile women undergoing ART treatment and assessed possible risk factors that lead to infertility among women in Windhoek, Namibia.

Of the 178 women that received ART treatment at the Cape Windhoek Fertility Clinic, the majority 119 (66.9%) did not fall pregnant as compared to 59 (33.1%) that fell pregnant. The findings of this study are relevant since fertility rate has been on the decline in Namibia for the past 20 years as more women have become aware of the use of contraceptives and are seeking career advancement through further education, hence delaying marriage and childbearing altogether (Indongo & Pazvakawambwa, 2012; Palamuleni, 2017). This is further evident by the fact that most (94.9%) women evaluated were of age (>30 years) and a large number (91.6%) of the women were employed, with the majority (46.1%) of them being in the business world, which can be demanding at times.

Although this subject has been understudied in Namibia, the prevalence of infertility (66.9%) among Namibian women obtained from this study is alarmingly high and this confirms the widely known high prevalence of infertility in the developing world as indicated by other studies (Mascarenhas et al., 2012; Masoumi et al., 2015; Okunola et al., 2017; Ombelet & Onofre, 2019; Pedro & Andipatin, 2014).

The data obtained in this study showed that the great majority (94.9%) of women seeking ART treatment, with the hope of conceiving were >30 years of age. Several studies (Shier et al., 2016; Smith et al., 2011; Somigliana et al., 2016) have well established that age plays a crucial role in one's fertility status, particularly among women, and that with increasing age (>35 years) so does pregnancy success rates decrease due to ageing and decreased ovarian reserve (Balasch, 2010). This is evident from our results as 64% of the women that did not fall pregnant after ART treatment were >30 years of age. Contrary to common knowledge about the relationship that exists between female age and fecundity rates (Balasch, 2010; Tan et al., 2014), it is worth noting that the highest (100%) successful pregnancy outcome after ART treatment was seen among women in the age group >49 years, even though the study showed that there was no correlation between the age of the patient and the pregnancy outcome (Chi-square; p-value=0.077). The high pregnancy success rates among this group (>49 years) could be attributed to the effectiveness of the ART treatment administered to these women since our statistics showed that there was a correlation between the pregnancy outcome and the ART treatment (Chi-square; p-value=<0.001) received. However, the younger women (20-29 years) had the second-highest (44.4%) pregnancy success rates instead. This could be attributed to a viable ovarian reserve given their age since female fertility has a 'best-before date' of 35 years and age remains a paramount determinant in a female's ability to conceive either naturally or by ART (Balasch, 2010).

The data of this study showed that there is a correlation between the pregnancy outcome and the ART treatment administered (Chi-square; p-value=<0.001). Of all the ART treatment modalities the women evaluated were exposed to, IVF/ET proved to be the most effective treatment with 6 (75%) successful pregnancy outcome as compared to 2 (25.0%) unsuccessful pregnancy outcome. The effectiveness of IVF could be attributed to the relationship between age and ovarian reserve since 94.6% of the women in this study were >30 years of age. This is further evidenced by a study conducted by Armstrong and Akande (2013), which showed that IVF with or without ICSI proved to be the most effective treatment for infertility in older women.

Of the 178 women evaluated, the majority 144 (80.9%) were Black women compared to women of Mixed race 23 (12.9%) and White women 11 (6.2%). These differences in the proportions of races seeking ART treatment could be attributed to cultural differences, motivation for having children, and the number of children desired, which reflects the demography of the Namibian

population. Although the study found no correlation between race and the pregnancy outcome (Chi-square; p-value=0.646), it is worth noting that White women had the highest (45.5%) successful pregnancy outcome as compared to women of Mixed race (34.8%) and Black women (31.9%).

It was also found that out of the 178 women seeking ART treatment, majority 163 (91.6%) of the women were employed in a variety of industries. Although there is no relationship between the work industry and the pregnancy outcome (Chi-square; p-value=0.132), it is clear from our data that the majority of these women were educated. This confirms what other studies (Indongo & Pazvakawambwa, 2012; Palamuleni, 2017) have found, women that delay marriage and child-bearing in pursuit of further education and career advancement spent more time seeking ART treatment than their counter-part who were less educated.

Of the risk factors assessed such as infections (e.g. HIV, HBsAg, HCV, and Syphilis) and lifestyle habits (e.g. smoking and alcohol consumption) in this study, it was found that they contributed little to nothing in the predisposition of the women being infertile, since most women were negative and the majority did not smoke or drink alcohol as seen in tables 4 and 2, respectively. This, however, is contrary to the findings of studies (Afolabi, 2018; Gimenes et al., 2014; Kjetland et al., 2012) previously conducted in developing countries that indicate the impact of STIs and lifestyle habits as contributing causative agents of infertility. Of the patients that tested positive, HBsAg positive women had the highest (61.5%) rate of successful pregnancy outcome compared to HIV and Syphilis positive women, (50.0%) and (33.3%) respectively. There is no correlation between the pregnancy outcome and the HIV and Syphilis status, (p=0.986) and (p=0.611) respectively. However, there is a correlation between the pregnancy outcome and the HBsAg status (p=0.024).

The relationship between HBsAg status and pregnancy outcome in women undergoing ART treatment remains a controversial subject (Wang et al., 2019). Some studies (Pirwany, Phillips, Kelly, Buckett, & Tan, 2004; Ye et al., 2014) have reported that HBsAg seropositive status of women tend to have negative implications on ART treatment outcomes and therefore reduced pregnancy outcome, while other studies (Shi et al., 2014; Lee, Ng, Yeung, & Ho, 2010) found that there was no significant difference in pregnancy outcome between HBsAg seropositve and seronegative women.

Contrary to what has been found in other studies, our study found that there was a higher (61.5%) successful pregnancy outcome in HBsAg seropositive women compared to HBsAg seronegative women (30.9%), which we did not anticipate. Those results were similar to the results found by another study (Lam et al., 2010) conducted at the Chinese University of Hong Kong.

Lam et al., (2010) showed that higher pregnancies and implantation rates were demonstrated among couples with wives being HBsAg seropositive but not among those with husbands being HBsAg seropositive. Because HBV can be found in cervical and vaginal secretion, the vertical transmission may occur from the HBsAg seropositive mother due to intrauterine exposure or transplacental transmission. Interestingly, HBV infection has been detected in new-borns of HBsAg seronegative mother. Because HBsAg had been detected in semen and spermatozoa DNA (Qian et al., 2005; Huang et al., 2002), proving the father to foetus transmission and confirmed by direct sequencing (Wang et al., 2003).

Of the 178 infertile women seeking ART treatment, most 145 (81.5%) were married compared to 33 (18.5%) single. This is clear evidence that most African cultures place a high premium on children and therefore marriage is seen as an ideal platform for women to showcase their ability to reproduce. It is as though the marriage is not complete without children. Therefore failure to reproduce is often implicated by several consequences as evidenced by several studies conducted in sub-Sahara Africa (Afolabi, 2018; Fledderjohann, 2012; Hess et al., 2018; Ibisomi & Mudege, 2014; Luk & Loke, 2015), in which women are often the victims of polygamous relationship and therefore risk of HIV acquisition, divorce, domestic abuse or maltreatment, loss of social security, lack of domestic support in the home, poverty or high dependency among elderly people, lack of respect and status in society, social isolation and humiliation. The outcome of this study showed that there was a correlation between the marital status and the pregnancy outcome (Chi-square; p-value=0.038) and that single women had a higher (48.5%) successful pregnancy outcome as compared to married women (29.5%). This difference in pregnancy outcome between the married and single women could be attributed to the fact that single women could be exposed to more than one man, easily change partner, or choose to use donor sperm as part of their ART treatment, thus increasing their chance of a successful pregnancy outcome. On the other hand, the low pregnancy outcome of married women can be regarded as cultural or an act of faithfulness or a mindset that married women want only the sperm of their spouse to be used for the ART. This leads us to postulate that the problem of unexplained infertility in Namibia can be originated from married males.

On the other hand, the growing number of single women 33 (18.5%) seeking ART treatment could be because more single women are now willing to seek motherhood alone without the need of a male partner.

The study found that of the 178 women who underwent ART treatment, the majority 130 (73%) only received 1 treatment cycle. Of those who received 1 treatment cycle, 89 (68.5%) did not fall pregnant although they had a pregnancy success rate of 31.5% (41). However, those who received 2 treatment cycles had the most successful pregnancy outcome 16 (42.1%). Those who received only 1 treatment cycle could be due to psychological, emotional and financial burden that comes with ART treatment and procedures, hence discontinuing seeking subsequent treatment cycles after failure to conceive on the first cycle (Lande et al., 2015). Contrary to the findings of studies (Chambers et al., 2014; Gameiro et al., 2013; Gnoth et al., 2011) conducted previously that confirmed that 2 or more treatment cycles results in higher pregnancy success rates, this study found that women who had undergone more than >2 ART treatment cycles had the highest 8 (80.0%) rate of unsuccessful pregnancy outcome compared to those who only had 1 treatment cycle and 2 treatment cycles, 89 (68.5%) and 22 (57.9%) respectively. The contributing factor of high unsuccessful pregnancy rates among the women that had more than 2 treatment cycles could be the effectiveness of the ART treatment administered to these patients in response to the individual characteristics of these patients (i.e. age, coital frequency, type of infertility, lifestyle habits, anovulatory disorders, etc.) as they may differ from woman to woman (Steiner & Jukic, 2016; Tan et al., 2014).

This study also found that there was a higher (53.9%) prevalence of primary infertility in comparison to secondary infertility (46.1%) among the women evaluated. This was similar to studies carried out in Morocco and Sudan (Benksim et al., 2018; Elhussein, Ahmed, Suliman, Yahya, & Adam, 2019). However, this finding differed with another study conducted in Nigeria, where secondary infertility was more predominant than primary infertility (Polis et al., 2017). The reason primary infertility was predominant in this study could be due to most patients not being predisposed to infections in comparison to studies with a higher prevalence of secondary infertility due to predisposition to infections such as STIs, postabortal and puerperal sepsis, PID and uterine tuberculosis (Afolabi, 2018; Apari, deSousa, & Müller, 2014).

It is also worth noting that patients with secondary infertility had a higher (37.8%) chance of conceiving than those with primary infertility (29.2%), and this could be attributed to the fact that they previously had children, hence easier to diagnose and treat. This is further evidenced by the fact that those patients who had >2 children had the highest (40.9%) successful pregnancy rates as opposed to those who had lesser children: 2 children (35.0%), 1 child (40%), and 0 children (29.2%).

For women to fall pregnant, they need to have functional ovaries, oviducts, and uterus. Therefore, any condition that affects any of these organs may lead to infertility. The data of this study showed that the predominant cause of complications that led to infertility among these women was defective ovulation (28.7%) and as per CDC, defective ovulation may be due to the following causes: endocrine disorders, physical disorders, ovarian disorders, and endometriosis (Anwar & Anwar, 2016). These findings were similar to several other previous studies conducted (Benksim et al., 2018; Elhussein et al., 2019). However, our findings differed from other studies (Afolabi, 2018; Ombelet & Onofre, 2019; Pedro & Andipatin, 2014) that attributed tubal factor as the most common cause of infertility due to STIs, infections and postpartum infections, particularly in Africa.

The TSH levels of the 178 participants that received ART treatment were measured and classified into 2 groups: low (0.6%) and normal (99.4%). There is no correlation between the outcome in pregnancy and the TSH result among the women who underwent ART treatment (p=0.480). It has long been established that thyroid disorders can lead to infertility and that is further evident by the 1 patient with low TSH (hyperthyroidism) that had no successful pregnancy outcome in this study.

The PRL levels of the 178 women seeking ART treatment were evaluated and grouped into 3 categories: low (5.1%), normal (88.8%), and high (6.2%). It is a known fact that high levels of PRL in the blood (hyperprolactinemia) cause infertility and gonadal disorders in males and females. Moreover, elevated levels of PRL in the blood serves as an indication of pituitary dysfunction. This study found that patients presenting with hyperprolactinemia had the highest (72.7%) rate of unsuccessful pregnancy outcome as compared to low (66.7%) and normal (66.5%) levels of PRL in the blood. Although there is no correlation between the PRL levels in the blood and the pregnancy outcome (Chi-square; p-value=0.913), it is evident that a functional endocrine system plays a vital role in a female's reproductive ability, hence any disorders in the endocrine system

has the potential to negatively affect the regulation of hormones that are significant in regulating female reproduction.

The AMH levels of the 178 women evaluated for ART treatment were grouped into 4 categories: negligible (11.2%), reduced (30.3%), normal (33.1%), and excessive (25.3%) response. AMH levels are useful in fertility assessment, as it provides guidance to ovarian reserve status and identifies women that may need to consider either egg freezing or trying for a pregnancy sooner rather than later if their long-term future fertility is poor. This study found that the highest rate of unsuccessful pregnancy outcome was among the patients that had a reduced ovarian response, 41 (75.9%), negligible ovarian response, 15 (75.0%), and excessive ovarian response, 29 (64.4%) as compared to those women who had a normal ovarian response (42.4%). These findings confirm what other studies (Ishii et al., 2019; Vaughan & Harrity, 2015) have concluded, that AMH could be the most sensitive marker of ovarian reserve and therefore serve to guide both doctors and patients on the likelihood of pregnancy success depending on the ART treatment modality implemented. Although there is no correlation between the AMH levels and the pregnancy outcome (Chi-square; p-value=0.173), one cannot overlook the usefulness of AMH in determining ovarian response when considering women for ART treatment.

5.2 Limitations

The outcome of this study may not give a clear indication of the prevalence of infertility among women in the entire Namibian nation due to the costs involved with ART treatment offered mainly at privately owned hospitals and/or clinics, thereby resulting in those who cannot afford treatment to be left out despite being infertile.

This study cannot conclusively determine the affordability of the entire Namibian women who may want to seek ART treatment since no data was collected on the average income earned by these women or the household, they are part of. Besides, the data was not classified according to regions.

The cost of ART procedures limited most participants in this study to one treatment cycle and thus we could not conclusively infer whether or not one or more cycles may be needed for a successful pregnancy to occur.

The risk factors (i.e. STIs) mostly attributed to the cause of infertility among women in developing countries could not be verified seeing that the majority of women in the study were negative when screened for STIs.

5.3 Conclusion

The severity of infertility in the developing world is worrisome in comparison to the developed world. Involuntary childlessness continues to have serious social and economic impacts on the lives of those implicated, particularly women in the developing world. It is the silent cry of many African women and for some, it has reached as far as a sense of loss of one's dignity and worth as a woman.

The high prevalence of infertile women (66.9%) in this study, calls for immediate remedial measures and interventions both at the national and continental level. However, access to affordable ART treatment remains a challenge in low-resource settings (i.e. Africa) seen that for the most part they are only offered by privately owned hospitals and/or clinics at costly fees.

The exorbitant cost of ART is the main barrier that hinders many from seeking it and getting the much-needed assistance to help them conceive and bear children. In 2007 a meeting conducted in Tanzania by the Walking Egg project established that one way to increase accessibility to fertility treatment, particularly in the developing world, was by lowering the cost of ART (Teoh & Maheshwari, 2014). As suggested by Ombelet and Onofre (2019), the successful implementation of low-cost infertility care in developing countries, particularly in public health facilities, would include the simplification of diagnostic and ART procedures, minimizing the complication rate of interventions, providing training-courses for health-care workers and incorporating infertility treatment into sexual and reproductive health-care programmes.

Moreover, preventative measures to curb the challenge of involuntary childlessness such as dealing with underlying causes that may eventually lead to fertility, i.e. untreated infections, STIs, could also be adopted and implemented. Lastly, educating the public about infertility would alleviate the stigma faced by women in their communities and make man aware that they could eventually be the cause of infertility thereby not shifting the entire blame on their female partners.

REFERENCES

Abbott Laboratories. (2013). Architect HBsAg qualitative (Reagent package insert). Lisnamuck, Longford: Author.

Abbott Laboratories. (2014a). Architect HIV Ag/Ab Combo (Reagent package insert). Lisnamuck, Longford: Author.

Abbott Laboratories. (2014b). Architect Anti-HCV (Reagent package insert). Lisnamuck, Longford: Author.

Abbott Laboratories. (2014c). Architect β-hCG (Reagent package insert). Lisnamuck, Longford: Author.

Abbott Laboratories. (2015). Architect TSH (Reagent package insert). Lisnamuck, Longford: Author.

Abbott Laboratories. (2016a). Architect FSH (Reagent package insert). Lisnamuck, Longford: Author.

Abbott Laboratories. (2016b). Architect Prolactin (Reagent package insert). Lisnamuck, Longford: Author.

Abbott Laboratories. (2017). Architect Syphilis TP (Reagent package insert). Lisnamuck, Longford: Author.

Afolabi, B. M. (2018). The Dilemma and Psycho-Social Challenges of Un-Intentional Infertility in Sub-Saharan Africa. *Open Access Journal of Translational Medicine & Research*, *1*(1), 1–2. https://doi.org/10.15406/oajtmr.2017.01.00002

Agenor, A., & Bhattacharya, S. (2015). Infertility and Miscarriage: Common Pathways in Manifestation and Management. *Women's Health*, *11*(4), 527–541. https://doi.org/10.2217/WHE.15.19

Ahmad, J., Priya, Dm., & Akhtar, N. (2015). Prevalence of hypothyroidism in infertile women and evaluation of response of treatment for hypothyroidism on infertility. *Indian Journal of Endocrinology and Metabolism*, *19*(4), 504. https://doi.org/10.4103/2230-8210.159058

Anwar, S., & Anwar, A. (2016). Scient Open Access Exploring the World of Science Infertility: A Review on Causes, Treatment and Management. *Womens Health & Gynecol*, *2*(6), 040. Retrieved from www.scientonline.org

Apari, P., de Sousa, J. D., & Müller, V. (2014). Why Sexually Transmitted Infections Tend to Cause Infertility: An Evolutionary Hypothesis. *PLoS Pathogens*, *10*(8), e1004111. https://doi.org/10.1371/journal.ppat.1004111

Armstrong, S., & Akande, V. (2013). What is the best treatment option for infertile women aged 40 and over? *Journal of Assisted Reproduction and Genetics*, *30*(5), 667–671. https://doi.org/10.1007/s10815-013-9980-6

Balasch, J. (2010). Ageing and infertility: an overview. *Gynecological Endocrinology*, *26*(12), 855–860. https://doi.org/10.3109/09513590.2010.501889

Benksim, A., Elkhoudri, N., Ait Addi, R., Baali, A., & Cherkaoui, M. (2018). Difference between primary and secondary infertility in morocco: Frequencies and associated factors. *International Journal of Fertility and Sterility*, *12*(2). https://doi.org/10.22074/ijfs.2018.5188

Bernardi, L. A., Cohen, R. N., & Stephenson, M. D. (2013). Impact of subclinical hypothyroidism in women with recurrent early pregnancy loss. *Fertility and Sterility*, *100*(5), 1326-1331.e1. https://doi.org/10.1016/j.fertnstert.2013.07.1975

Binita, G., Suprava, P., Mainak, C., Koner, B. C., & Alpana, S. (2009). Correlation of prolactin and thyroid hormone concentration with menstrual patterns in infertile women. *Journal of Reproduction & Infertility*, *10*(3), 207–212. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3719326&tool=pmcentrez&rendertype=abstract

Bishop, M., Fody, E., & Schoeff, L. (2010). *Clinical Chemistry - Techniques, Principles, Correlations*. *Techniques, Principles, Correlations*. https://doi.org/10.1017/CBO9781107415324.004

Bishop, M. L., Fody, E. P., & Schoeff, L. E. (2010). *Clinical chemistry : techniques, principles, correlations*. Wolters Kluwer Health/Lippincott Williams & Wilkins.

Chambers, G. M., Paul, R. C., Harris, K., Fitzgerald, O., Boothroyd, C. V, Rombauts, L., … Jorm, L. (2014). and New Zealand : cumulative live birth rates as measures of success, 114–118. https://doi.org/10.5694/mja16.01435

Cho, M. K. (2015). Thyroid dysfunction and subfertility. *Clinical and Experimental Reproductive Medicine*, *42*(4), 131–135. https://doi.org/10.5653/cerm.2015.42.4.131

Cox, K. L. (2011). Immunoassay Development, Optimization and Validation Flow Chart. *ImmunoAssay Methods*, (Md), 1–38.

Dave, J. A., Klisiewicz, A., Bayat, Z., Mohamed, N. A., Stevens, Z., Mollentze, W. F., & Kinvig, T. (2015). SEMDSA/ACE-SA guideline for the management of hypothyroidism in adults. *Journal of Endocrinology, Metabolism and Diabetes of South Africa*, *20*(2), 18–26. Retrieved from http://www.embase.com/search/results?subaction=viewrecord%7B&%7Dfrom=export%7B&%7Did=L605828213

Dunn, D., & Turner, C. (2016). Hypothyroidism in Women. *Nursing for Women's Health*. https://doi.org/10.1016/j.nwh.2015.12.002

Dyer, S. J., & Patel, M. (2012). The economic impact of infertility on women in developing countries - a systematic review. *Facts, Views & Vision in ObGyn*, *4*(2), 102–109. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24753897%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3987499

Elhussein, O. G., Ahmed, M. A., Suliman, S. O., Yahya, leena I., & Adam, I. (2019). Epidemiology of infertility and characteristics of infertile couples requesting assisted reproduction in a low-resource setting in Africa, Sudan. *Fertility Research and Practice*, *5*(1), 7–11. https://doi.org/10.1186/s40738-019-0060-1

Emokpae, M. A., Osadolor, H. B., & Ohonsi, O. A. (2011). Sub-clinical hypothyroidism in infertile Nigerian women with hyperprolactinaemia. *Nigerian Journal of Physiological Sciences*, *26*(1), 35–38.

Eniola, W., Adetola, A., & Abayomi, T. (2012). A review of Female Infertility; important etiological factors and management. *Journal of Microbiology and Biotechnology Research Scholars Research Library J. Microbiol. Biotech. Res*, *2*(3), 379–385. Retrieved from http://scholarsresearchlibrary.com/archive.html

Evers, A. S. (2012). Paracrine interactions of thyroid hormones and thyroid stimulation hormone in the female reproductive tract have an impact on female fertility. *Frontiers in Endocrinology*, *3*(MAR), 1–8. https://doi.org/10.3389/fendo.2012.00050

Fields, E., Chard, J., James, D., & Treasure, T. (2013). Fertility (update): summary of NICE guidance. *BMJ : British Medical Journal*, *346*, f650. https://doi.org/10.1136/bmj.f650

Fledderjohann, J. J. (2012). "Zero is not good for me": Implications of infertility in Ghana. *Human Reproduction*, *27*(5), 1383–1390. https://doi.org/10.1093/humrep/des035

Franklyn, J. A. (2013). Hypothyroidism. *Medicine*, *41*(9), 536–539. https://doi.org/10.1016/j.mpmed.2013.06.003

Fupare, S., Jambhulkar, R. K., & Tale, A. (2016). Correlation of Fsh , Lh and Prolactin Infertility in Reproductive Age Group Women. *European Journal of Molecular Biology and Biochemisrty*, *3*(1), 16–21.

Gabriel, A. N. (2008). Thyroid function profile of infertile women in enugu, awka and nnewi municipalities.

Gaitonde, D. Y., Rowley, K. D., & Sweeney, L. B. (2012). Hypothyroidism: An update. *American Family Physician*, *86*(3), 244–251. https://doi.org/10.1080/20786204.2012.10874256

Gameiro, S., Verhaak, C. M., Kremer, J. A. M., & Boivin, J. (2013). Why we should talk about compliance with assisted reproductive technologies (ART): a systematic review and meta-analysis of ART compliance rates, *19*(2), 124–135. https://doi.org/10.1093/humupd/dms045

Gardner, D. G., Shoback, D. M., Greenspan, F. S. (Francis S., Beers, Mark H., ed.Berkow, Robert, ed. Bogin, Robert M., ed. Fletcher, Andrew J., ed. Merck Rahman, M. I. M. H. B. ; R. B., Schaffer, Alexander J.Avery, Mary Ellen Finberg, Laurence Markowitz, M., Ferrero, Narciso A., dir.Debaisi, Gustavo Ferrero, Fernando C. Gil, Stella Maris Mazzucchelli, María Teresa Nizzo, Dante D. Ossorio, María Fabiana Veber, S. E., … Hoskins, J. D. (2011). *Greenspan's Basic and Clinical Endocrinology*. *McGraw Hill*.

Gimenes, F., Souza, R. P., Bento, J. C., Teixeira, J. J. V., Maria-Engler, S. S., Bonini, M. G., & Consolaro, M. E. L. (2014). Male infertility: A public health issue caused by sexually transmitted pathogens. *Nature Reviews Urology*, *11*(12), 672–687. https://doi.org/10.1038/nrurol.2014.285

Gnoth, C., Maxrath, B., Skonieczny, T., Friol, K., Godehardt, E., & Tigges, J. (2011). Final ART success rates : a 10 years survey, *26*(8), 2239–2246. https://doi.org/10.1093/humrep/der178

Greve, B. (2019). What is it all about? *Welfare, Populism and Welfare Chauvinism*, *11*(1), 1–16. https://doi.org/10.2307/j.ctvhrd13m.6

Hess, R. F., Ross, R., & Gililland J.L., J. (2018). Infertility, psychological distress, and coping strategies among women in Mali, West Africa: A mixed-methods study. *African Journal of Reproductive Health*, *22*(1), 60–72. https://doi.org/10.29063/ajrh2018/v22i1.6

Hiraoka, T., Wada-Hiraike, O., Hirota, Y., Hirata, T., Koga, K., Osuga, Y., & Fujii, T. (2016). The impact of elevated thyroid stimulating hormone on female subfertility. *Reproductive Medicine and Biology*, *15*(2), 121–126. https://doi.org/10.1007/s12522-015-0221-9

Huang, J., Huang, T., Qiu, H., Fang, X., Zhuang, T., Qiu, J. (2002). Studies on the integration of

hepatitis B virus DNA sequence in human sperm chromosomes. Asian J Androl, 4:209–12.

Ibisomi, L., & Mudege, N. N. (2014). Childlessness in Nigeria: perceptions and acceptability. *Culture, Health and Sexuality*. Taylor & Francis. https://doi.org/10.1080/13691058.2013.839828

Indongo, N., & Pazvakawambwa, L. (2012). Determinants of fertility in Namibia. *African Journal of Reproductive Health*, *16*(4), 50–57.

Inhorn, M. C. (2003). Global infertility and the globalization of new reproductive technologies: Illustrations from Egypt. *Social Science and Medicine*, *56*(9), 1837–1851. https://doi.org/10.1016/S0277-9536(02)00208-3

Inhorn, M. C., & Patrizio, P. (2014). Infertility around the globe: New thinking on gender, reproductive technologies and global movements in the 21st century. *Human Reproduction Update*, *21*(4), 411–426. https://doi.org/10.1093/humupd/dmv016

Ishii, R., Tachibana, N., Okawa, R., Enomoto, M., Asami, M., Toriumi, R., … Taketani, Y. (2019). Different anti - Műllerian hormone (AMH) levels respond to distinct ovarian stimulation methods in assisted reproductive technology (ART): Clues to better ART outcomes, (January), 263–272. https://doi.org/10.1002/rmb2.12270

Jose-Miller, A. B., Boyden, J. W., & Frey, K. A. (2007). Infertility. *American Family Physician*.

Khandelwal, D., & Tandon, N. (2012). Overt and Subclinical Hypothyroidism. *Drugs*, *72*(1), 17-33 17p. https://doi.org/10.2165/11598070-000000000-00000

Kjetland, E. F., Leutscher, P. D. C., & Ndhlovu, P. D. (2012). A review of female genital schistosomiasis. *Trends in Parasitology*, *28*(2), 58–65. https://doi.org/10.1016/j.pt.2011.10.008

Krassas, G. E., Poppe, K., & Glinoer, D. (2010). Thyroid Function and Human Reproductive Health. *Endocrine Reviews*, *31*(5), 702–755. https://doi.org/10.1210/er.2009-0041

Lam, P. M., Suen, S. H., Lao, T. T., Cheung, L. P., Leung, T. Y., & Haines, C. (2010). Hepatitis B infection and outcomes of in vitro fertilization and embryo transfer treatment. *Fertility and Sterility*, *93*(2), 480–485. https://doi.org/10.1016/j.fertnstert.2009.01.137

Lande, Y., Seidman, D. S., Maman, E., Baum, M., & Hourvitz, A. (2015). Why do couples discontinue unlimited free IVF treatments? *Gynecological Endocrinology*, *31*(3), 233–236. https://doi.org/10.3109/09513590.2014.982082

Lee, V. C. Y., Ng, E. H. Y., Yeung, W. S. B., & Ho, P. C. (2010). Impact of positive hepatitis B surface antigen on the outcome of IVF treatment. *Reproductive BioMedicine Online*, *21*(5), 712–717. https://doi.org/10.1016/j.rbmo.2010.06.036

Lindsay, T. J., & Vitrikas, K. R. (2015). Evaluation and treatment of infertility. *American Family Physician*, *91*(5), 308–314.

Luk, B. H. K., & Loke, A. Y. (2015). The Impact of Infertility on the Psychological Well-Being, Marital Relationships, Sexual Relationships, and Quality of Life of Couples: A Systematic Review. *Journal of Sex and Marital Therapy*, *41*(6). https://doi.org/10.1080/0092623X.2014.958789

Mascarenhas, M. N., Flaxman, S. R., Boerma, T., Vanderpoel, S., & Stevens, G. A. (2012). National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys. *PLoS Medicine*, *9*(12), 1–12. https://doi.org/10.1371/journal.pmed.1001356

Masoumi, S. Z., Parsa, P., Darvish, N., Mokhtari, S., Yavangi, M., & Roshanaei, G. (2015). An epidemiologic survey on the causes of infertility in patients referred to infertility center in

Fatemieh Hospital in Hamadan. *Iranian Journal of Reproductive Medicine*, *13*(8).

Medenica, S., Nedeljkovic, O., Radojevic, N., Stojkovic, M., Trbojevic, B., & Pajovic, B. (2015). Thyroid dysfunction and thyroid autoimmunity in euthyroid women in achieving fertility. *European Review for Medical and Pharmacological Sciences*, *19*(6), 977–987.

Molnar, C., & Gair, J. (2012). *Concepts of Biology-1st Canadian Edition* (1st ed.). OpenStax College. Retrieved from https://opentextbc.ca/biology/front-matter/preface-to-the-1st-canadian-edition/

Murto, T., Bjuresten, K., Landgren, B.-M., & Stavreus-Evers, A. (2013). Predictive value of hormonal parameters for live birth in women with unexplained infertility and male infertility. *Reproductive Biology and Endocrinology*, *11*(1), 61. https://doi.org/10.1186/1477-7827-11-61

Nazarpour, S., Tehrani, F. R., Simbar, M., & Azizi, F. (2015). Thyroid dysfunction and pregnancy outcomes. *Iranian Journal of Reproductive Medicine*, *13*(7), 387–396.

Nupur, H., Andaleeb, F., Premlata, M., Nisha, S., & Swati, G. (2015). Correlation of Prolactin and Thyroid Hormone Levels in Infertile Women, *3*(Table 1), 1–2. https://doi.org/10.4172/2161-0681.1000304

Ogbera, A. O., & Okosieme, O. E. (2014). Thyroid International 1· 2014.

Okunola, T., Ajenifuja, K. O., Loto, O. M., Salawu, A., & Omitinde, S. O. (2017). Follicle stimulating hormone and anti-müllerian hormone among fertile and infertile women in Ile-Ife, nigeria: Is there a difference? *International Journal of Fertility and Sterility*, *11*(1), 33–39. https://doi.org/10.22074/ijfs.2016.4645

Ombelet, W. (2011). Global access to infertility care in developing countries: a case of human rights, equity and social justice. *Facts, Views & Vision in ObGyn*, *3*(4), 257–266. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24753875%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3987469

Ombelet, Willem. (2014). Is global access to infertility care realistic? the Walking Egg Project. *Reproductive BioMedicine Online*. https://doi.org/10.1016/j.rbmo.2013.11.013

Ombelet, Willem, Cooke, I., Dyer, S., Serour, G., & Devroey, P. (2008). Infertility and the provision of infertility medical services in developing countries. *Human Reproduction Update*, *14*(6), 605–621. https://doi.org/10.1093/humupd/dmn042

Palamuleni, M. (2017). Determinants of fertility decline in Namibia: an analysis of the proximate determinants, *14*(2), 41–63.

Pedro, A., & Andipatin, M. (2014). A Qualitative Exploration of South African Women's Psychological and Emotional Experiences of Infertility. *Open Journal of Preventive Medicine*, *04*(05), 327–337. https://doi.org/10.4236/ojpm.2014.45040

Pfeifer, S., Butts, S., Dumesic, D., Fossum, G., Goldberg, J., Gracia, C., … Widra, E. (2015). Subclinical hypothyroidism in the infertile female population: A guideline. *Fertility and Sterility*, *104*(3), 545–553. https://doi.org/10.1016/j.fertnstert.2015.05.028

Pirwany, I. R., Phillips, S., Kelly, S., Buckett, W., & Tan, S. L. (2004). Reproductive performance of couples discordant for hepatitis B and C following IVF treatment. *Journal of Assisted Reproduction and Genetics*, *21*(5), 157–161. https://doi.org/10.1023/B:JARG.0000031248.44180.0a

Polis, C. B., Cox, C. M., Tunçalp, Ö., McLain, A. C., & Thoma, M. E. (2017). Estimating infertility prevalence in low-to-middle-income countries: An application of a current duration approach to Demographic and Health Survey data. *Human Reproduction*, *32*(5), 1064–

1074. https://doi.org/10.1093/humrep/dex025

Poppe, K., Velkeniers, B., & Glinoer, D. (2007). Thyroid disease and female reproduction. *Clinical Endocrinology*, *66*(3), 309–321. https://doi.org/10.1111/j.1365-2265.2007.02752.x

Qian, W.P., Tan, Y.Q., Chen, Y., Peng, Y., Li, Z., Lu, G.X., ... Shing, L. (2005). Rapid quantification of semen hepatitis B virus DNA by real-time polymerase chain reaction. World J Gastroenterol, 11:5385–9.

Saran, S., Gupta, B., Philip, R., Singh, K., Bende, S., Agroiya, P., & Agrawal, P. (2016). Effect of hypothyroidism on female reproductive hormones. *Indian Journal of Endocrinology and Metabolism*. https://doi.org/10.4103/2230-8210.172245

Sarmah, H. K., & Hazarika, B. B. (2012). Importance of the Size of Sample and its Determination in the Context of Data Related to the Schools of Greater Guwahati. *Bulletin of the Gauhati University Mathematics Association*, *12*(1), 16. Retrieved from https://www.researchgate.net/profile/Hemanta_Sarmah3/publication/306099484_Importance_of_the_size_of_Sample_and_its_determination_in_the_context_of_data_related_to_the_schools_of_greater_Guwahati/links/57b14a5c08aeb2cf17c47650.pdf

Sharma, S., Mittal, S., & Aggarwal, P. (2009). Management of infertility in low resource countries. *BJOG: An International Journal of Obstetrics and Gynaecology*, *116*(SUPPL. 1), 71–76. https://doi.org/10.1111/j.1471-0528.2009.02312.x

Shi, L., Liu, S., Zhao, W., Zhou, H., Ren, W., & Shi, J. (2014). Hepatitis B virus infection reduces fertilization ability during in vitro fertilization and embryo transfer. *Journal of Medical Virology*, *86*(7), 1099–1104. https://doi.org/10.1002/jmv.23908

Sidibé, E. H. (2007). [Thyroid diseases in sub-Saharan Africa]. *Santé (Montrouge, France)*, *17*, 33–39. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17897900%5Cnhttp://www.jle.com/e-docs/00/04/34/EE/vers_alt/VersionPDF.pdf

SJ, D. (2007). The value of children in African countries: Insights from studies on infertility. TT - . *Journal of Psychosomatic Obstetrics and Gynecology*, *28*(2), 69–77. https://doi.org/http://dx.doi.org/10.1080/01674820701409959

Smith, J. F., Eisenberg, M. L., Millstein, S. G., Nachtigall, R. D., Sadetsky, N., Cedars, M. I., & Katz, P. P. (2011). Fertility treatments and outcomes among couples seeking fertility care: Data from a prospective fertility cohort in the United States. *Fertility and Sterility*, *95*(1), 79–84. https://doi.org/10.1016/j.fertnstert.2010.06.043

Somigliana, E., Paffoni, A., Busnelli, A., Filippi, F., Pagliardini, L., Vigano, P., & Vercellini, P. (2016). Age-related infertility and unexplained infertility: An intricate clinical dilemma. *Human Reproduction*. https://doi.org/10.1093/humrep/dew066

Steiner, A. Z., & Jukic, A. M. Z. (2016). Impact of female age and nulligravidity on fecundity in an older reproductive age cohort. *Fertility and Sterility*, *105*(6), 1584-1588.e1. https://doi.org/10.1016/J.FERTNSTERT.2016.02.028

Sule, J. O., Erigbali, P., & Eruom, L. (2008). Prevalence of infertility in women in a Southwestern Nigerian community. *African Journal Biomedical Research*, *11*(2), 225–227. https://doi.org/10.4314/ajbr.v11i2.50716

Tabong, P. T., & Adongo, P. B. (2013). Infertility and Childlessness : A Qualitative Study of the Experiences of Infertile Couples in Infertility and Childlessness : a Qualitative Study of the Experiences of Infertile Couples in Northern Ghana. *BMC Pregnancy and Childbirth*, *13*(1), 1. https://doi.org/10.1186/1471-2393-13-72

Tan, T. Y., Lau, M. S. K., Loh, S. F., & Tan, H. H. (2014). Female ageing and reproductive outcome

in assisted reproduction cycles. *Singapore Medical Journal*, *55*(6), 305–309. https://doi.org/10.11622/smedj.2014081

Teng, W., Shan, Z., Patil-Sisodia, K., & Cooper, D. S. (2013). Hypothyroidism in pregnancy. *The Lancet Diabetes and Endocrinology*. https://doi.org/10.1016/S2213-8587(13)70109-8

Teoh, P. J., & Maheshwari, A. (2014). Low-cost in vitro fertilization: Current insights. *International Journal of Women's Health*, *6*(1), 817–827. https://doi.org/10.2147/IJWH.S51288

Unuane, D., Tournaye, H., Velkeniers, B., & Poppe, K. (2011). Endocrine disorders & female infertility. *Best Practice & Research. Clinical Endocrinology & Metabolism*, *25*(6), 861–873. https://doi.org/10.1016/j.beem.2011.08.001

van Balen, F., & Trimbos-Kemper, T. C. M. (1995). Involuntarily Childless Couples: Their Desire to have Children and Their Motives. *Journal of Psychosomatic Obstetrics & Gynecology*, *16*(3), 137–144. https://doi.org/10.3109/01674829509024462

Vander Borght, M., & Wyns, C. (2018). Fertility and infertility: Definition and epidemiology. *Clinical Biochemistry*, *62*, 2–10. https://doi.org/https://doi.org/10.1016/j.clinbiochem.2018.03.012

Vaughan, D. A., & Harrity, C. (2015). Anti-Müllerian hormone levels and ART outcomes. *Fertility and Sterility*, *104*(3), e251. https://doi.org/10.1016/j.fertnstert.2015.07.791

Wang, L., Li, L., Huang, C., Diao, L., Lian, R., Li, Y., … Zeng, Y. (2019). Maternal chronic hepatitis B virus infection does not affect pregnancy outcomes in infertile patients receiving first in vitro fertilization treatment. *Fertility and Sterility*, *112*(2), 250-257.e1. https://doi.org/10.1016/j.fertnstert.2019.03.039

Wang, S., Peng, G., Li, M., Xiao, H., Jiang, P., Zeng, N., ... Wang, Z. (2003). Identification of hepatitis B virus vertical transmission from father to fetus by direct sequencing. Southeast Asian J Trop Med Public Health, 34: 106–13.

Warren, E. (2014). Thyroid disease. *Clinical Cases in Anesthesia*. https://doi.org/10.1093/bja/85.1.15

Welsh, K. J., & Soldin, S. J. (2016). How reliable are free thyroid and tot0al t3hormone assays? *European Journal of Endocrinology*, *175*(6), R255–R263. https://doi.org/10.1530/EJE-16-0193

WHO | Iodine supplementation in pregnant and lactating women. (2015). *WHO*. Retrieved from http://www.who.int/elena/titles/guidance_summaries/iodine_pregnancy/en/

World Health Organisaion. (2012). WHO | Global prevalence of infertility, infecundity and childlessness. *WHO*.

Wu, A. K., Elliott, P., Katz, P. P., & Smith, J. F. (2013). Time costs of fertility care: The hidden hardship of building a family. *Fertility and Sterility*, *99*(7), 2025–2030. https://doi.org/10.1016/j.fertnstert.2013.01.145

Ye, F., Liu, Y., Jin, Y., Shi, J., Yang, X., Liu, X., … Zhang, L. (2014). The effect of hepatitis B virus infected embryos on pregnancy outcome. *European Journal of Obstetrics and Gynecology and Reproductive Biology*, *172*, 10–14. https://doi.org/10.1016/j.ejogrb.2013.10.002

Yusuf, L. (2016). Depression, anxiety and stress among female patients of infertility; A case control study. *Pakistan Journal of Medical Sciences*, *32*(6). https://doi.org/10.12669/pjms.326.10828

Zegers-Hochschild, F., Adamson, G. D., Dyer, S., Racowsky, C., de Mouzon, J., Sokol, R., … van der Poel, S. (2017). The International Glossary on Infertility and Fertility Care, 2017.

Fertility and Sterility, *108*(3). https://doi.org/10.1016/j.fertnstert.2017.06.005

NAMIBIA UNIVERSITY OF SCIENCE AND TECHNOLOGY

Faculty of Health and Applied Sciences

Department of Health Sciences

Annexure A: Informed consent to participate in the study

Dear Participant,

My name is Adão Francisco Lucas, a post-graduate student at Namibia University of Science and Technology (NUST). I am conducting a study as a requirement for the Master of Health Sciences qualification. The title of my study is **"Fertility and pregnancy outcome among women undergoing assisted reproductive technology treatment in Windhoek, Namibia."**

I would like you to request your thoughtful consideration to participate in this study after sharing briefly with you what the study entails.

The primary aim of this study is to determine the prevalence of successful pregnancy outcomes among infertile women undergoing ART treatment at the Cape Windhoek Fertility Clinic in Windhoek, Namibia.

This study will not only add knowledge to the medical field in terms of reproductive Biology within the Namibian context but it will also help stakeholders such as the Ministry of Health and Social Services (MoHSS) develop intervention measures such as educating the Namibian women at an earlier stage regarding fertility.

I would like to bring to your attention the following ethical considerations which will guide your participation:

1. Your participation in this study is entirely voluntary and that there are no direct personal benefits for participating in this study.
2. After you read through, please feel free to ask questions and seek any explanation and/or clarification regarding the study before deciding to be part of it.
3. All information that you will provide will be treated as strictly confidential.
4. You are free to withdraw from the study, if you wish to do so at any point in time and it will not have any negative effect on the outcome of your treatment/consultation.
5. This study has been reviewed by the NUST ethic committee and has also been approved by the MoHSS. The details are accessible if you wish to know more.
6. I can be contacted on my mobile 0813674338 or via email (Adaofranciscolucas@gmail.com), if you have any further questions regarding this study.

I, _____have read the foregoing information or it has been read to me. I had the opportunity to ask questions and all my questions have asked have been answered to my satisfaction. I, therefore, voluntarily consent to participate in this research.

Signature: _____ **Date: _____**

NAMIBIA UNIVERSITY OF SCIENCE AND TECHNOLOGY

Faculty of Health and Applied Sciences

Department of Health Sciences

Research topic: Fertility and pregnancy outcome among women undergoing assisted reproductive technology treatment in Windhoek, Namibia.

Annexure B: Research Questionnaire

1. What is your date of birth?

   _____

2. What is your marital status?

   Single ☐ Married ☐ Divorced ☐

3. What is your occupation?

   _____

4. Please indicate your appropriate race.

   Black ☐ Mixed ☐ White ☐ Other ☐

5. Are you on any contraceptives? If yes, specify (how long and which type).

   _____

6. When last did you have your menstrual period (LMP)?

   _____

7. Do you smoke cigarettes?

   _____

8. Do you drink alcohol? If yes, how much and how often?

   _____

9. Are you currently on any medication? If yes, specify (Name of medication and how long have you been taking them).

   _____

10. Have you been screened or treated for infertility before?

    _____

11. Do you have or is there any history of any medical condition in your family? If yes, please specify.

    _____

12. Have you ever had any surgeries? If yes, please specify.

    _____

13. Have you ever fallen pregnant? If yes, how many children do you have?

    _____

14. Have you lost any pregnancy before? If yes, specify (cause of loss).

    _____

15. For how long have you been trying to conceive?

    _____