Evaluating the feasibility of low cost sperm preparation methods within a prospective intrauterine insemination programme in Gabon

By

Lionel Wildy Moungala

(12232310)

Submitted in fulfilment of the requirements for the degree
Masters of Science (Reproductive Biology)
In the Faculty of Health Sciences
University of Pretoria

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DEDICATION

Every challenging work needs self-effort as well as guidance of elders, especially those who are very close to our heart.

I dedicate my humble effort to my sweet and loving God, the Reproductive Biology Laboratory members, my mother (Constance Moungala) and my dad (William Moungala) whose affection, love, encouragement and prayers make me able to complete this journey

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DECLARATION BY CANDIDATE

'I hereby declare that the dissertation submitted for the degree MSc Reproductive Biology, in the Faculty of Health Sciences, University of Pretoria is my own original work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references.'

LIONEL WILDY MOUNGALA

Name of student **Signature**

Date

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ETHICAL CLEARANCES

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 20 Oct 2016.
- IRB 0000 2235 IORG0001762 Approved dd 13/04/2011 and Expires 13/04/2014.

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Faculty of Health Sciences Research Ethics Committee

27/03/2014

Approval Certificate
New Application

Ethics Reference No.: 52/2014

Title: Evaluating the feasibility of low cost sperm preparation methods within a prospective IUI program in Gabon

Dear Mr. Lionel Wildy Moungala

The **New Application** as supported by documents specified in your cover letter for your research received on the 30/01/2014, was approved, by the Faculty of Health Sciences Research Ethics Committee on the 26/03/2014.

Please note the following about your ethics approval:
- Ethics Approval is valid for 1 year.
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We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed.
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

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26/03/2015

Approval Certificate
Amendment
(to be read in conjunction with the main approval certificate)

Ethics Reference No.: 52/2014

Title: Evaluating the feasibility of low cost sperm preparation methods within a prospective IUI program in Gabon

Dear Mr.Lionel Wildy Mbungala

The **Amendment** as described in your documents specified in your cover letter dated 20/02/2015 received on 24/02/2015 was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 25/03/2015.

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Ethics amendment is subject to the following:
- The ethics approval is conditional on the receipt of 6 monthly written Progress Reports, and
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SUMMARY

Introduction:

Intrauterine insemination (IUI) is frequently used as the first-line treatment for infertility, due to the relative simplicity and cost-effectiveness of the procedure. Even though, several sperm processing techniques are used for IUI, semen samples need to be washed using a single and appropriate method prior to insemination. Implementing low-cost sperm preparation methods for IUI would benefit those seeking help in developing countries such as Gabon, where treatment for infertility is currently virtually non-existent.

The first section of this research was aimed towards the evaluation of a sperm swim-up method (SW-10) while in the second section the feasibility of establishing an IUI programme in Gabon was explored.

Methods:

The initial section involved three sets of experiments. Semen samples were obtained from patients and donors participating in the Reproductive Biology Laboratory's research donor registry programme. For experiment (i), semen samples (n=25) were divided into 3 equal aliquots and processed using three syringes with volumes of 5 ml (SW-5),10 ml (SW-10) and 20 ml (SW-20), respectively. During experiment (ii), semen samples (n=20) were split into 2 equal volumes and processed using either the SEP-D kit or the SW-10 method. In experiment (iii) (n=20) two sperm preparation methods i.e. the single layer centrifugation (SLC) and the density gradient centrifugation (DGC) were compared. For post-processing analyses, concentration, motility and total motile sperm count (experiments (i), (ii) and (iii)), as well as morphology, viability and DNA integrity (experiment (ii)) were evaluated.

Information on the feasibility of establishing an IUI programme in Gabon in the second section was obtained from a questionnaire completed by gynaecologists practising in Gabon.

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Results:

In the **first section**, experiment (i) indicated a significant increase in motility and concentrations in spermatozoa processed using the SW-10 method when compared to SW-5 and SW-20 ($p<0.05$). Experiment (ii) revealed that the SW-10 method yielded spermatozoa with significant superior motility and concentration when compared to that obtained using the SEP-D kit ($p<0.05$). The SW-10 yielded a statistically significant larger number of spermatozoa with intact plasma membranes, DNA content and normal morphology ($p<0.05$). In experiment (iii), semen processed using SLC resulted in spermatozoa with statistically significant higher concentrations. However spermatozoa obtained from the DGC had superior motility.

The **second section** comprised a gynaecological survey conducted in Gabon. Seventeen (85%) of the 20 registered gynaecologists participated in the survey. Gynaecologists were particularly interested in a basic infertility treatment and training programme, as well as in the establishment of an ART unit in Libreville. All participants were in agreement that ART services would improve both diagnostic and therapeutic patient services in Gabon.

Discussion and conclusion:

This study indicated that spermatozoa recovered from the simplified sperm swim-up method had statistically significantly higher sperm parameters when compared to those from the SEP-D kit. Processing semen using SLC resulted in significantly higher sperm concentration when compared to that of the DGC, which yielded a higher percentage of progressively motile spermatozoa.

The need to establish an IUI programme in Gabon was proved in the second section of this study. In conclusion, the simplified swim-up method can possibly be an effective low-cost alternative in the preparation of semen samples for IUI procedures. The implementation of low-cost sperm preparation methods can be important in developing countries such as Gabon.

Keywords: *Density gradient centrifugation (DGC) - Gabon - Intrauterine insemination - SEP-D kit - Single layer centrifugation (SLC) - Sperm - Swim-up method*

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LIST OF ABBREVIATIONS

AIDS:	Acquired immunodeficiency syndrome
ALH:	Amplitude lateral head displacement
ART:	Assisted reproductive technology
BMI:	Body mass index
CASA:	Computer-aided sperm analysis
CI:	Confidence limit
DNA:	Deoxyribonucleic acid
DGC:	Density gradient centrifugation
HIV:	Human immunodeficiency virus
ICSI:	Intra-cytoplasmic sperm injection
IMC:	Inseminating motile count
IUI:	Intrauterine insemination
IVF:	*In vitro* fertilization
P:	Probability value
pH:	Potential of hydrogen
RBL:	Reproductive biology laboratory
SLC:	Single layer centrifugation
SOP:	Standard operating procedure
STI:	Sexually transmitted infection
SW-5:	Swim-up method using the 5 ml syringe
SW-10:	Swim-up method using the 10 ml syringe
SW-20:	Swim-up method using the 20 ml syringe
TMC:	Total motile sperm count
TVOA:	Trans-vaginal oocyte aspiration
tWE:	the Walking Egg
VAP:	Average path velocity
VCL:	Curvilinear velocity
VSL:	Straight-line velocity
WOB:	Wobble
WHO:	World Health Organization

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CHAPTER 1: OVERVIEW OF STUDY

1.1 Motivation for study

Infertility is defined as the inability to achieve a pregnancy within at least one year of adequate sexual exposure without the use of contraceptives[1]. Absolute childlessness is referred to as primary infertility, and secondary infertility can be seen as the inability to conceive an additional child[2]. Infertility affects both men and women with almost equal frequency[1]. Even though the world population is increasing, globally 48.5 million couples experienced infertility related problems in 2010[3]. Sub-Saharan Africa has been reported as having the highest incidence of infertility in the world[3]. The geography of infertility in Africa shows specific zones, with low fertility rates evident in West Africa from Senegal, Mali, Burkina Faso to Niger, and in central Africa, from Cameroon, Congo, Gabon to Sudan[3]. In sub-Saharan Africa over 50% of visits to gynaecology clinics concern infertility related issues[4].

Gabon, officially called the Gabonese Republic is located on the west coast of central Africa (see Figure 1). Located on the equator, Gabon is bordered by Equatorial Guinea to the Northwest, Cameroon to the North, the Republic of the Congo on the East and South, and the Atlantic Ocean's Gulf of Guinea to the West[5]. A study performed in Gabon in 2012, indicated an increase in the infertility rates during the past 20 years[5], with more than 40% of couples presenting with secondary infertility[6]. In 2013, the Department of Health in Gabon voiced their concern regarding the increasing rate of infertility, specifically in the eastern parts of the country where mining activity is high[7]. The only reproductive laboratory in central Africa is located in Cameroon (Clinique Odysee and Clinique de l' Aeroport)[8]. Subsequently sub-fertile couples with the financial means residing in countries such as Burundi, central African Republic, Democratic Republic of the Congo, Chad,

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Equatorial Guinea, and Gabon, are obliged to travel for assisted reproductive technology (ART) treatment[9]. Therefore the need for access to basic diagnoses for ART procedures (such as intrauterine insemination (IUI)) in sub-Saharan Africa is thus evident.

Infertility may not be a public health priority in both developed and developing countries, however it is an issue in the lives of the numerous affected individuals. Fertility and parenthood are valued in Africa to the extent that procreation is generally considered to be the major purpose of marriage[10]. The negative impact of childlessness on couples is experienced to a more serious degree in developing countries when compared to the effect in Western societies[11]. In many African cultures a woman is defined by motherhood, with the woman usually bearing the brunt of a couple's inability to conceive[12]. Childless women are frequently disgraced with isolation, neglect, depression, unhappiness, domestic violence and polygamy forced upon them[13-15]. Apart from the psychological and social stigmatization associated with infertility, there are serious economic consequences of childlessness in developing countries. In the absence of social security systems, older individuals without children have no familial support to depend on for economic provision[16]. Consequently the need to assist couples who struggle to conceive is crucial in developing countries.

Various ART treatments or procedures are available in some industrial and non-industrial countries[17]. Evidence shows that the IUI procedure is the first-line treatment for moderate male-factor sub-fertility, and is considered to be the most cost-effective and least invasive ART method available[18]. Access to clinics in Africa is limited and treatment is mostly restricted to private settings[19]. A high standard of infertility management seems more accessible in Anglophone[A] than Francophone[B] Africa[20]. Various obstacles to establishing accessible infertility clinics in Francophone countries exist[9]. These include funding, geographical barriers and a lack of infrastructure required for ART (skills, resources, equipment, supplies, electricity

[A] English speaking countries
[B] French speaking countries

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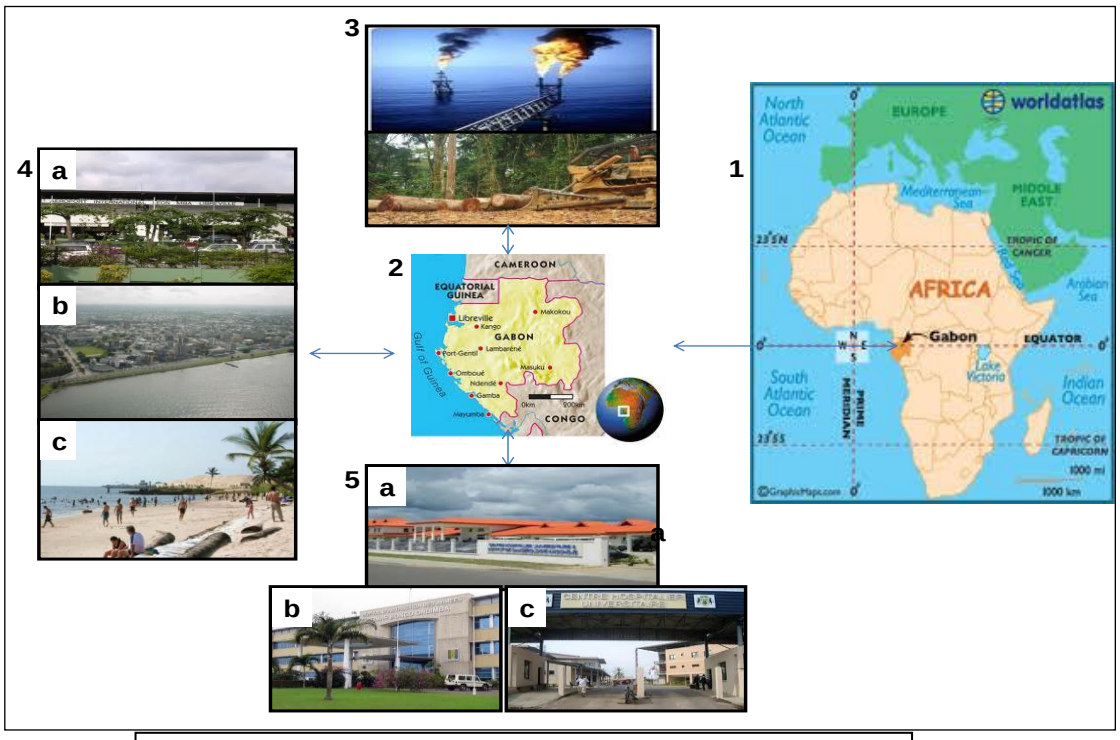
etc.). Owing to political stability, Gabon has the potential for promoting medical technological advanced procedures, which could include basic to advanced ART procedures[21].

This study is aimed at modifying and optimizing a simplified method of sperm preparation for developing countries with minimal facilities, and to evaluate the feasibility of establishing an IUI programme in Gabon.



1) Location of country within Africa;
2) Map of Gabon;
3) Major industries;
4.a) International airport of Libreville;
4.b & c) Overview of Libreville (political capital of Gabon;
5.a) Oncology and Gynaecology Academic Hospital of Agondje;
5.b) Military Hospital of Libreville;
5.c) General Hospital of Libreville;

Figure 1: Gabon: Geographic location and a broad overview of the country[22]

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1.2 Research questions

1.2.1 Is the SW-10 method of sperm preparation appropriate for IUI with respect to sperm yield?

1.2.2 Can the SW-10 method give similar sperm yield and quality results, as those of the commercially available SEP-D kit?

1.2.3 Will the single layer centrifugation (SLC) yield results comparable to the density gradient centrifugation?

1.2.4 Is there any window of opportunity for the establishment of an IUI programme in Gabon?

1.3 Hypotheses

1.3.1 H_0: The SW-10 method of sperm preparation is suitable for IUI with reference to sperm yield.

H_A: The SW-10 is not suitable for IUI with reference to sperm yield.

1.3.2 H_0: The SW-10 method will yield results comparable to those of the commercially available SEP-D kit with respect to motile sperm yield and quality.

H_A: The SW-10 method will yield results inferior to those of the commercially available SEP-D kit with respect to motile sperm yield & quality.

1.3.3 H_0: A SLC will yield results comparable to those of the DGC with respect to motile sperm yield and morphology.

H_A: A SLC will yield results inferior to those of the DGC with respect to motile sperm yield and morphology.

1.3.4 H_0: There is a window of opportunity for the establishment of an IUI programme in Gabon.

H_A: There is no window of opportunity for the establishment of an IUI programme in Gabon.

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1.4 Objectives

Primary objective: To design a simplified sperm preparation method applicable to a developing country with minimal resources (such as Gabon); i.e.

i) modifying a standard sperm swim-up method;
ii) comparing the modified sperm swim-up technique to the commercially available SEP-D kit, with respect to motile sperm yield and quality; and
iii) evaluating the SLC and DGC methods, with respect to motile sperm yield and quality.

Sperm parameters to be analysed could include:

- motility parameters using computer assisted sperm analysis (CASA);
- concentration via Makler and Neubauer counting chambers
- plasma membrane integrity using the Hypo-osmotic swelling test and eosin-nigrosin
- DNA fragmentation via the Halosperm® G2 kit
- morphology evaluation (World Health Organization guidelines,2010)

Secondary objective: To evaluate the feasibility of establishing an IUI programme combined with basic sperm preparations in Gabon by:

i) establishing the need for semen analyses and preparation of samples of male partners from sub-fertile couples without children (questionnaire completed by practicing gynaecologists in Gabon); and
ii) determining whether the aetiology of female partners from sub-fertile couples complies with IUI procedures (questionnaire completed by practising gynaecologists in Gabon)

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Mankind originated in Africa, with the continent known as the cradle of mankind[23]. Research done in 2012 confirmed that the development of Homo sapiens began in Africa 200,000-100,000 years ago[24]. Africa is a vast continent (12,000,000 square miles), which is larger than North America[25]. According to the African Union and the United Nations in 2012, a total of fifty-five countries are recognized on the African continent. With a supply of untapped natural resources in Africa, industrialized countries are continually in competition to gain access to the region's reserves[26].

Most of the countries in Africa are considered to be developing nations. Developing countries (or less developed countries), according to the World Bank in 2013, are nations that have an income per capita of between US$1,000 and US$12,000. These countries include Argentina, Chile, Mexico, Brazil and most of the African countries[27]. In comparison to developing countries, developed nations are most often termed industrialized countries. These countries are characterized by high levels of income per capita, rated at more than US$12,000 per annum and in 2010 countries such as the United States, Canada, Japan, Republic of Korea, Australia and New Zealand qualified[27]. However, considerable economic differences are evident between developing countries. Three African countries are known to be more developed than the other developing countries, due to their higher gross domestic product. These are South Africa, Nigeria and Egypt[28]. There are significant differences in healthcare systems between developed and less developed countries. In general, developing countries or less developed countries are faced with severe financial constraints, limited budgets and infrastructure particularly when trying to access infertility care[29]. Ombelet *et al*. (2008) pointed out that infertility treatment options are almost non-existent and

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expensive in most developing countries. The review highlighted inadequate professional experience among medical staff in developing countries due to limited resources[11].

Infertility is considered to be one of the most common, long lasting and costly diseases, touching all communities and races[30]. Absolute childlessness is referred to as primary infertility, while secondary infertility is the inability to conceive after having a previous pregnancy[31]. Most couples seeking infertility treatment are sub-fertile, which implies a decreased monthly conception rate, with natural pregnancy being possible[32]. Approximately one-third of infertility cases are due to male factors, one-third to female factors, and the remaining third to idiopathic origins[1]. More than 180 million couples in developing countries suffer from primary or secondary infertility[33]. In 2010, almost 1.9% of women aged 20 to 44 presented with primary infertility, and 10.5% secondary infertility[34]. Subfertility affects approximately 15% of all couples, which imposes a significantly medical and social burden on those individuals affected[35]. More than 7% of men are estimated to have reproductive complications[36]. Infertility management, especially in Africa, is still a huge challenge. Assisted reproductive technology (ART) facilities are generally not widely accessible and in most of the cases are not affordable in Africa[11].

The literature review will focus predominantly on the incidence, impact, main causes and treatment options relating to infertility in Africa.

2.2 Overview of infertility in Africa

2.2.1 Prevalence of childlessness

A large majority of childless couples reside in developing countries[37]. The World Health Organization (WHO) expressed the viewpoint to increase research efforts in Africa[38], since Africa is reported to have the highest incidence of infertility in the world[39]. Differences in infertility rates are observed between more developed and less developed countries. The incidence of primary infertility in Africa is approximately 3%, in

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contrast high levels of secondary infertility are observed in most African countries. The incidence of secondary infertility among women aged 20 to 44 ranges from 5% in Togo to 23% in the Central African Republic[40]. The dissimilarities in secondary infertility prevalence rates observed in sub-Saharan African countries can be attributed to the varying rates of sexually transmitted infections (STI) and also the impact of the human immunodeficiency virus (HIV)[40]. An overview of regional prevalence of infertility in Africa will be presented in the following section.

In **northern African** societies, family and childbearing practices are considered to be very important[41]. A decrease in fertility rate has been reported in the northern region of Africa. The average number of children per woman has declined from 7 in 1960 to 3 in 2006[41]; In Algeria, 6 children per woman decreased to 2; in Morocco, 6.5 children per woman decreased to 2, and in Libya 7.5 children per woman decreased to 3[42]. The decrease in fertility rate was initially observed in a few countries including Egypt and Tunisia. These two countries were the first to introduce policies to limit population growth in 1992 by promoting the use of contraceptives[43]. Tunisia, indicated as the country with the highest use of contraceptives, has the lowest fertility rates of all the northern African countries **(Addendum A)**. Statistics from Tunisia disclose that more than 15% of couples are unable to conceive naturally, with 89% of these women suffering from depression[44]. Sudan, a northern African country, is also experiencing a decline in fertility rates. This country is facing internal political instability which began in 2013. Previous literature on infertility prevalence in Sudan revealed that on average, 3% of women presented with primary infertility and 16% secondary infertility[44]. More recent studies show that 79.5% of infertile couples have primary infertility while 20.5% have secondary infertility[45]. **Addendum A** indicates that Sudan is the northern African country where the number of children per woman is highest and the use of contraceptives is lowest. From this a conclusion can be drawn that the use of contraceptives as well as political unrest in Sudan also probably influences fertility rates.

The incidence of infertility in Africa is comparatively widespread, with high rates (22.44%) being reported in the **central African countries** such as Cameroon, Central African Republic and Gabon, and low rates (11.79%) in the eastern and western African

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countries in 2000[40]. A study conducted in 2000 with a follow-up study in 2003 revealed that central Africa has the highest infertility rates, ranging between 3.1- 6.9% (primary infertility) and 18.9-26.5% (secondary infertility) among women aged 20 to 44[46]. Figure 2.1 illustrates the African "infertility belt" which stretches across central Africa from Tanzania in the east to Gabon in the west. The prevalence of infertility in this region of Africa can be explained partly by the high incidence of HIV infections, tuberculosis and STIs[47]. Decreased fecundity was reported in HIV infected individuals in Africa[31]. Dhont *et al*. (2011) concluded that increased HIV incidence and promiscuous sexual behaviour were frequently observed in women suffering from secondary infertility in Rwanda[48].

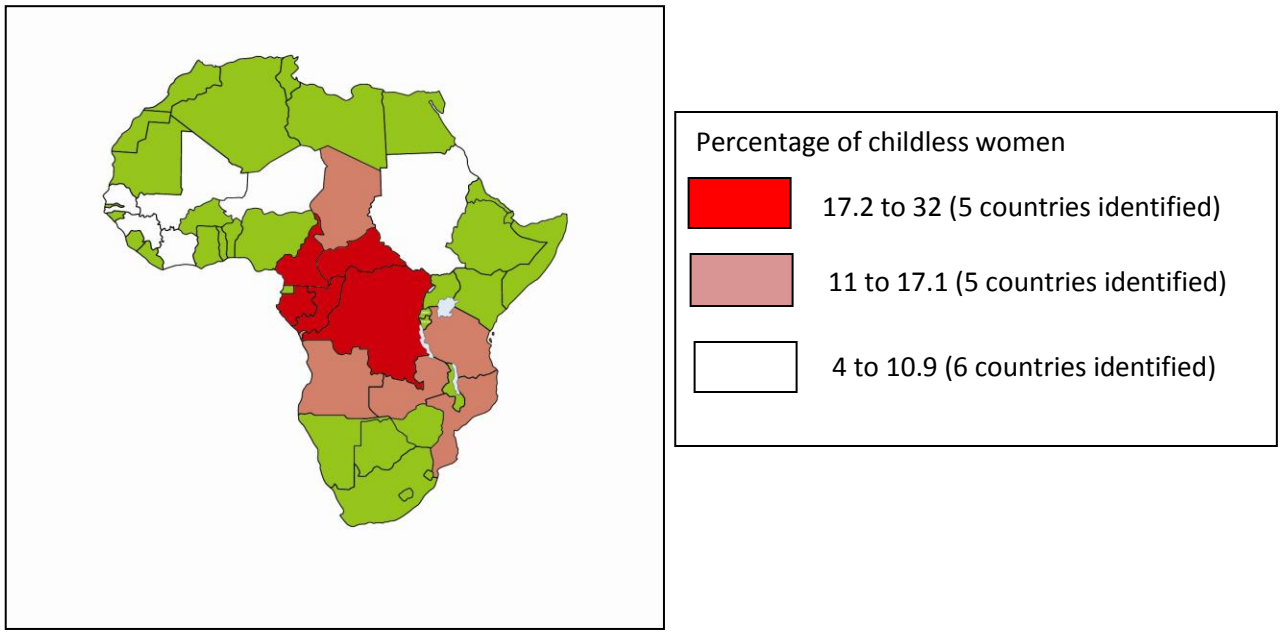

Figure 2.1: Infertility distribution in 16 sub-Saharan African countries[49]

In 2012, statistics on central African countries (see Table 2.1) showed that Gabon had the highest rates of childless women aged between 15 and 44 (32%)[50]. Approximately 25% of women in Cameroon suffered from infertility in 2011[51]. Even though Cameroon has a relatively low percentage of childless women compared to other central African countries, infertility in this country remains a major concern. The infertility rate in Congo is 20.5% (Table 2.1), lower than in Gabon (32%), but higher than the rate in Cameroon and the Central African Republic (17.2%). The Congo that is the second largest country on the African continent by area has the largest total population of all central African countries[52]. The total fertility rate in Congo decreased from 7.3% in 1995 to 6.3% in

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2007[53]. This decline was confirmed by Romaniuk in 2011, who reported elevated rates of infertility in central Congo specifically among the Mongo, Azande and Maniema, who are considered to be the largest groups in the country[54].

Table 2.1: Prevalence of infertility amongst women aged between 15 and 44 in central African countries in 2012[50]

Countries	Infertility rate
Cameroon	17.2
Central African Republic	17.3
Congo (Democratic Republic)	20.5
Gabon	32
Equatorial Guinea	25.2

In **southern African** countries**,** secondary infertility is more predominant in Malawi in comparison to Lesotho, Zimbabwe, Zambia Namibia and Madagascar (Table 2.2). The average number of children per woman in South Africa declined from 6 in 1990 to 3 in 2002[3].

Table 2.2: Infertility prevalence in southern African countries[3]

Countries	Infertility ranges (%)	Infertility rates (%)	
		Secondary infertility	**Primary infertility**
Madagascar	16.2-20.4	**10.7**	8.9
Lesotho	17.1-21.5	**11.6**	4.0
Zimbabwe	16.8-22.4	**12.7**	2.8
Zambia	13.8-17.5	**13.0**	1.4
Namibia	16.2-20.8	**14.7**	2.8
Malawi	12.2-15.0	**15.8**	1.1

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A decline in fertility rate was observed the past four decades, dropping from a total fertility rate of about 6,7 children per woman during the late 1960s to about 2,8 children per woman in 2001[55]. Human immunodeficiency virus (HIV) is a predominant factor in the decrease in fertility rates in South Africa. Studies done in South Africa, during 2010 and 2011 demonstrated that infertility prevalence among the HIV infected population was between 10.6-20%[56,57],with the majority of HIV cases in the productive age group (15-44 years old)[57].

In 2003, in the **western African** country Nigeria, around 800 000 couples were struggling to conceive, with more than 30% due to female factors[3]. Although male factors in Nigeria represent almost 40% of all causes, it is a neglected reproductive health aspect in the country[58]. Table 2.3 indicates the prevalence of infertility in western African countries.

Table 2.3: Childlessness prevalence in western African countries[3]

Countries	Infertility ranges	Infertility rates	
		Secondary infertility	**Primary infertility**
Ghana	10.1-13.5	**5.2**	1.6
Niger	8.9-12.0	**10.2**	1.9
Benin	14.0-16.8	**10.3**	3.9
Burkina Faso	16.6-17.2	**10.4**	3.1
Senegal	13.7-16.7	**10.4**	5.2
Cote D'ivoire	11.5-14.8	**11.3**	5.0
Nigeria	10.5-14.6	**13.6**	4.0
Togo	10.7-15.8	**14.4**	2.9
Mali	13.7-16.7	**16.1**	3.1

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Eastern Africa is reported to have the lowest infertility prevalence rates, with an average of between 8-13%[3]. Eastern African countries include the Republics of Burundi, Kenya, Rwanda, Uganda, Botswana and the United Republic of Tanzania (Table 2.4).

Table 2.4: Incidence of primary and secondary infertility in eastern African countries[3]

Countries	Infertility ranges (%)	Infertility rates (%)	
		Secondary infertility	Primary infertility
Rwanda	9.3-12.0	**9.6**	0.8
Burundi	8.6-11.5	**10.5**	1.3
Uganda	9.9-13.5	**11.7**	5.3
Kenya	13.7-16.7	**13.2**	2.7
Tanzania	10.7-12.0	**13.7**	4.1

There was a modest decline in infertility rates of these countries between 1990 and 2012, and the prevalence was much lower than in other sub-Saharan regions. Uganda has one of the highest fertility rates in the world with an average total fertility rate of 7.1 children[62]. However, between 1980 and 2000, a decrease of 10% in total fertility rate was observed in this country[63]. Similarly to Uganda, a decrease in fertility rate was observed in Kenya between 1998 and 2010[62]. Tanzania is the eastern African country with the highest incidence of secondary infertility (Table 2.4). Primary infertility accounted for one third of infertility cases in Tanzania, with the remaining two thirds due to secondary infertility in 2001[64]. Female related infertility factors in Tanzania were as high as 65.9% and male related factors at only 8.8%. A combination of male and female

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factors was estimated to be 13.2% and unexplained infertility was rated at 12% in Tanzania[64].

2.2.2 Impact of infertility

Infertility has a major effect on the quality of life and health of affected couples. Although the negative consequences of childlessness are much more pronounced in developing countries when compared to those in western societies, interest from the international community and local health care providers is nearly non-existent[62,64,65]. Research has shown that infertility prevalence among men and women is usually the same[66]. However, African societies perceive infertility as the woman's burden[67]. Women are more prepared to disclose their infertility than men[68]. Social and psychological consequences of infertility are commonly observed in these women[69].

Socially, in the African culture a woman is valued once married and fertile, because she loses dignity outside marriage. In the Macua culture in Madagascar, infertility is the "inability of the man and woman's blood to mix"[70]. The commonly held belief that the wife is solely responsible for infertility often leads to polygamy[71,72]. Infertile women are returned to their families after failure to conceive, bringing dishonour and humiliation on the families. In the broader picture, in Africa, divorced women are most likely to be infertile[41]. Husbands present with feelings of victimization and disappointment, believing the women and their families will have known about the infertility status[73]. Male infertility in Africa is mostly considered a taboo topic, therefore it is rarely discussed. The social impact of male infertility is less severe than for women. Some societies offer the use of natural remedies to overcome infertility. In Zimbabwe, "chiramu", a traditional practice, is used to treat male infertility in couples. This practice involves using husband's brother or any close relative to impregnate the wife[74]. If this is unsuccessful, the wife is punished and returned to her family[74]. Male infertility in Egypt is based on the belief that "the sperm cells are weak", and women are most often experiencing procreative blame[75,76].

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In addition to social consequences, psychological pain and shock are experienced by man or woman, as a result of insults levelled by the spouse, relatives, and neighbours. Those couples who can afford the treatment of infertility have to go through physically and emotionally demanding ART procedures[77]. Experiencing infertility is extremely stressful for women[78,79]. Even though the desire to have a child has been reported as being the same for men and women[80], the psychological impact of infertility is probably more devastating to women than men[80]. A negative attitude and a feeling of worthlessness and insufficiency, as well as anger and depression have been reported amongst most barren women[70]. Male infertility, although defined as a drawback, is probably less psychologically devastating[80]. However the condition has a huge impact on masculinity and loss of control and is more stigmatized than female infertility[81]. Masculinity is reportedly associated with self-confidence, self-control and respect[82]. Clinical depression was reported in more than 30% of infertile males in Zimbabwe in 2005[83]. Even though a study by Wischmann *et al*. (2012) disclosed that the treatment of infertility has an impact on self-assurance only, it does not affect any other component of quality of life. Nevertheless, challenges such as sexuality, relationships and job environment need to be addressed to provide psychological assistance to those couples who suffer from childlessness[84].

Reduced fecundity in HIV-infected individuals has been described[85]. Marital instability and polygamy that is result of a secondary infertility may in turn increase the spread of HIV-1 infection. The virus has been found to be three times more prevalent in infertile couples in Rwanda, when they are compared to fertile couples in the same population[48]**.** As both conditions are more common in resource-poor countries, stigmatization and isolation that could result are strongly influenced by socio-cultural and economic conditions[86,87].

As treatment options for HIV and infertility are expensive, in both cases the final result is a decrease in the population. With HIV treatment being in general more effective, available and affordable, this however is not the case with infertility treatment[88]. Awareness, attention to, documentation of and research on the HIV epidemic are much

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more vigorously pursued than infertility. Public measures are being implemented to treat HIV, while infertility treatments are most often available in the private sector only. Infertile couples need to avail themselves of medical procedures such as ART[89].

2.3 Factors associated with infertility

2.3.1 Anatomical and pathological causes of infertility

Female factor infertility comprises one third of global infertility cases and Fallopian tube obstruction is one of the main causes of female infertility in Africa[90]. In Zambia in 2012 more than 61% of infertile women were diagnosed with tubal obstruction[91]. Tubal factor infertility can be the result of either female genital mutilation or pelvic inflammatory disease (PID)[92]. Female genital mutilation, also known as female circumcision, is a traditional practice involving the removal of the female reproductive organs for non-medical reasons. This procedure is practised in more than 30 African countries, with approximately 132 million females being mutilated in 1998[93]. Pelvic inflammatory disease is a reproductive tract infection which can also result in occlusion of the Fallopian tubes[94]. A study by Khan *et al*. (2014) reveals that PID is also the main cause of tubal infertility in industrialized countries[95].

Ovulatory disorders are responsible for almost 50% of all documented infertility cases[96] and were diagnosed in 33% among infertile women residing in developing countries[97]. Hormones are biological compounds synthesized by endocrine glands and released into the blood[98]. Various hormones, proteins and steroids are involved to ensure the ability of humans to reproduce. These include the luteinizing hormone (LH), follicle stimulating hormone (FSH), estrogens, androgens, progestogen and gonadotropin-releasing hormone (GnRH)[99]. The luteinizing hormone, FSH, GnRH, progesterone, and oestrogen are involved in the menstrual cycle pathway[100]. The luteinizing hormone is essential for the growth and maturation of follicles and subsequently the oocytes[101]. Furthermore, this hormone initiates ovulation and the production of androgen and progesterone. Insufficient LH production can cause

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anovulation, early oocyte formation and abnormal menstruation that could lead to infertility[102]. A deficiency in GnRH results in hypothalamic amenorrhoea which can lead to infertility[96]. Hypothalamic amenorrhoea is a condition manifesting an absence of menstruation for numerous months. Oestrogen plays also an important role in the libido levels of a female. A decrease in oestrogen production can lead to dyspareunia which is a medical condition characterised by painful intercourse[103]. Thyroid-stimulating hormone also has an impact on a woman's ability to sustain a pregnancy. A meta-analysis by Thangaratinam *et al*. (2011) reported an association between miscarriages and preterm births due to thyroid autoimmunity[104].

Endometriosis and uterine fibroids are two gynaecological abnormalities that display similar symptoms, but have different epidemiological aspects. Endometriosis is a perplexing condition, which is characterized by abnormal development of endometrial cells located in the outer layer of the uterus, resulting in pain and infertility[105]. The incidence of endometriosis increases with age and has been found to occur more in Caucasian women, but an increase in severe cases has been reported in African women[105]. Uterine fibroids are benign tumours that develop from fibroid cells in the uterus, leading to a decrease in pregnancy rates[106]. A review by Khan *et al*. (2014) revealed that between 20 and 40% of females experience to have uterine fibroids during their reproductive life[107]. The development of uterine fibroids can be induced by hormonal imbalances. An imbalance of oestrogen and progesterone has been reported to have an impact on the incidence of fibroids and endometriosis[108,109]. A study by Kim *et al.* (2013) disclosed that progesterone stimulates the growth of fibroid tissues while a deficiency in progesterone could conceivably increase the risk of endometrial cancer[109]. Race can be a contributing factor when studying the epidemiology of uterine fibroids since the prevalence tends to be higher among black African females compared to Caucasian females[110]. A study by Moorman *et al*. (2013) concluded that African American women have a higher prevalence of uterine fibroids compared to white American women[111]. Genetic factors and family history could possibly explain the racial difference between the incidence of endometriosis and uterine fibroids[108]. Age could possibly be a determinant in the incidence of fibroids. Bulun *et al.* (2013) reported that

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the conditions occur in more than 70% of women aged above 30[112]. Medical options for the treatment of fibroids are discussed in a review by Chabbet *et al.* (2014)[113].

Even though **male infertility** is seldom openly discussed in African societies[114], half of all infertility cases worldwide are male factor related[115]. The condition can be classified as pre- and post-testicular disorders, influencing sperm count as well as motility, sperm DNA fragmentation, sperm-zona (oocyte) binding potential, acrosin activity and ejaculation dysfunction[116]. **Pre-testicular disorders** refer to conditions impeding the adequate production of sperm by the testes[117]. These disorders are associated with hormonal abnormalities. Pre-testicular disorders can be sorted in two principal categories: hypogonadotrophic hypogonadism (HH) and coital disorders. Hypogonadotrophic hypogonadism can be caused by a deficiency of FSH and LH, or can be due to a decrease in GnRH production[118]. The condition can be congenital (hormonal imbalance) or acquired (trauma or tumour). Congenital disorders can be treated with gonadotrophins[20]. Testosterone administration can also be used as a treatment for congenital HH in some patients[119]. Coital disorders include erectile dysfunction and premature ejaculation. In general, the condition occurs less frequently than HH[20]. Anejaculation and retrograde ejaculation are two manifestations of ejaculatory disorders[20]. During ejaculation the neck of the bladder closes to prevent semen from flowing back. Retrograde ejaculation occurs when the neck of the bladder does not close, causing a reflux of semen into the bladder[120]. **Post-testicular disorders** are genital abnormalities occurring after production of sperm by testes. These include any defects in the reproductive tract and any complications that occur during the ejaculation process, as indicated in a review by Olooto *et al.* (2012). Post-testicular disorders include vas deferens obstruction, inherent absence of the vas deferens, blockage in the seminal pathway and autoimmune diseases. Other conditions such as prostatis and hypospadias are classified as post-testicular disorders[117]. Blockage of the ejaculatory duct is the only form of obstruction within the male reproductive organ[121]. The incidence of vas deferens or epididymis blockage, following infections, is the cause of 32.2% of male infertility in Nigeria and 40% in Ghana[122].

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2.3.2 Sexually transmitted infections and Human immunodeficiency virus

Sexually transmitted infections (STIs) comprise between 50% and 85% of all infertility cases in sub-Saharan Africa[123,124]. Infections in males have been known to cause obstructive azoospermia and severe sperm abnormalities, resulting in sub-fertility[125]. The most common STIs that impact infertility include *Chlamydia trachomatis* and *Neisseria gonorrhoea*[126,127]. These two STIs, together with HIV, can be important determinants of pregnancy outcome in Africa due to the high prevalence rates in the continent[128] (Figure 2.2).

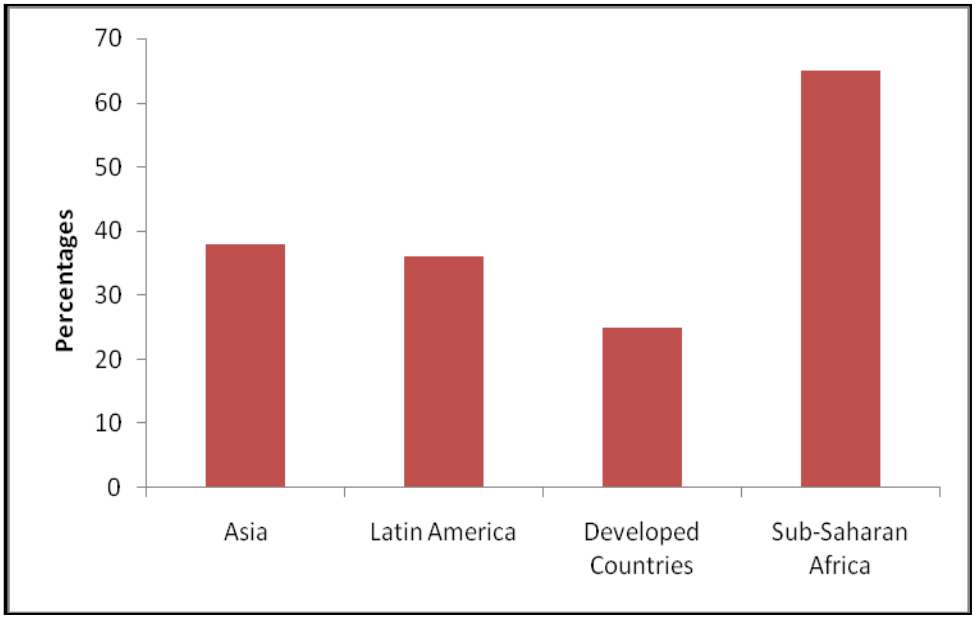

Figure 2.2: Percentage of infertile women with infection-related diagnoses by region[129]

As *Chlamydia trachomatis* is generally asymptomatic it is difficult to diagnose and treat[130]. *Neisseria gonorrhoea* is a major public health issue, the reason being that the pathogen has developed resistance to several antimicrobials currently in use to manage the infection[131]. Ectopic pregnancies and tubal occlusions are two consequences of these infections[132]. As indicated in Figure 2.2, sub-Saharan Africa has the highest infertility-related infection rates, when compared to those of Asia, Latin America and the developed countries in 1987. A review by Agnès *et al*. (2005) revealed that a minimum

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of 30% of pregnancies in Africa are not planned of which 12% are terminated. This highlights the important role of clinical care to prevent post-abortal infections in women in developing countries[133]

The highest incidence of HIV in the world is found in Africa[134]. A review by Baral *et al.* (2012) estimated that 22.9 million people are living with HIV/AIDS in sub-Saharan Africa[134]. Infertility and HIV are associated in various ways, with both conditions having a severe psychological and socio-cultural impact, and also the difficulty in treatment options or the absence thereof, especially in developing countries[135]. The retrovirus has been reported to impact human fecundity significantly[136]. A high incidence of menstrual irregularities and persistent amenorrhoea were found in HIV positive females[137]. Added to these conditions, there exists a strong correlation between tubal abnormalities and HIV positive patients[135]. The study disclosed that 52% of HIV positive patients were diagnosed with tubal irregularities compared to 26% of HIV negative patients[135]. In infected male patients, development of hypogonadism (decrease in testosterone levels) and a decline in semen quality, secondary to the development of immunodeficiency, were observed[138]. Semen abnormalities such as oligozoospermia or azoospermia, morphologically abnormal sperm and leukocytospermia were found to be more prevalent in HIV-infected males[138].

2.3.3 Behavioural and lifestyle factors

Human reproduction is influenced by behavioural and lifestyle factors. A decrease in fertility due to **obesity** is reported by Kathryn *et al*. (2014)[139]. The impact of **alcohol, caffeine** and **smoking** on reproductive performance is discussed in a review by Homan *et al*. (2007)[140]. The negative influence of **illicit drugs** on human reproductive ability was highlighted in a review by Fronczak *et al*. (2012)[141]. In addition to these lifestyle factors, various chemicals that are known to interfere with the endocrine system are classified as **environmental factors** that induce infertility[142].

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Obesity is defined as having a body mass index (BMI) equal or greater than 30 kg/m^2. In 2000, the condition affected approximately 5% of the population in developing countries and around 30% in developed countries[143]. A study performed by Chavarro *et al.* (2010) indicated a decrease in serum testosterone and sex hormone binding globulin levels following an increase in BMI[144]. Koning *et al.* (2010) showed that obesity is the main cause of anovulation in over 65% of patients suffering from polycystic ovarian syndrome[145]. The condition has also been found to increase miscarriage rates in western European societies[145].

Alcohol, caffeine, and nicotine are the most commonly consumed stimulant drugs in the world[146]. In 2004, alcohol was consumed by more than 2 billion people in the world with almost 76 million being diagnosed with alcohol disorders[147]. A study by Parry (2000) concluded that alcohol intake has increased in developing countries[148]. Foetal alcohol syndrome (FAS) that refers to clinical abnormalities found in newborn children is caused by frequent exposure to alcohol during the prenatal period[149]. In 2010 Africa was the continent with the highest alcohol consumption per drinker in the world[150].An elevated rate of FAS (65.2-74.2 per 1,000 children) was reported in South Africa, in 2005[151]. A review by Mohammadzadeh (2014) reveals that between 70 and 80 per 1000 children in South Africa are born with FAS[152]. Alcohol is associated with a decrease in reproductive health. In females, the increased consumption of alcohol can lead to anovulation, imbalance of hormonal secretion, and in some cases even foetal loss[153]. In men, frequent alcohol consumption can have a negative impact on semen quality. Reduced sperm concentration, motility and abnormal sperm morphology have been observed mostly in men who abuse alcohol[154]. Consumption of **caffeine** and the use of **nicotine** products have increased in developing countries in the last decade[155]. Caffeine which is the most commonly consumed active substance in the world is found in many foods and beverages[156]. A caffeine intake of >400 mg/daily can have adverse effects on a pregnant female and can lead to spontaneous abortion[157]. Even though an intake of more than 50 mg of caffeine a day has been proved to decrease *in vitro* fertilization (IVF) pregnancy rates[158], a moderate consumption of caffeine (<200–300 mg/daily) has no impact on female health during pregnancy[159]. Nicotine is one of the most frequently

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used stimulant substances which are associated with a decrease in fertility capacity[160], and can negatively impact the quality of embryos *in vitro*[161]. An increase, from 4.96 to 6.25 trillion, in cigarette sales worldwide was recorded between 1980 and 2012[162]. The use of illicit drugs such as anabolic steroids, marijuana, cocaine and methamphetamines, can negatively impact human fertility[141]. Fronczak *et al*. (2012) reported a decrease in sperm function and a deterioration on sperm structure associated with the use of these drugs[141]. The consumption of these substances has increased in Africa since 2005 according to the United Nation report in 2012[163] where a population of 28 million people are known as drug users[40]. The high prevalence of the use of these drugs can be explained by the almost inexistent border control observed in most of sub-Saharan Africa countries[40].

Toxicants such as arsenic, lead, solvents, pesticides, as well as phthalates are **environmental factors** impacting on human health[142]. These chemicals can be present in plastic products such as solvents, cosmetics products (lotions, perfume and nails polish), vinyl gloves, children's toys and household items (such as shower curtains, upholstery and table cloths)[164]. An assessment of state of science regarding endocrine disruptors published by a group of experts for the United Nations Environment Programme and World Health Organization (2012), indicated that new sources of exposure to endocrine disrupting chemicals (EDC) are increasingly found in food products, interior decor, electronic salvaging and dumpsites[99]. Exposure to these chemicals is reported to have a negative impact on fertility especially in the developing world[115]. A study by Aneck-Hahn *et al*. (2007) disclosed a decrease in semen parameters of young South African men due to exposure to EDC[165]. The effect of toxicants on fertility is estimated to be higher in industrialized countries. However, two studies performed in Sweden and England disclosed that increased industrial activities do not impact on human fertility[166]. Poor semen quality, low sperm count and abnormal morphology, due to exposure to toxicants were reported by Sharpe *et al.* (2010)[167]. A review by Wong *et al.* (2011) reveals an induction of oxidative stress in the testes because of the effect of environmental chemicals[142]. Lead, a well-known toxicant that affects female reproduction negatively. A decrease in female fertility due to lead

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exposure is reported by Chang *et al*. (2006). The study revealed a decrease in fertility ability associated with a low blood concentration of lead in female[168]. Jobling *et al.* (2013) summarized various epidemiological studies on the impact of endocrine disruptors on puberty in humans[169]. Phthalates are used as plasticizers and it is estimated that 18 billion pounds per annum of these compounds are used[170]. A study by Gupta *et al*. (2010) reported a decrease in fertility due to exposure to these compounds[171]. Caserta *et al*. (2011) confirmed the adverse effect of phthalates on reproductive health[172].

2.4 Sperm preparation methods

Different sperm preparation techniques have been developed based on the considerable increased need for ART treatment over the last twenty years[173]. A cardinal contributing factor required for successful ART treatment is the preparation of morphologically normal, motile sperm[174]. Human semen is a fluid containing spermatozoa, seminal plasma, white blood cells, reactive oxygen species (generated from damaged spermatozoa and/or presence of leukocytes), biomolecules, seminal bacteria, intra-cellular and non-cultivable micro-organism i.e. *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, as well as seminal viruses[175]. Semen preparation methods for IUI or *in vitro* fertilization are performed in assisted reproduction in order to separate sperm cells (with normal motility and morphology) from the seminal plasma products which can compromise fertilization ability[176]. A survey (between 2007-2010) by Fourie *et al*. (2012) found positive bacterial cultures in 50% of semen samples[177]. Another study by Esfandiari *et al*. (2002) detected positive bacterial culture in 48% of semen samples from infertile men[178]. The elimination of bacteria or the decontamination of semen during sperm preparation is a preventive measure in HIV-serodiscordant couples[179].

A perfect sperm preparation technique should be gentle in order to minimize damage of spermatozoa as well as maximize the recovery of a high number of functional and morphological normalspermatozoa[180]. Although, three processing methods are described by the World Health Organization laboratory manual (2010) for the

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examination and processing of human semen[181], the most commonly used techniques are the swim-up and the density gradient centrifugation (DGC)[182]. In the following section the standard sperm swim-up, the single layer centrifugation (SLC) and the DGC methods will be discussed together with the commercially available SEP-D kit.

2.4.1 Standard swim-up method

The standard sperm swim-up method entails layering liquefied semen under the culture media and allowing the motile spermatozoa to migrate into the culture medium. The harvested motile sperm are subsequently used for insemination (Figure2.3).

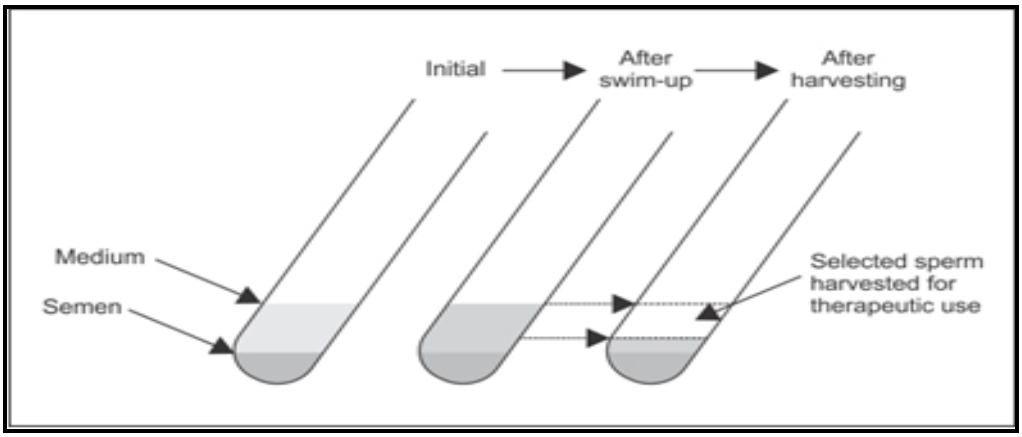

Figure 2.3: Diagrammatic illustration of the standard swim-up method of sperm preparation (Reproductive Biology photo imaging library)

Although this technique yields a lower number of spermatozoa, it selects sperm according to their motility[181]. This method has also been reported to yield spermatozoa with low percentage of apoptosis and good deoxyribonucleic acid (DNA) integrity[175,183]. Although the standard swim-up technique is suitable to process semen samples of good quality, the method can probably be used for oligoasthenozoospermic samples, when adequate motile sperm have been observed during the diagnostic phase. However in case of severe oligozoospermic, centrifugation methods are recommended[184,185]. This method can be used for the preparation of samples for IVF depending on semen quality[186].

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2.4.2 SEP-D kit

The SEP-D kit (SureLife Media Technologies, Singapore, cat no: SL 001) is used for the preparation of sperm prior to IUI at a current price tag of R 347.47(€ 24.06)[C] per device (including the catheter). The SEP-D is commercially available in a set of five syringes filled with a Hepes buffered medium, which can be used for the preparation of five individual semen samples. Semen is aspirated into the syringe and incubated for 30 minutes to allow the progressively motile sperm to migrate into the medium (Figure 2.4).

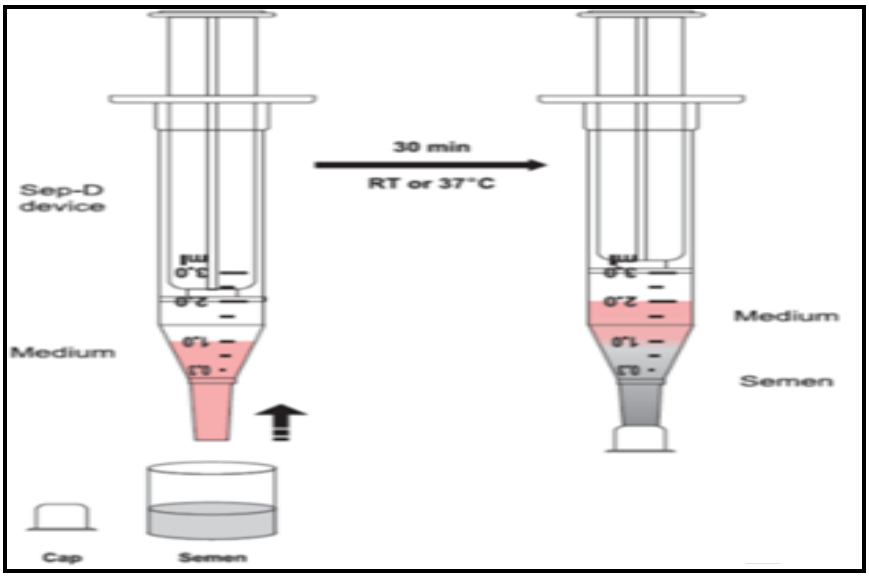

Figure 2.4: Sketch demonstrating the SEP-D kit method for sperm preparation (Reproductive Biology photo imaging library)

Subsequently the semen is expelled, leaving a fraction of highly motile sperm that can be inseminated into the uterus by attaching an insemination catheter to the device. The technique has been reported to be easier and simpler when compared to the standard swim-up and density gradient methods[187]. Gentis *et al*. (2012) reported a significant increase in IUI pregnancy rates when spermatozoa were processed using the SEP-D kit, rather than the standard swim-up method. However, no significant differences were found with regard to sperm motility and morphology[187].

[C]All costs were obtained in ZAR, with an exchange rate of € 1 to ZAR 14, 44 (30/06/2014). All fiscals include 14% Value Added Tax (VAT).

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2.4.3Single layer centrifugation (SLC)

Single layer centrifugation (SLC) is a technique used to improve sperm quality prior to IVF and IUI. The procedure for SLC is similar to density gradient centrifugation (DGC), except for the use of a single gradient of relative high density. Liquefied semen is layered on top of the gradient and then centrifuged. This method selects spermatozoa that have normal morphology and DNA integrity[188]. Compared to DGC, SLC is cost-effective and less time consuming. SLC is also more adaptable than DGC, since the technique can be maximized to a larger size of sample[189]. A study has demonstrated that SLC could have better sperm recovery level than the sperm swim-up method[190]. The SLC has shown to be effective in the removal of seminal plasma proteins and also cholesterol from the surface of sperm cells[191].

2.4.4 Density gradient centrifugation (DGC)

Density gradient centrifugation is a procedure which separates spermatozoa according to their density or specific gravity, mass per unit volume[192]. This technique consists of centrifuging the seminal plasma over density gradients (see Figure 2.5). Discontinuous gradient centrifugation that is used to prepare sample for IVF and ICSI, is the method of choice for teratozoospermic samples as well as for cases of unknown infertility[193]. Compared to the standard swim-up method, DGC is easier to standardize and yields more consistent results[192]. This method enables the selection of motile sperm cells with a good morphology and high DNA integrity when compared to single layer centrifugation[194,195].

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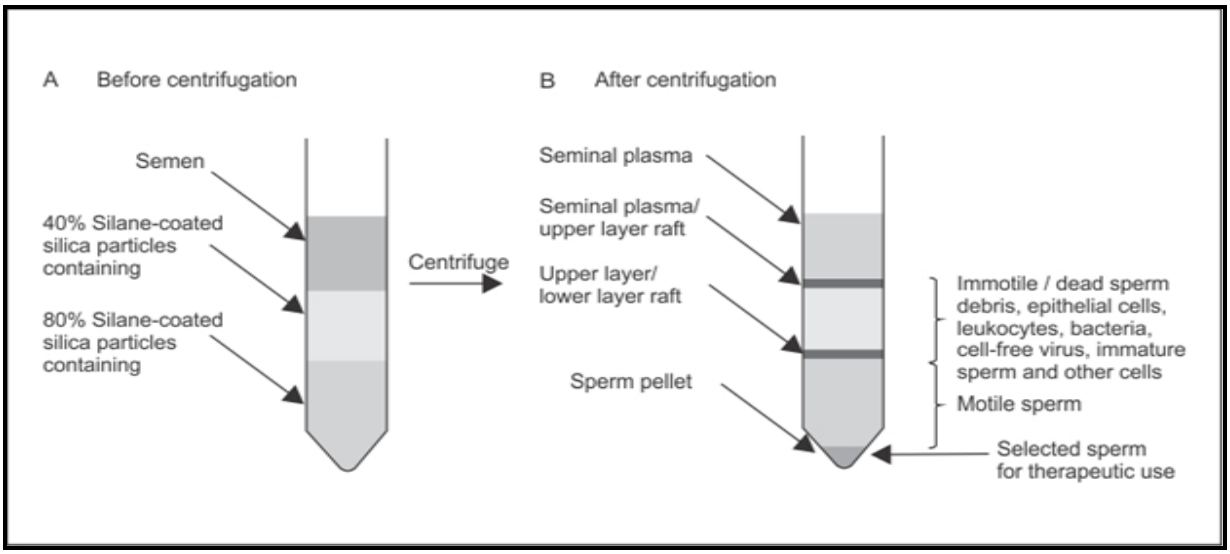

Figure 2.5: Density gradient centrifugation: illustration of the layers before and after centrifugation (Reproductive Biology photo imaging library)

This DGC technique which has been reported to be more efficient for the removal of antisperm antibodies in the semen[199], also decreases the levels of reactive oxygen species[196]. Brahem *et al*. (2011) and Xue *et al*. (2014) indicated a decrease in the percentage of fragmented DNA following DGC[197,198].

2.4 Assisted Reproductive Technology (ART) treatments

2.5.1 Intrauterine insemination (IUI)

The procedure is considered to be a first-line treatment option[199] that is suitable for use in cases of unexplained infertility[200], couples presenting with no female factors, mild male factor subfertility[201], cervical hostility, as well as minimal or mild endometriosis[202-204]. This method is less invasive and more cost-effective than either IVF or ICSI. The pregnancy rate using IUI varies according to the patient profile, different fertility factors, the stimulation procedure used and the total number of cycles completed and the method of sperm preparation[205].

For IUI, a minimum threshold of 1 million motile spermatozoa is needed for successful conception, irrespective of the type of sperm preparation method used[206]. Two sperm factors, i.e. the post-processed insemination motile count (IMC) and sperm morphology

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are reported to be the best criteria to determine the outcome of IUI[207]. Intrauterine insemination is generally performed with controlled ovarian hyperstimulation (OH). An increase in pregnancy rate has been observed when IUI is performed in conjunction with OH than without OH[208]. Intrauterine insemination (IUI) is an ART technique which involves introducing washed sperm inside the uterine cavity by means of a catheter (Figure 2.6)[209].

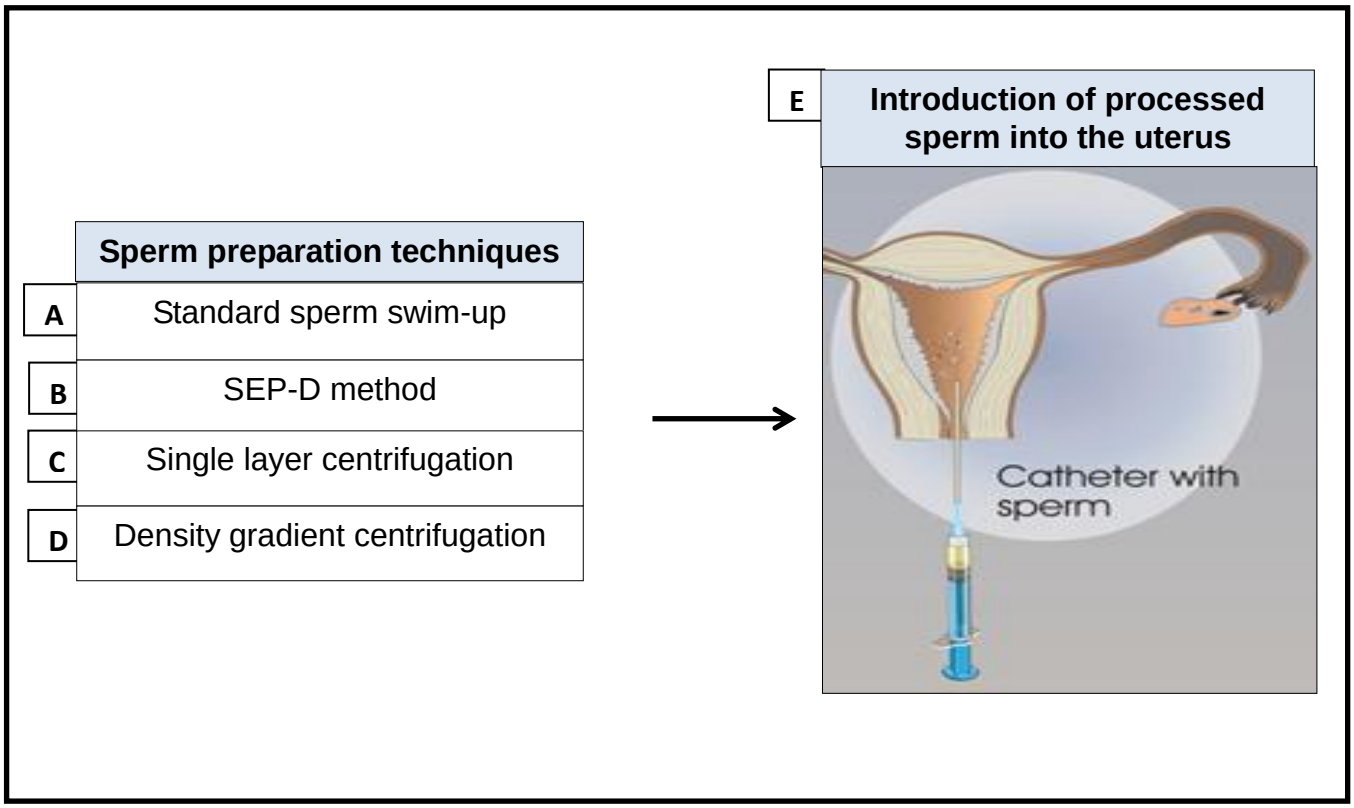

Figure 2.6: Diagrammatic illustration of the IUI procedure with various sperm preparation techniques using A: standard swim-up method; B: SEP-D kit; C: single layer centrifugation; D: density gradient centrifugation; E: processed sperm is introduced into the uterus via a catheter (Adapted from the Reproductive Biology photo imaging library)

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2.5.3 *In vitro* fertilization (IVF)

In vitro fertilization is an infertility treatment in which female and male gametes are combined in a culture dish prior to fertilization (Figure 2.7). Spermatozoa are prepared using the sperm preparation methods described in Section 2.2.1 (Figure 2.7. A) and oocytes are obtained by trans-vaginal oocyte aspiration (Figure 10.B). After fertilization (Figure 2.7.E), the development of each embryo is observed (from 2 cells to blastocyst), as illustrated in Figure 2.7.F.

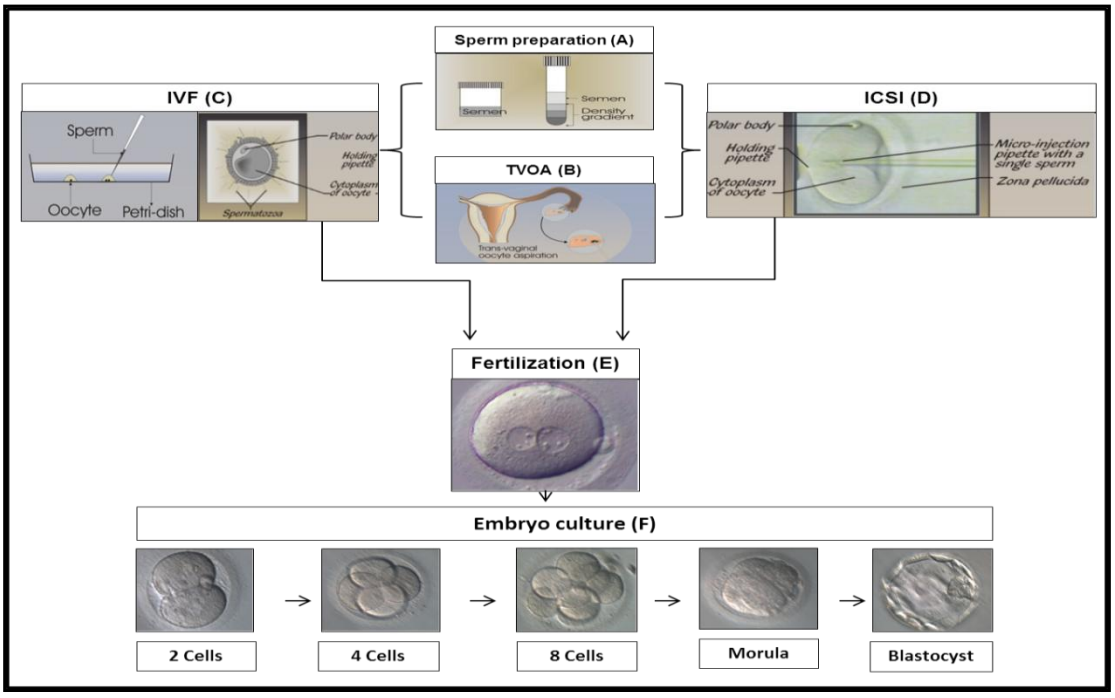

Figure 2.7: Graphical representation of A: sperm preparation; B: TVOA; C: *in vitro* fertilization (IVF); D: intra-cytoplasmic sperm injection (ICSI); E: fertilization and F: embryo development. *TVOA: trans-vaginal oocyte aspiration (Adapted from the Reproductive Biology photo imaging library)

According to a study by Malizia *et al*. (2009) the success rate of IVF after six controlled ovarian stimulation cycles is estimated to be 72%[210]. Irrespective of the origin of the infertility, IVF is the ART treatment with the highest pregnancy rate per cycle[211]. The IVF procedure was initially performed on females diagnosed with tubal occlusion or absence of Fallopian tubes, and then has become the treatment of choice for severe endometriosis and unexplained infertility after IUI[212].

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2.5.4 Intra-cytoplasmic sperm injection (ICSI)

Intra-cytoplasmic sperm injection (ICSI) is an ART treatment where a single sperm is injected into the oocyte to support fertilization (refer to Figure 2.7). The ICSI procedure that was introduced in 1992 was originally for severe male infertility[213]. Currently ICSI is used in cases of severe male factor infertility as well as in fertilization failure during previous IVF cycles[209]. This procedure has made possible a more consistent fertilization of cryopreserved oocytes[214], and is also considered to be the method of choice during pre-implantation genetics diagnosis[215]. The ICSI technique requires more expensive equipment than IUI and IVF, consequently the cost of ICSI is higher than IUI and IVF. In 2012, the average cost for ICSI treatment in South Africa ranged between € 3,302 ±€ 625[D](R 47,680) compared to IVF (€ 3,255 ±€ 576; R 47,002) and IUI (€ 542 ±€ 159; R 7,826)[216].

2.6 Access to infertility treatment in Africa

Infertility has been acknowledged as a public health concern by the WHO. Although reproductive health education and prevention of infertility are priorities, the need for accessible diagnostic procedures and reproductive technology is crucial. After a period of more than 30 years of IVF, only a small part of the world population has benefited from the new technologies[216]. In 2001, during a WHO international meeting in Geneva (Switzerland), Dr Mahmoud Fathalla, chairperson of the WHO Advisory Committee on Health Research, focused his introductory speech on how to increase access to treatment for infertility[217]. In 2006 the International Committee of Monitoring of ART (ICMART), reported very low levels of infertility treatments in developing countries compared to those in developed countries[218].

The need for infertility diagnosis and treatment is more serious in developing countries than in developed countries. Infertile couples (56,1%) in developing countries and 51,2% in developed countries are seeking infertility treatment[219]. Access to treatment in

[D] All costs were obtained in ZAR, with an exchange rate of € 1 to ZAR 14, 44 (30/06/2014). All fiscals include 14% Value Added Tax (VAT).

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Africa presents a challenge since health care facilities are limited. Few public hospitals in Africa offer infertility treatments. In those countries where infertility management is accessible in the public sector, the quality of the treatments is generally poor or of low quality. Alternatively, females are obliged to access private health care[64]. In countries such as Ghana and Nigeria, where private health care is available at a high cost, ART treatment is almost not accessible to the general population[220]. Infertility treatment is probably the most neglected and underestimated health care facet in developing countries[65]. In 2007, there were 8 medical schools in South Africa with only 3 providing infertility treatment[220]. A considerable number of countries in Africa have no access to infertility treatment. In the central African region (Gabon, Congo, Cameroon, Guinea, and the Central African Republic) there are no public hospitals providing infertility treatment, only 2 private clinics in Cameroon providing ART (see **Addendum B1**). This region is known to have a higher incidence of secondary infertility[221].Limited access to infertility treatment in these countries is attributed to inadequate equipment and a lack of qualified personal. A concerted effort needs to be made to improve the existing infrastructure and to train medical staff in the necessary procedures.

Advances in infertility care are reported in some sections of Africa. In the eastern and western regions, numerous infertility clinics are in existence. In the eastern African countries, Tanzania has the highest number of infertility clinics followed by Uganda. **(Addendum B1)**. In western Africa, Nigeria is the most advanced country having more than 10 clinics/hospitals specializing in infertility care **(Addendum B2).** In 2005, infertility treatment was available at 14 teaching hospitals, 22 general hospitals and numerous private clinics in Nigeria[222]. In North Africa, Egypt is the country with the highest number of IVF centres (see **Addendum B3**). In 2003, 40 IVF units existed in Egypt with a single public IVF centre at the University of Alexandria[115]. Studies in Nigeria and Egypt demonstrate that innovative ART treatments can be implemented with the medical skills and knowledge currently available in African countries[40]. With more than 30 IVF practices, South Africa reflects a high number of infertility treatment centres in Africa (**Addendum B4),** with most of them located in the private sector[223]. The first fertility clinics in South Africa were established in 1982, in Pretoria and Cape-

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town (Tygerberg Hospital) respectively[224], followed by the Fertility and Endocrine Unit at Steve Biko and the Groote Schuur Hospital Fertility Clinic. This country published the first results obtained from the South African Register of Assisted Reproductive Techniques (SARA), between 2009 and 2012 and involving approximately 70% of ART units in South Africa. The report indicated a total of 4,512 aspirations and 3,872 embryo transfers, resulting in 1,303 clinical pregnancies following IVF and ICSI[225].

2.7 Conclusion

Africa is the continent where high infertility rates are prevalent with secondary infertility a leading cause of childlessness[40]. There is however considerable disparity in access to infertility treatment between developed and developing countries. The relation between the cost of ART and the access to treatments should be emphasized[226]. Quality ART care in government facilities often is almost non-existent with treatment costs in private hospitals to be covered by patients. Sallam (2008), highlighted the need to establish three levels of ART units in developing countries, with level 1 offering basic infertility treatments, level 2 providing advanced ART treatments and level 3 as a tertiary infertility unit[227]. The success and sustainability of infertility treatments in resource-poor settings to a large extent depends on the ability to optimize these techniques in terms of availability, affordability and efficacy[228].

Implementing low cost assisted reproduction is possible if methods of treatment are simplified in such a way that they remain effective, safe and affordable. Intrauterine insemination is the most affordable treatment compared to IVF and ICSI. Gabon is one of the sub-Saharan African countries experiencing a high infertility rate, without any infrastructure offering infertility management. This central African country, which is politically stable, is extending its public hospitals network considerably and can be viewed as a positive indicator with regards to the health sector[229]. The Gabonese government expenditure on the health system increased from 4% in 2003 to 7% in 2007[230]. Gabon, and more precisely Libreville, could be seen as a favourable location to initiate an affordable IUI programme as part of an accessible reproductive health initiative.

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CHAPTER 3: MATERIALS AND METHODS

3.1 Background

The student was pre-trained for twelve months at the Reproductive Biology Laboratory, Steve Biko Academic Hospital in basic concepts of assisted reproductive technology, while registered at the Health Professions Council of South Africa as a student medical scientist (no: MSS 0002615). The training consisted of observing spermatology procedures followed by practical laboratory tests under supervision (i.e. macroscopic assessments: seminal pH, viscosity, volume and cellular content; microscopic evaluations including sperm motility, concentration, morphology, DNA fragmentation, flow cytometry, computer-aided sperm analysis (CASA), supravital tests, as well as interpretations of microbiology evaluations). Basic embryology procedures (including intrauterine insemination, *in vitro* fertilization and intra-cytoplasmic sperm injection) were also observed together with laboratory management practices such as quality control, equipment maintenance and procurement of assisted reproduction disposables.

3.2 Sampling and semen collection

Semen samples were obtained from non-smoking medical students at the University of Pretoria and from patients participating in the Assisted Reproduction Programme at Steve Biko Academic Hospital. The donors were all in good health and their semen profiles adhered to the minimum requirements stipulated by the World Health Organization (WHO) guidelines (2010)[181]. Experiment (i) required 25 semen samples, and each experiment (ii) and (iii) included 20 semen samples.

Each semen sample had a minimum volume of 1.5 ml, a minimum concentration of $15x10^6$ sperm/ml, with a sperm morphology of more than, or equal to, 4% with progressive motility of 40% or more. The semen samples were collected according to the standard operating procedure (SOP) employed by the Reproductive Biology

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Laboratory, no: F1.18.1. After liquefaction at 37ºC for 30 minutes, semen parameters were assessed according to the WHO (2010) criteria[181].

The study was conducted in line with the Declaration of Helsinki for medical research and institutional approval was granted (Faculty of Health Sciences Research Ethics Committee, University of Pretoria, no: 54/2014) for the study.

3.3 Experimental set-up

3.3.1 Experiment (i): Development of a simplified sperm swim-up method to obtain motile sperm with minimum equipment

The main objective of this experiment was to evaluate sperm motility parameters after semen has been processed using three different volume disposable syringes: 5 ml (cat no: 161031) and 20 ml (cat no: 161071) from Promex™ (Sekunjalo Healthcare, Sandton, South Africa, www.mbendi.com) and 10 ml (cat no: 9999810101) from Kendall monoject™ (Massachusetts, United States, www.vitalitymedical.com) (Figure 3.1). The syringes that are sterile, non-pyrogenic and latex-free are made of polypropylene plastic (www.vitalitymedical.com). A 24 hour sperm survival test (SOP no: F1.13.1 FC, Reproductive Biology Laboratory) was performed using the syringes to test the safety and toxicity of the plastic syringes. A result of > 80% for all syringes validated the use of the syringes.

Semen samples were divided into three aliquots and each sample was prepared according to the direct swim-up method described by the WHO, 2010[185]. A volume of 1 ml PureSperm Wash® (Nidacon International, Mölndal, Sweden, www.nidaconinternational.com. cat no: PSW 100) was aspirated into the syringe, followed by equal volume of the semen sample, thereby creating a double layer. The syringes were placed at a 45º angle to increase the surface area between the medium and the semen, subsequently improving the ability of the sperm to migrate into the medium, before being incubated for 60 minutes at 37ºC (see Figure 3.2 for the layout of the experiment). After incubation, the seminal fluid was expelled drop by drop and the

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remaining medium (0.3 ml) was used to determine the post-processed sperm parameters (concentration and motility).

Figure 3.1: Disposable Promex™ (5 and 20 ml) and from Kendall monoject™ (10 ml) syringes used for the current study

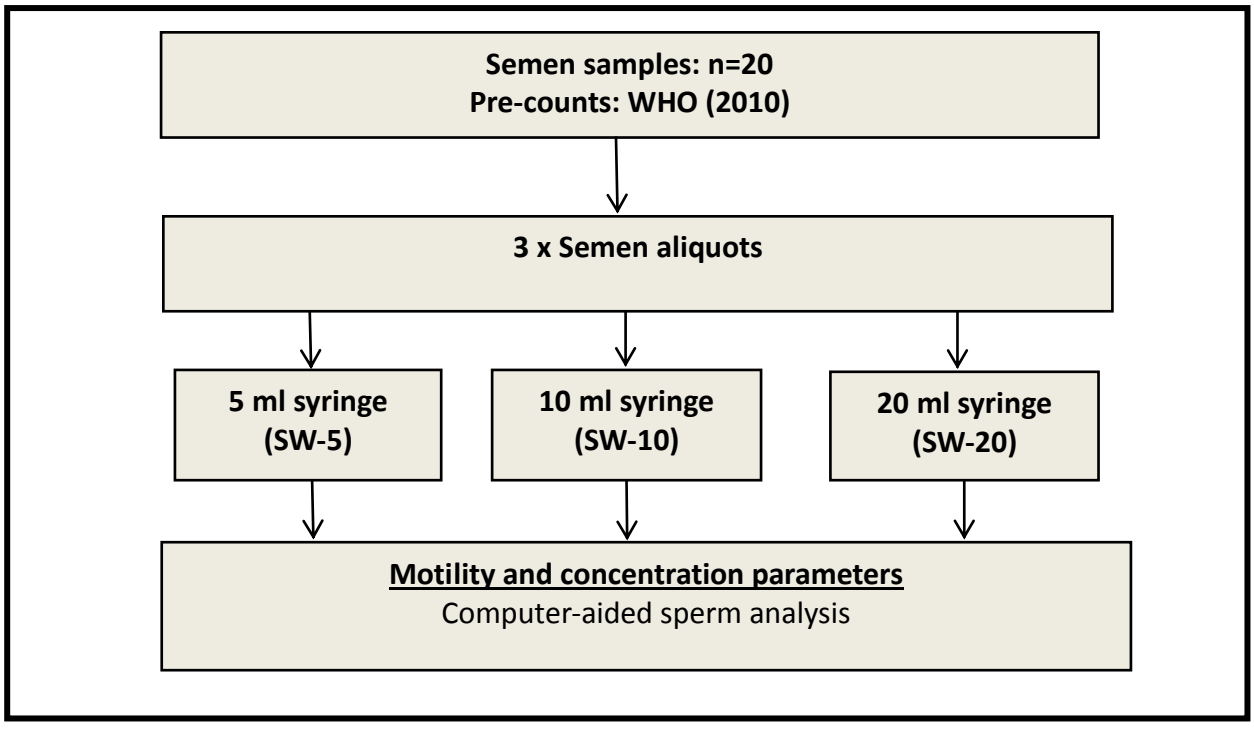

Figure 3.2: Diagram of the simple sperm swim-up experimentation method using syringes of different volumes

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3.3.2 Experiment (ii): Comparison between the simplified sperm swim-up (SW-10) method and the commercially available SEP-D kit

Semen samples were divided into two equal aliquots and processed using either the simple sperm swim-up (SW) method as performed in experiment (i)[181] or the SEP-D kit, following the manufacturer's guidelines. The syringe volume that yielded the highest total motile count (from experiment (i)) was used for the second experiment and compared to the SEP-D kit. The SEP-D kit (SureLife, Franklin, Singapore, www.surelifeivf.com. cat no: SEP-D 05) that is commercially available, consists of a syringe pre-filled with this medium (Figure 3.3). The SEP-D medium contains an HEPES buffer, glucose, sodium bicarbonate, ethylenediaminetetra-acetic acid, glycine, gentamicin, human serum albumin, and phenol red (Product specifications: www.surelifeivf.com).

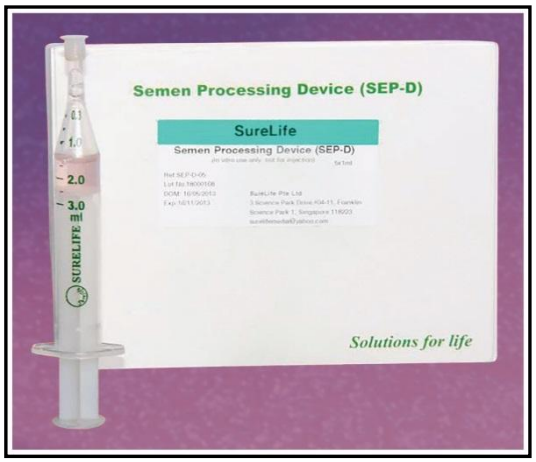

Figure 3.3: SEP-D kit used for semen processing in the current study

A volume of 1 ml liquefied semen was aspirated slowly, without the formation of air bubbles, into the SEP-D syringe and incubated at a 45º angle, at 37ºC for 60 minutes. After incubation the cap of the syringe was removed and the semen was gently expelled followed by the culture medium until only 0.3 ml of culture medium remained. Sperm samples recovered by these preparation techniques were assessed for motility, concentration, vitality, morphology and deoxyribonucleic acid (DNA) fragmentation. Experiment (ii) is illustrated in Figure 3.4. Solutions used for the experiment are listed in **Addendum C**.

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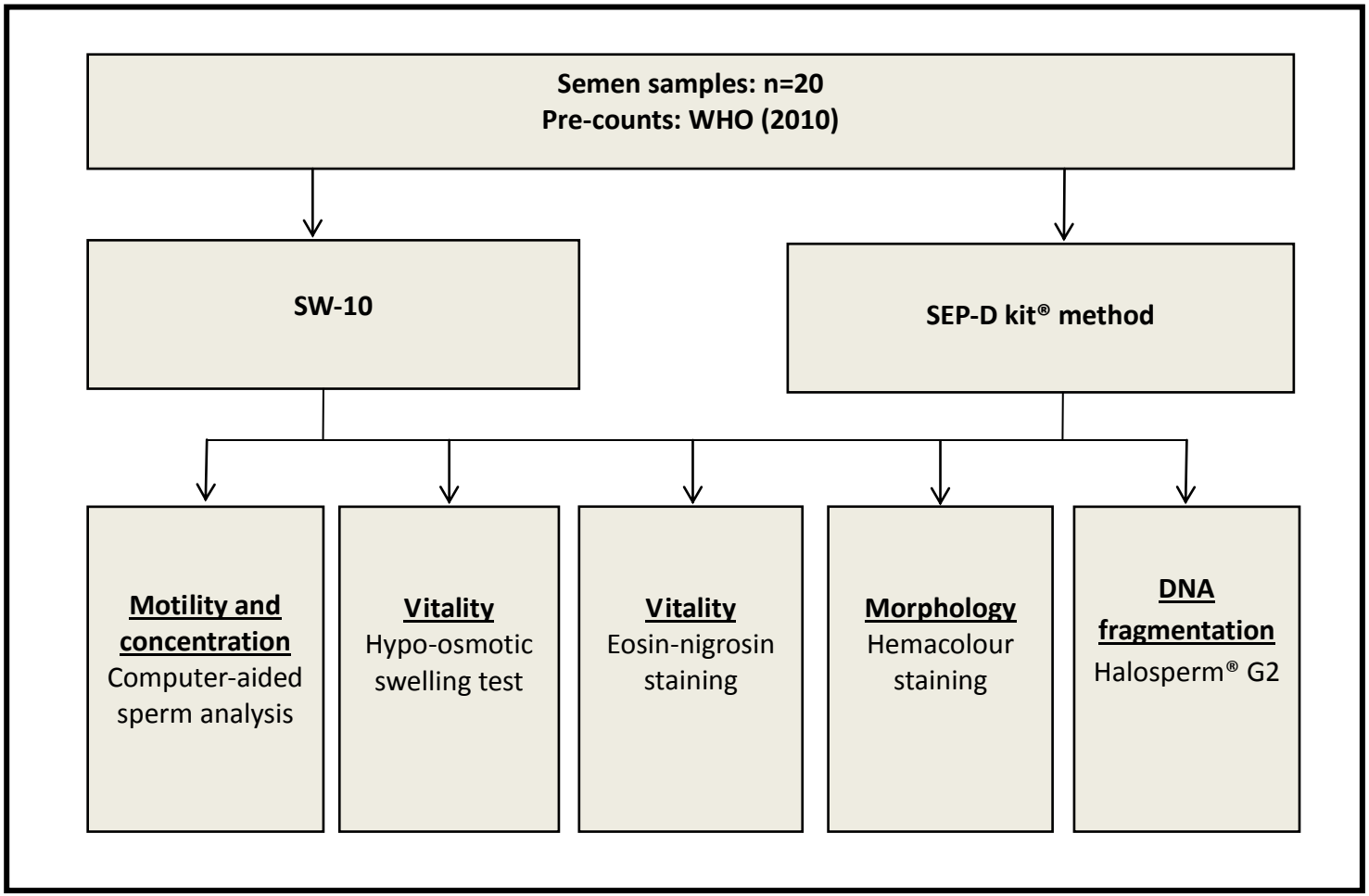

Figure 3.4: Flow diagram illustrating the comparison between the simplified sperm swim-up (SW-10) method and the SEP-D kit

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3.3.3 Experiment (iii): Comparison between the single layer and density gradient centrifugation, based on post-processed sperm parameters

Semen samples were split into two equal aliquots and processed by means of single layer centrifugation (SLC) or density gradient centrifugation (DGC) using 80% PureSperm® (Nidacon, cat no: PS80-100) and 40%PureSperm® (Nidacon, cat no: PS40-100), according to the manufacturer's guidelines. The SLC was prepared by layering 1 ml of the liquefied semen sample on top of 2 ml of 80% PureSperm®. The DGC was prepared by layering 2 ml of 40% PureSperm® over 2 ml of 80% PureSperm®, and with 1 ml of the liquefied semen layered on top of the gradients. The preparations were centrifuged (5810R; Eppendorf®, Hamburg, Germany) for 20 minutes at 300 g. After the supernatant had been removed, the pellet was re-suspended in 5 ml PureSperm® Wash. Subsequently, the solutions were centrifuged for 10 minutes at 500 g, the supernatant was discarded and 300 µl of the sperm pellet was used to evaluate motility and concentration (see Figure 3.5).

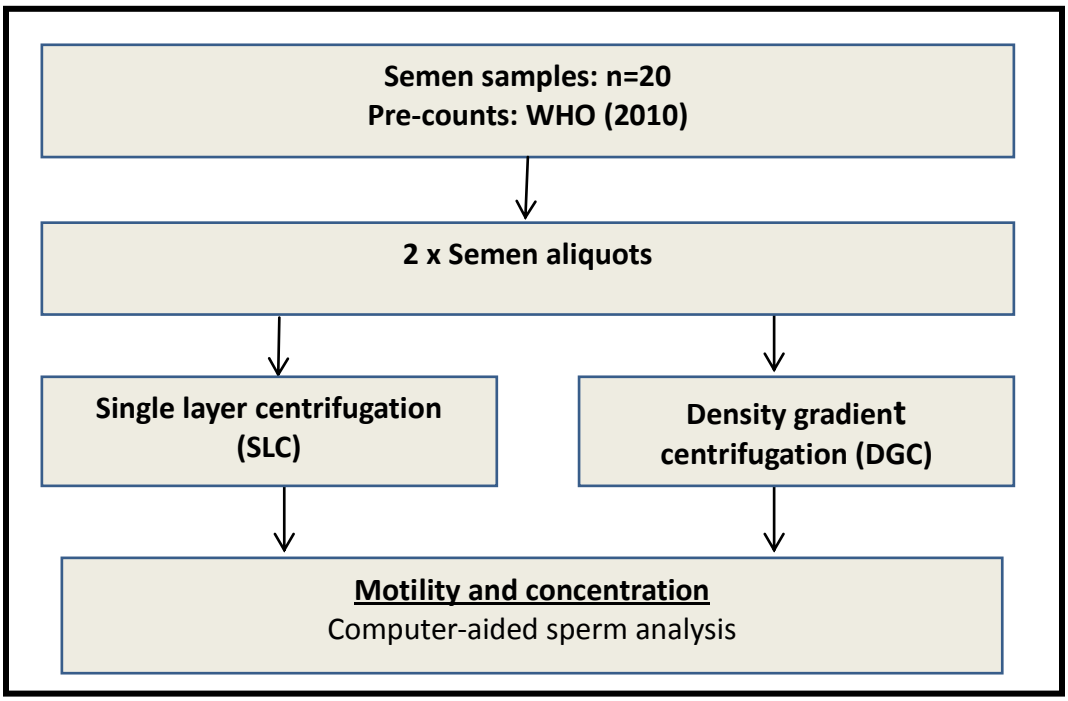

Figure 3.5: Flow diagram illustrating the comparison between single layer centrifugation and the density gradient centrifugation

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3.4 Sperm analyses

For all analyses, all samples were counted single blindly by a medical scientist (investigator) at the Reproductive Biology Laboratory, Steve Biko Academic Hospital and compared to the student readings for quality control. If too large difference (5% or more) was observed, a re-evaluation would be required. A second evaluation was only required for vitality tests (HOS test and eosin-nigrosin).

3.4.1 Motility (Total motile count): Experiment (i), (ii) and (iii)

Spermatozoa recovered from the preparations described in experiment (i), (ii) and (iii) were microscopically examined using a computer-aided sperm analysis (CASA) (medeaLab CASA; MTG-GmbH, Altdorf, Germany) at 200 times (200x) magnification (Axioscope 40; Carl Zeiss, Göttingen, Germany) (Figure 3.6) according to the SOP no: A1.22.1.1 (CASA system usage, Reproductive Biology Laboratory, Steve Biko Academic Hospital). Figure 3.6 is a representation of the MTG-GmbH computer-aided sperm analysis (version 5.4) screenshot. Sperm cell movement was tracked and the motility was evaluated during a live video recording. A total of 10 fields or 200 sperm cells were assessed per chamber (WHO, 2010)[181].

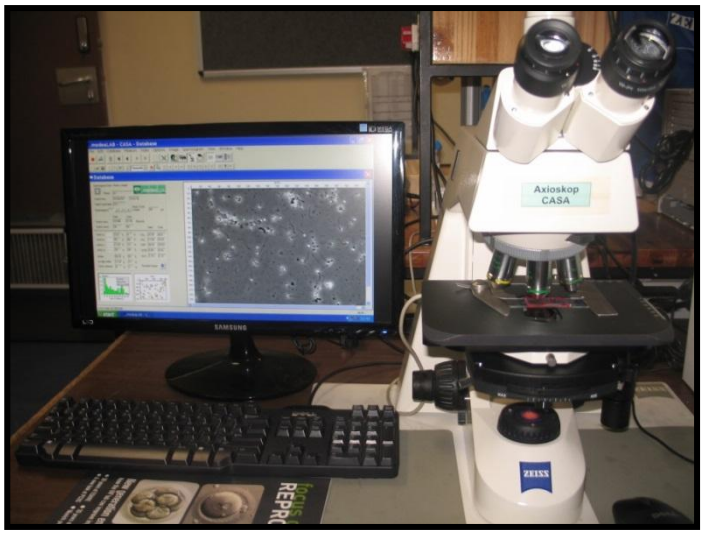

Figure 3.6: The MTG-GmbH computer-aided sperm analysis system at Reproductive Biology Laboratory, Steve Biko Academic Hospital

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The CASA system is a precise, standardized system used for sperm analyses (concentration and motility)[231]. This system classifies the spermatozoa into four motility groups (a, b, c and d) and five velocity parameters[181] (Figure 3.7). The motility parameters are:

i) a: rapid progressive
ii) b: slow progressive
iii) c: non progressive
iv) d: immotile

The velocity parameters determined by the CASA system are classified as:

i) VCL: Curvilinear path velocity
ii) VSL: Straight-line path velocity
iii) VAP: Average path velocity
iv) WOB: Wobble
v) ALH: Amplitude lateral head displacement

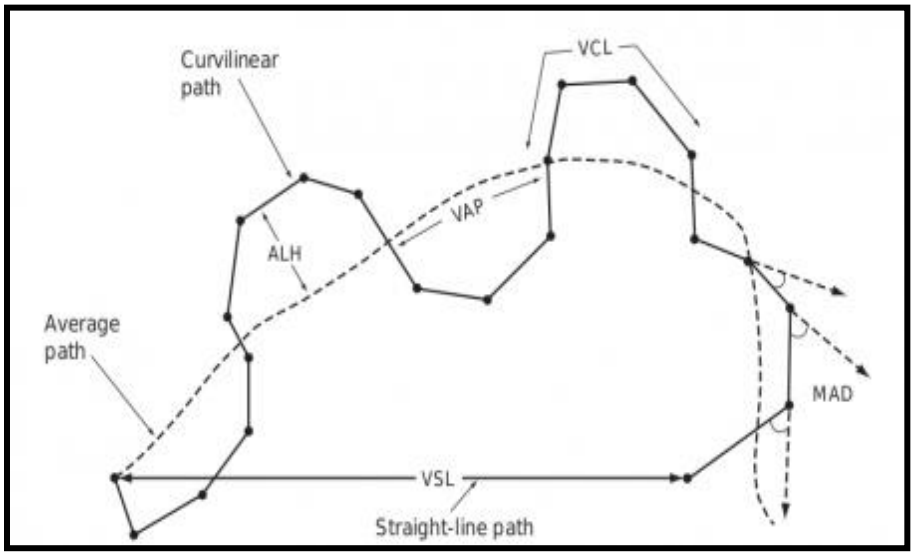

Figure 3.7: Sperm velocity parameters measured using the CASA system[181]

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The post-processed sperm concentration and motility (obtained via the CASA system) were assessed in line with the World Health Organization guidelines, 2010[181]. Post-washed total motile count (TMC) was calculated following a study by Ok *et al.* (2013), who defined TMC as "the product of the concentration of sperm (total sperm count) by the rapid progressive motility (percent motile sperm) by the volume (inseminating) after processing"[232].

TMC (After washing) = Total sperm count x rapid progressive motile sperm x volume

The CASA analyses were performed using Leja® counting chambers (Two chambers slides, 20 µm deep; Leja®, Nieuw-Vennep, Netherlands). Slides were initially warmed to 37ºC and a volume of 5 µl of the semen sample was loaded into each micro-chamber[181]. For quality control purposes, two separate chambers were loaded with the same sample and video recordings of at least 10 random representative fields per chamber were recorded for 30 seconds. Internal quality control was performed by Prof DR Franken (University of the Free State, WHO Semenology expert) prior to experimentation.

3.4.2 Sperm vitality: Experiment (ii)

3.4.2.1 Eosin-nigrosin

An evaluation of membrane integrity was done to determine the percentage of live spermatozoa. The one-step eosin-nigrosin vitalscreen kit (FertiPro, FP12VI02, Beernem, Belgium, www.fertipro.com) was used adhering to the manufacturer's stipulations and as discussed in the SOP (Supravital stain, F1.13.1 FC, Reproductive Biology Laboratory, Steve Biko Academic Hospital).

In this technique dead sperm cells absorb the eosin dye, while intact cells prevent the dye from entering the cytoplasm. The nigrosin stain creates a dark background against which dead cells and intact cells can be clearly identified[233]. The eosin-nigrosin kit

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contains two solutions: 0.67% eosin Y (red solution) and 10% nigrosin (black solution). A sperm vitality assessment was done 30 minutes after semen collection and consisted of leaving two drops of eosin Y solution in 50 µL of semen for 30 seconds, followed by 3 drops of nigrosin for 30 seconds. One drop of the semen mixture was transferred to a microscope slide and a smear was made. A minimum of 200 spermatozoa were evaluated under a 100 times (100x) oil immersion bright field microscope (Axiostar **plus;** Carl Zeiss, Göttingen, Germany**).** Unstained (live) sperm will be white whereas stained (dead sperm) will appear to be pink or red (Figure 3.8)[233].

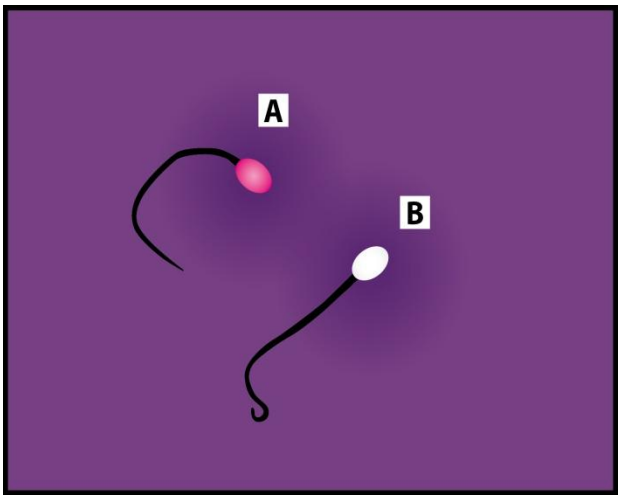

Figure 3.8: Schematic illustration of eosin-nigrosin staining: A: dead spermatozoa (stained pink or red), B: live spermatozoa (unstained) (Reproductive Biology photo imaging library)

3.4.2.2 Hypo-osmotic swelling test

The hypo-osmotic swelling (HOS) test was used in conjunction with eosin-nigrosin staining to evaluate sperm vitality. Sperm membrane integrity is an important determinant of good fertilization, since it plays a major role in sperm motility, capacitation, acrosome reaction and binding of spermatozoa to the egg surface[234]. The HOS test was performed according to the SOP F1.5.1, Reproductive Biology Laboratory, Steve Biko Academic Hospital.

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This technique consists of immersing spermatozoa for 5 minutes in a hypo-osmotic medium. Spermatozoa with intact membranes will have curly tails due to a controlled swelling, while spermatozoa with damaged membranes will display an uncontrolled swelling leading to straightening of the tails[235]. The HOS solution was prepared by dissolving 0.735 g of sodium citrate dehydrate and 1.351 g of D-fructose in 100 ml of purified water. The solution was warmed before the semen sample was mixed. A volume of 100 µL of semen was transferred into the swelling solution and incubated at 37ºC for 30 minutes. Then 10 µL of spermatozoa was placed on a clean slide and covered with a 22 mm x 22 mm cover slip. The slide was analyzed using phase-contrast optics microscope (Axioskop 40**;** Carl Zeiss, Göttingen, Germany) at 200 times (200x) or 400 times (400x) magnification. A total of 200 spermatozoa were evaluated. The number of unswollen (dead) and swollen (vital) spermatozoa was determined. A positive control was performed by exposing spermatozoa in a very low temperature for 30 minutes.

3.4.3 DNA fragmentation

The DNA fragmentation was analysed using the Halosperm® G2 assay and by following the manufacturer's guidelines (www.halotechdna.com). The Halosperm® G2 (Halotech DNA SL, Spain) is a kit consisting of an agarose cell support (ACS), super-coated slides (SCS), 10 units of Eppendorf tubes (EPT), a denaturant agent, a lysis solution, a float and two staining solutions (A and B). This method involves the immersion of unfixed spermatozoa in an agarose microgel, followed by DNA denaturation in those sperm with fragmented DNA, using an acid solution. Nuclear proteins are then removed by a lysis solution. While nucleoids with large haloes of spreading DNA will be produced in sperm with less DNA denaturation, minimal or no dispersion nucleoids haloes will be observed in sperm with fragmented DNA (Figure 3.9).

The agarose gel was placed in a 90-100ºC water bath for 5 minutes. Subsequently 50 µL of the agarose gel was incubated at 37ºC for 5 minutes using an Eppendorf tube. A volume of 25 µL of semen was added to the incubated 50 µL agarose gel followed by

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the transfer of 8 µL of the mixture onto the Halosperm-coated slide. The slide was then left at 3ºC for 5 minutes to allow the spermatozoa to be fixed into the gel. The slide was treated with the denaturation acid solution (solution A) and incubated for 7 minutes before being drained. Following denaturation, the lysis solution (solution B) was applied to the slide and incubated for 20 minutes. The slide was then washed for 5 minutes using distilled water and dehydrated in 70% and 100% ethanol for 2 minutes. The slide was allowed to dry. The preparation was treated with solution C then solution D each for 7 minutes. Finally the slide was analyzed under a bright field microscope **(**Axioskop 40**;** Carl Zeiss, Göttingen, Germany**)**. A minimum of 300 spermatozoa were evaluated.

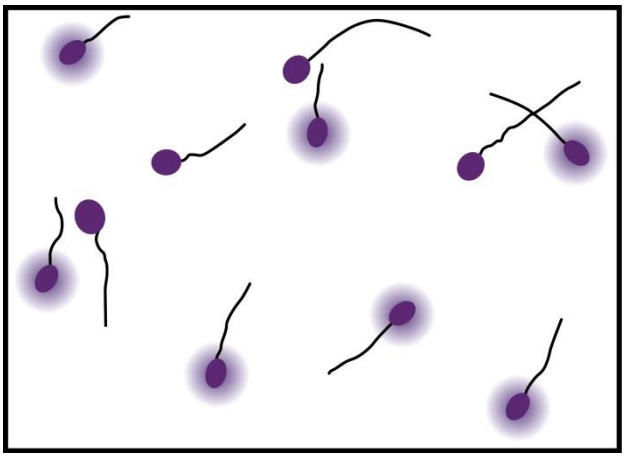

Figure 3.9: Graphical depiction of spermatozoa after processing using Halosperm® G2 stain (Reproductive Biology photo imaging library)

3.4.4 Sperm morphology

Sperm morphology can be a predictor of sperm function[236]. Morphology was assessed using the method described by Björndahl *et al*. (2010)[237]. This technique involved the preparation of semen smears, fixation and staining of the smears before visualization. The preparation of smears consisted of pipetting 10 µL on a slide and performing the feathering or the apposed slide techniques. After this the smears were left to air dry and then fixed. The fixation consisted of immersing the slides in methanol for 15 seconds. Following fixation, the slides were treated in eosin solution for 25 seconds, and then placed in haematoxylin for 20 seconds, which stains the nucleus blue. The slide was

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rinsed in tap water to remove excess haematoxylin. After, the slide was mounted using Entalin before being left overnight to dry. Normal and abnormal spermatozoa were examined under immersion oil, with light microscopy (Axiostar plus, Carl Zeiss, Göttingen, Germany) at 100 times (100x) magnification. Classification of morphological normal and abnormal spermatozoa was evaluated according to the strict (Tygerberg) criteria for evaluation of sperm morphology[238]. A morphologically normal spermatozoon had an oval head and an acrosome covering 40%–70% of the head area. A normal spermatozoon had no neck, midpiece, tail abnormalities or cytoplasmic droplets larger than 50% of the sperm head.

3.5 Evaluation of the feasibility of establishing an IUI programme in Libreville (Gabon)

Gynaecologists (n=20) who are members of the Gabonese Society of Obstetricians Gynaecologists and Reproduction (SGGOR) were invited to participate in the survey during the student's visit to Gabon (July 2013-December 2014). A questionnaire (**Addendum D**), which included 10 multiple-choice questions that were dichotomous and rating-related, was personally handed to gynaecologists. The questionnaire focused on assessing the level of infertility assistance available and the feasibility of establishing an IUI programme in Libreville, as well as designed to obtain patient's related statistics.

While undertaking the survey, the student received enquiries from Gabonese citizens requesting basic information on (in)fertility and contact numbers of assisted reproduction clinics in southern Africa. The student interacted with four Gabonese nationals who shared their personal history of infertility. The student obtained verbal consent from the participants to take notes during the conversations and summarized the content of the exchanges as case studies. The interactions outline the cultural difficulties and beliefs encountered during each individual's journey to conceive and have their own biological children. Permission from the University of Pretoria ethics

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committee was additionally obtained (Ethics no: 54/2014) to incorporate the information into the dissertation.

3.6 Statistical analyses

3.6.1 Experiment (i), (ii) and (iii)

Data were computerized using an Excel spreadsheet and statistical analyses were carried out by Prof P Becker (Biostatistics Unit, Research Office, Faculty of Health Sciences, University of Pretoria). To obtain a 95% power for clinical relevance, 25 samples were selected for experiment (i), and 20 samples for experiments (ii) and (iii). Data-sets were analysed using a Wilcoxon signed ranks tests. Standard deviations, geometric means values and 95% confidence intervals were determined. A random effect together with the generalized least squares was calculated. The significance level was set at $P<0.05$ using Stata Release 11 (www.xlstat.com).

3.6.2 Feasibility of establishing an IUI programme in Libreville (Gabon)

An Excel spreadsheet was compiled with the responses obtained from the questionnaire. All variables were identified and listed, as well as questions were categorized and analysed according to the types of answers. Statistical analyses were conducted on all the questions in which the categories were coded. The Stata Release 13 software program was used for data analyses. Responses to the questionnaire survey were recorded as frequencies expressed as percentages.

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CHAPTER 4: RESULTS

4.1 Introduction

The results obtained from this study are reported in two sections:

The **first section** comprises of three experiments; i.e.

Experiment (i) consisted of developing a **simplified sperm swim-up method (SW)**, by comparing sperm yield (motility and concentration) obtained after processing using 5 ml (SW-5), 10 ml (SW-10) and 20 ml (SW-20) syringes.

Experiment (ii) compared the syringe which yielded the best motility and concentration in experiment (i) to the commercial SEP-D kit in respect of sperm motility, concentration, morphology, plasma membrane integrity and DNA fragmentation.

Experiment (iii) involved a comparison between the single layer and double layer density gradient centrifugation.

The second section of the study contained the results obtained from a questionnaire completed by gynaecologists practising in Gabon. Data are illustrated graphically in tables and figures to highlight important comparisons.

Two addenda are incorporated for additional information:

Semen parameters (volume, pH, concentration, progressive motility and morphology) of donors used in the first section of the study are presented graphically in **Addendum E.**

The mean volume of samples was 2.4 ml, with an average pH of 7.5, a sperm concentration of 41 x10^6/ml, and 8% normal morphology obtained from Experiment (i).

The mean volume of semen samples used in Experiment (ii) was 2.8 ml, with an average pH of 7.55, a sperm concentration of 38 x10^6/ml and a 7% normal morphology.

Semen samples used in Experiment (iii) presented with a mean volume of 2.1 ml, a pH of 7.5, sperm concentration of 44 x10^6/ml and normal sperm morphology of 9%.

Information obtained during interaction with Gabonese nationals who sought assisted reproductive assistance is conveyed in case studies in **Addendum F**.

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4.2 Section 1: Development of a simplified swim-up method

4.2.1 Experiment (i): Total motile count of spermatozoa obtained after processing using 5 ml (SW-5), 10 ml (SW-10) and 20 ml (SW-20) syringes

Table 4.1 indicates the sperm concentration, progressive motility and total motile count after processing with the **5 ml (SW-5), 10 ml (SW-10) and 20 ml (SW-20) syringes**. Table 4.2 reflects the post-processed velocity parameters of spermatozoa using the mentioned syringes. The tables include statistical relevancies with regards to the p-values, confidential limits (CI) and the mean standard deviation values for the comparative study. Figure 4.1 shows graphically the differences in sperm concentration and progressive motility using the 5 ml, 10 ml and 20 ml syringes as represented by the dashed line in Table 4.1.

Spermatozoa processed using the SW-10 yielded significantly ($p<0.05$) higher progressive motility (77.54%; ±9.02), when compared to that yielded by SW-5 (50.62%; ±10.58) and SW-20 (46.52%; ±10.27). The SW-10also yielded a significantly ($p<0.05$) higher sperm concentration (28.47 $x10^6$/ml) than that of SW-5 (15.97 $x10^6$/ml; ±8.04) and SW-20 (19.16 $x10^6$/ml; ±9.12) (Figure 4.1). As a consequence, the total motile count of sperm was found to be significantly higher ($p<0.001$) when using SW-10 (662.27 $x10^6$/ml; ±12.46) (Table 4.1). Despite no significant differences ($p<0.479$) noted in the total motile count of the harvested post-processed sperm samples obtained from the **SW-5 and the SW-20** (242.52 $x10^6$/ml and 267.39 $x10^6$/ml, respectively); statistical differences were observed in progressive motility and concentration ($p<0.015$ - Table 4.1). A lower population of purified sperm was harvested with the SW-5. The harvested sample, however, displayed higher rapid progressive sperm motility (50.62%; ±10.58) and total motility (74.17%; ±12.40), than in corresponding samples obtained from the SW-20 treatment ($p<0.014$ and $p<0.018$, respectively). The latter mentioned syringe also yielded a significantly higher concentration of spermatozoa (19.16 $x10^6$/ml; ±9.12) when compared to that of the 5 ml syringe (15.97 $x10^6$/ml; ±8.04) (Table 4.1).

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Table 4.1: Comparison of sperm samples obtained after swim-up method using the 5, 10, 20 ml syringes

Parameter	5 ml (a) (SW-5)	10 ml (b) (SW-10)	20 ml (c) (SW-20)	p-value (95% CI)		
	Mean (Standard deviation)			(a *vs* b)	(a *vs* c)	(b *vs* c)
Concentration (10^6/ml)	**15.97 (8.04)**	**28.47 (8.83)**	**19.16 (9.12)**	**<0.001** (0.173;0.312)	**0.012** (0.022;0.165)	**<0.001** (0.105;0.157)
Rapid progressive motility (%)	**50.62 (10.58)**	**77.54 (9.02)**	**46.52 (10.27)**	**<0.001** (0.151;0.351)	**0.014** (0.0261;0.043)	**<0.001** (0.243;0.441)
Total motility (%)	**74.17 (12.40)**	**83.12 (10.43)**	**67.03 (12.87)**	**0.014** (0.016;0.143)	**0.018** (-0.146;-0.012)	**<0.001** (0.092;0.131)
TMC (x10^6)	**2.42 (14.05)**	**6.62 (12.46)**	**2.67 (14.88)**	**<0.001** (0.244;0.401)	**0.479** (-0.049;0.106)	**<0.001** (0.115;0.171)

Total motile count after sperm preparation (TMC) = concentration (x10^6/ml) x rapid progressive motility x insemination volume (0.3 ml)

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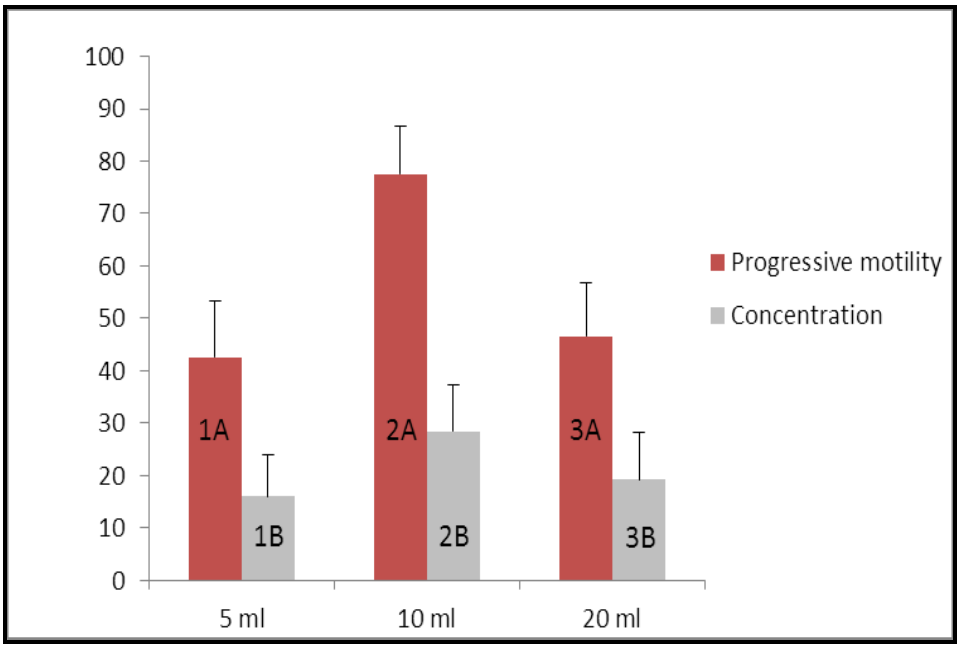

Figure 4.1: Graphical presentation of progressive motility and concentration parameters after processing spermatozoa using 5 ml (AB), 10 ml (AB) and 20 ml (AB) syringes

p<0.001: 1A vs 2A; 1B vs 2B; 2A vs 3A; 2B vs 3B

p<0.05: 1A vs 3A; 1B vs 3B

Significant differences were observed with regards to velocity parameters between treatments (Table 4.2). The curvilinear velocity (VCL) and the straight-line velocity (VSL) were significantly higher in post-processed sperm in the **10 ml syringe** (115.7 µm/s; ±9.43, for VCL and 81.5 µm/s; ±4.02, for VSL) when compared to that in the SW-5 (82.54 µm/s; ±9.06 for VCL and 71.6 µm/s; ±3.89 for VSL), ($p<0.05$). Similarly, significantly higher average path velocity (VAP) (92.8 µm/s; ±6.12) and wobble (WOB) (3.5 µm/s; ±0.76) velocity parameters were measured in purified sperm samples obtained following the SW-10 treatment. The amplitude of lateral head displacement (ALH) was significantly higher in the latter treatment sample (2.3 µm; ±0.65) while it was lower in the SW-5 (1.42 µm; ±0.32) and SW-20 (1.57 µm; ±0.28) samples. A similar pattern was observed when the SW-10 was compared to the SW-20 treatment.

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Table 4.2: Computer-aided post-processed sperm motility parameters of semen samples processed using the 5, 10 and 20 ml syringes

Parameters	5 ml (a) (SW-5)	10 ml (b) (SW-10)	20 ml (c) (SW-20)	p-value (95% CI)		
	Mean (Standard deviation)			(a *vs* b)	(a *vs* c)	(b *vs* d)
VCL (µm/s)	**82.54 (9.06)**	**115.7 (9.43)**	**88.43 (9.32)**	**<0.001** (-1.43;2.21)	**0.32** (-1.54;3.76)	**<0.001** (-2.76;1.43)
VSL (µm/s)	**71.6 (3.89)**	**81.5 (4.02)**	**73.8 (4.1)**	**<0.05** (-3.67;2.15)	**0.54** (-1.82;2.31)	**<0.05** (-3.65;2.55)
VAP (µm/s)	**78.4 (6.4)**	**92.8 (6.85)**	**82.3 (6.12)**	**<0.001** (-2.53;1.87)	**0.63** (-2.21;2.58)	**<0.001** (-2.33;2.88)
WOB (µm/s)	**1.5 (0.54)**	**3.5 (0.76)**	**2.5 (0.87)**	**<0.001** (-3.65;2.41)	**<0.05** (-1.47;3.02)	**<0.001** (-1.49;3.52)
ALH (µm)	**1.42 (0.32)**	**2.3 (0.65)**	**1.57 (0.28)**	**<0.05** (-2.19;3.32)	**0.43** (-3.65;1.76)	**<0.05** (-3.66;2.74)

VCL=curvilinear velocity, VSL= straight-line velocity, VAP= average path velocity, WOB= wobble, ALH=amplitude of lateral head displacement

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4.2.2 Experiment (ii): Sperm parameters obtained from the simplified sperm swim-up (SW-10) method and the commercial SEP-D kit

As spermatozoa harvested through the SW-10 treatment procedure had the best post-processed sperm parameters (motility and concentration) in experiment (i), the result was subjected to further testing in experiment (ii). This treatment is referred to as "*the swim-up (SW-10) method*". Sperm parameters (concentration, motility, morphology, plasma membrane integrity and DNA fragmentation) resulting from the comparison between the **SW-10** method and the **SEP-D kit** are depicted in Table 4.3 (standard deviations, p-values and confidential limits are presented in the table).

The sperm sample obtained through the SW-10 method displayed significantly ($p<0.001$) higher total motility (87.05%; ±4.18) with a concurrent higher sperm concentration (17.10 $x10^6$/ml; ±5.95) than that in the harvested sperm sample applying the commercial SEP-D kit procedure (75.35%; ±4.86 and 14.35 $x10^6$/ml; ±4.35). The SW-10 method yielded a sperm sample with a slightly higher morphological normal spermatozoa (9.75%; ±2.83), compared to the SEP-D kit (8.10%; ±1.66) ($p=0.42$). Assessment of the membrane integrity of the harvested sperm samples indicated that the SW-10 treatment resulted in sperm samples with more viable cells. The hypo-osmotic-swelling (HOS) test indicated that 79.47% (±6.31) of SW-10 sample's sperm membranes were intact when compared to 70.05% (±9.98) in the SEP-D treatment sample ($p<0.001$). Similar results were observed using the dye exclusion test (eosin-nigrosin): 82.31% (±5.15) for the SW-10 method versus 72% (±8.56) for the SEP-D kit. These results provided the average percentages of vital spermatozoa ($p<0.001$). A larger number of spermatozoa showed significant DNA-fragmentation in the SEP-D group (23.2%; ±6.77) when compared to that of the SW-10 group (13.70%; ±3.85).

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Table 4.3: Summary of sperm parameters (motility, concentration, morphology, plasma membrane integrity and DNA fragmentation) using the SW-10 method and the SEP-D kit

Parameters	Motility (%)	Concentration (10^6/ml)	Plasma membrane tests (%)		Morphology (%)	DNA fragmentation (%)
			HOS	Eosin-nigrosin		
SW-10 Mean (Standard deviation)	**87.05 (4.18)**	**17.10 (5.95)**	**79.47 (6.31)**	**82.31 (5.15)**	**9.75 (2.83)**	**13.70 (3.85)**
SEP-D kit Mean (Standard deviation)	**75.35 (4.86)**	**14.35 (4.35)**	**70.05 (9.98)**	**72.00 (8.56)**	**8.10 (1.66)**	**23.20 (6.77)**
p-value (95% CI)	**p<0.0001** (0.111;0.163)	**p<0.0001** (0.123;0.177)	**p<0.0001** (0.114;0.172)	**p<0.0001** (0.125;0.176)	**p=0.42** (0.116;0.164)	**p<0.0001** (0.098;0.162)

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Table 4.4: Velocity parameters of spermatozoa obtained after processing using the SW-10 method and SEP-D kit

Parameters	SW-10	Sep-D kit	p-value (95% CI)
	Mean (Standard deviation)		
VCL (µm/s)	**116.09 (0.36)**	**87.60 (2.31)**	**p<0.0001** (-29.854; -27.141)
VSL (µm/s)	**86.40 (0.68)**	**75.21 (0.47)**	**p<0.0001** (-12.670; -9.698)
VAP (µm/s)	**86.14 (0.51)**	**74.96 (0.59)**	**p<0.0001** (-12.550; -9.810)
WOB (µm/s)	**2.55 (0.13)**	**1.35 (0.08)**	**p<0.0001** (-1.456; -0.943)
ALH (µm)	**2.50 (0.03)**	**1.45 (0.02)**	**p<0.0001** (-1.144;-0.953)

VCL=curvilinear velocity, VSL= straight-line velocity, VAP= average path velocity, WOB= wobble, ALH=amplitude of lateral head displacement

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Computer-aided sperm analysis (CASA) parameters of spermatozoa, including curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), wobble (WOB) and amplitude of lateral head displacement (ALH) are illustrated in Table 4.4. The SW-10 method differed significantly ($p<0.0001$) in respect of the velocity parameter vectors. The VCL and VSL parameters obtained with the CASA system for the SW-10 (116.09 µm/s ±0.36 and 86.40 µm/s ±0.68 respectively) are significantly higher than those of the SEP-D kit group (87.60 µm/s ±2.31 and 75.21µm/s ±0.47 respectively). Similar results were obtained for ALH and VAP, indicating 86.14 µm/s ±0.51 (VAP); 2.55 µm/s ±0.13 (WOB) for the SW-10 method, and 74.96 µm/s ±0.59 (VAP); 1.35 µm/s ±0.88 (WOB) for the SEP-D kit. Finally, results of the ALH were highly significant ($p<0.0001$) in spermatozoa processed by means of the SW-10 method (2.50 µm/s ±0.03) when compared to those obtained from the SEP-D procedure (1.45 µm/s ±0.02).

4.2.3. Experiment (iii): Concentration and motility parameters of spermatozoa after processing using single layer centrifugation (SLC) and double layer density gradient centrifugation (DGC)

Post-processed mean sperm parameters (concentration, motility and total motile count) obtained after single layer centrifugation (SLC) and double layer density gradient centrifugation (DGC) are summarised in Table 4.5, with progressive motility and concentration parameters of spermatozoa illustrated in Figure 4.2. The CASA variables are depicted in Table 4.6. Standard deviations, p-values and confidential limits (CI) are indicated in Tables 4.5 and 4.6.

Significantly ($p<0.001$) higher sperm concentrations were observed when using the SLC (32.64 $x10^6$/ml; ±6.35) compared to those obtained using the DGC (11.36 $x10^6$/ml; ±7.05). Spermatozoa recovered from the SLC displayed a significantly ($p<0.001$) higher total motile count (6.02 $x10^6$/ml) (see Table 4.5).

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Table 4.5: Post-processed parameters of sperm after single layer (SLC) and double layer density gradient centrifugation (DGC)

Parameters Mean (Standard deviation)	SLC	DGC	p-value (95% CI)
Concentration (10^6/ml)	**32.64 (6.35)**	**11.36 (7.05)**	**<0.001** (-0.407;-0.281)
Rapid progressive motility (%)	**61.53 (8.76)**	**79.52 (9.53)**	**<0.001** (0.134;0.257)
Total motility (%)	**72.81 (12.71)**	**83.36 (14.54)**	**0.0005** (0.049;0.141)
TMC (x10^6/ml)	**6.02 (14.43)**	**2.71 (14.87)**	**<0.001** (-0.314;-0.182)

Total motile count after sperm preparation (TMC) = concentration x rapid progressive motility x insemination volume (0.3 ml)

Progressive motility of sperm cells recovered from the DGC was significantly higher (79.52%; ±9.53) when compared to those obtained from the SLC method (72.81%; ±12.71) (Table 4.5 and Figure 4.2). All motions parameters were significantly higher for spermatozoa recovered from the DGC (see Table 4.6). The curvilinear velocity and straight-line velocity of spermatozoa processed using the DGC were 110.3 µm/s (±7.89) and 79.5 µm/s (±5.08), which results statistically higher than spermatozoa processed using the SLC (85.67 µm/s; ±7.45 and 68.43 µm/s; ±5.84). Similar results were obtained for average path velocity (VAP) and wobble (WOB), indicating90.2 µm/s (±3.8) and 2.5 µm/s (±0.53) respectively, for the DGC, and 74.7 µm/s (±3.54) and 1.5 µm/s (±0.67) respectively for the SLC. The amplitude of lateral head displacement of spermatozoa was significantly higher in the DGC group (2.3 µm; ±0.65) when compared to the SLC group (1.42 µm; ±0.32).

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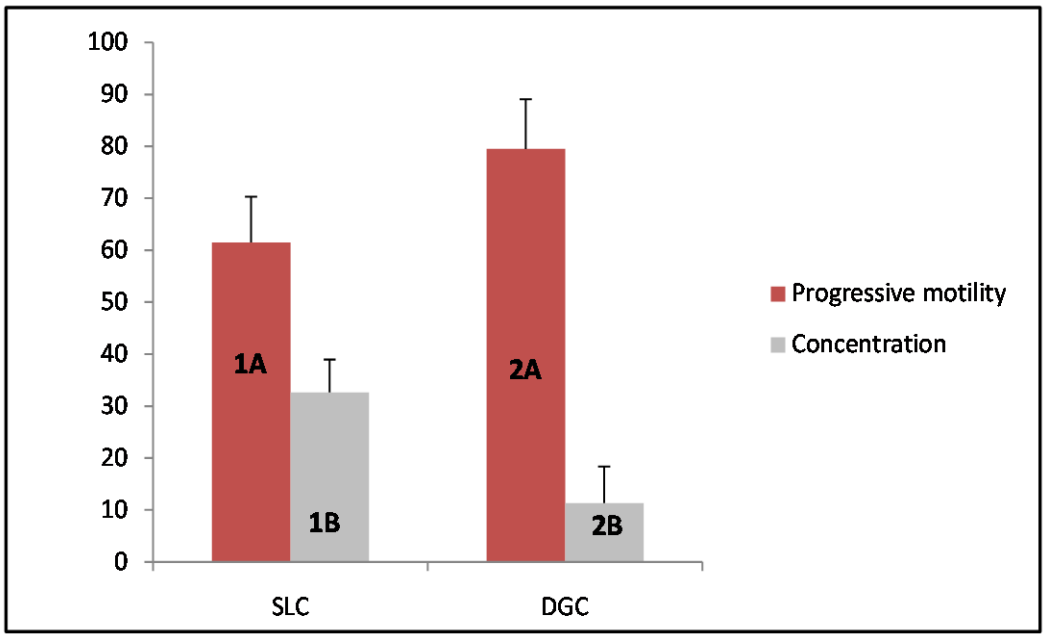

Figure 4.2: Progressive sperm motility and concentration parameters obtained after centrifugation techniques

p<0.05: 1A vs 2A and 1B vs 2B

Table 4.6: CASA velocity parameters of spermatozoa obtained after processing using the SLC and DGC methods

Parameters	SLC	DGC	p-value (95% CI)
	Mean (Standard deviation)		
VCL (µm/s)	**85.67 (7.45)**	**110.3 (7.89)**	**<0.001** (-0.85;0.32)
VSL (µm/s)	**68.43 (5.84)**	**79.5 (5.08)**	**<0.05**(-0.54;0.65)
VAP (µm/s)	**74.7 (3.54)**	**90.2 (3.8)**	**<0.001**(-0.67;0.43)
WOB (µm/s)	**1.5 (0.67)**	**2.5 (0.53)**	**<0.05** (-0.32;0.81)
ALH (µm)	**1.02 (0.92)**	**1.63 (0.75)**	**<0.05**(-0.94;0.12)

VCL=curvilinear velocity, VSL= straight-line velocity, VAP= average path velocity, WOB= wobble, ALH=amplitude of lateral head displacement

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4.3 Section 2: Gynaecologists' opinions on the diagnosis and treatment of infertility in Gabon

Responses to the questionnaire survey are represented as frequencies and expressed in percentages. Seventeen (85%) of the 20 gynaecologists practising in Libreville completed the questionnaire. All participants were from public hospitals with 5 also in private practices. Due to Gabon being a French-speaking country, not all gynaecologists were able to understand specific phrases (n=4), whereby the investigator had to interpret and explain some of the questions.

4.3.1 Gynaecologists' access to abdominal/vaginal ultrasound scans and an estimate of infertility-related consultations

Information on the access to vaginal and abdominal ultrasound scans was sought from gynaecologists, as well as the percentage of infertility-related consultations. Responses to these questions indicated that all the specialists have access to abdominal and vaginal ultrasound scans in their practices. Participants consulted with more than 50 patients monthly, of these 45% of consultations were related to infertility.

4.3.2 Male infertility diagnosis and treatment

To obtain information on the treatment of male infertility the following question was posed, "Are you able to: diagnose, or treat and/or refer male patients for infertility treatment". This question also served to provide information on laboratory investigations into male infertility and related diagnoses. Basic sperm analyses (excluding microbiological evaluations) were performed at four different pathology laboratories including Laboratoire 2000, Laboratoire National, Cabinet d'Analyses Medicale and Laboratoire d' Agondje, in Libreville. These analyses enabled gynaecologists to explain spermiogramme results to patients and point out possible causes of infertility during consultations. Seventy-five percent of respondents utilised these services while the others referred male patients directly to laboratories outside the country. Due to a lack

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of ART facilities in Gabon, 76.47% (13 out 17) of the gynaecologists advised couples to seek fertility assistance outside the country.

4.3.3 Referral of patients

Countries to which female and male patients were most frequently referred for further ART treatments, were identified by participants (Figure 4.3). Cameroon was the destination of choice, with 50% of all patients being referred to two ART units, situated in Douala and Yaounde, followed by 17% of patients being referred to an ART unit in Accra (Ghana). As 33% of respondents do not refer couples at all, these patients have to find clinics abroad at their own expense.

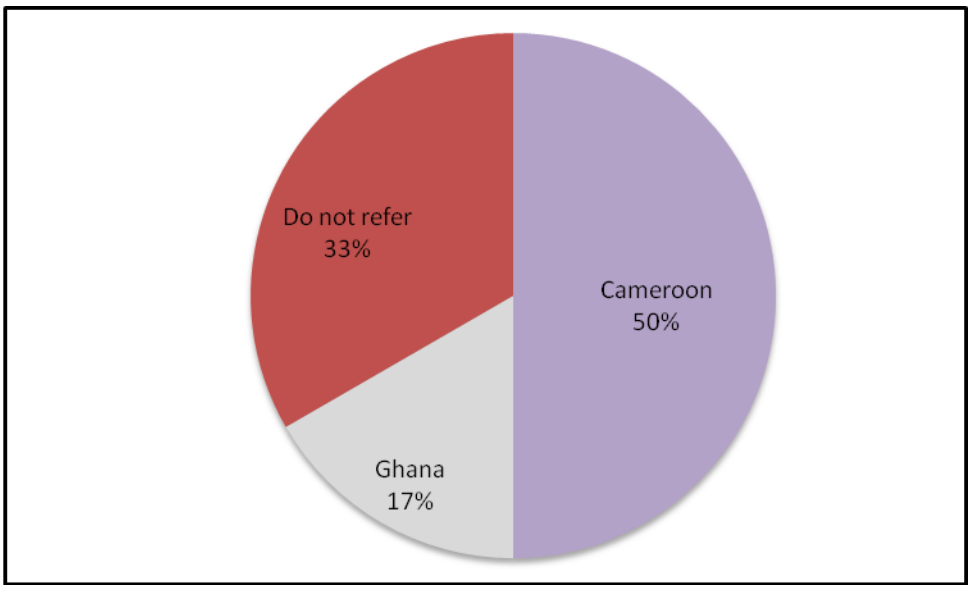

Figure 4.3: Referral options provided by gynaecologists to patients seeking ART

4.3.4 Estimated number of patients that could be referred to an ART unit in Gabon

Gynaecologists were requested to provide an estimate of the number of male patients that could benefit from a pathology laboratory providing semen analyses and services to couples that would possibly be referred for potential IUI treatments monthly. All respondents estimated that more than 50 patients would be referred to a pathology

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laboratory for semen evaluations, with 58% indicating that between 25-50 couples are likely to be referred for IUI treatment monthly (Figure 4.4).

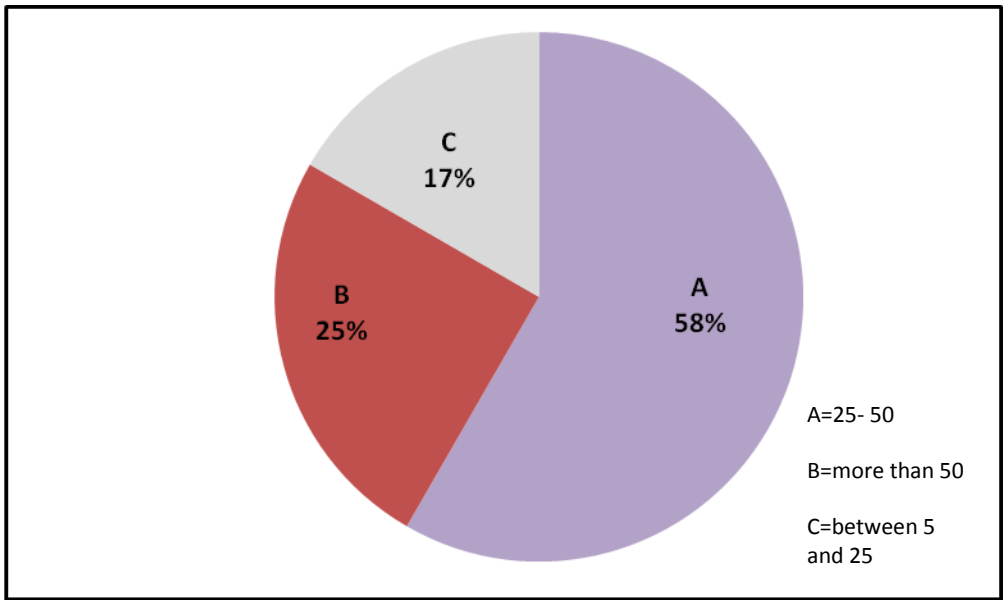

Figure 4.4: Percentages of number of patients that could be referred for monthly IUI treatments

4.3.5 Interest of gynaecologists in a potential IUI programme being undertaken in Gabon

A personal opinion section was included in the questionnaire. Gynaecologists were asked whether an ART unit in Gabon would benefit patients who visited their practices to seek diagnostic and therapeutic assistance. Respondents' interests in attending a training programme on how to perform basic fertility diagnosis/treatments could be assessed, as well as their willingness to participate in setting-up an ART unit in Libreville.

All participants were of opinion that ART services would be beneficial for both diagnostic and therapeutic patient services in Gabon, and indicated that these services should include screening for sexually transmitted diseases; semen diagnostic

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evaluation; hysteroscopy and endoscopy; as well as IUI together with *in vitro* fertilization and intra-cytoplasmic sperm injection procedures.

4.3.6 Interaction with Gabonese nationals seeking information assisted conception (Addendum F)

Due to the nature of the current study and the interaction of the investigator with Gabonese gynaecologists, while undertaking the survey the investigator was contacted by several Gabonese citizens requesting basic information on ART and contact numbers of assisted reproduction clinics in southern Africa.

The addendum provides a glimpse into the personal experiences of some of these Gabonese nationals who contacted the investigator, while documenting the role of cultural taboos and beliefs in individuals' journeys to conceive and have their own children. During the interaction the investigator made notes of all relevant information. Two females (case studies A & B), a single male (case study C) and a couple (case D1 and D2) interacted with the investigator during his visit to Gabon between July and August 2014. All the accounts are anonymous, while written information and ART related costs incurred were provided by case D.

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CHAPTER 5: DISCUSSION AND CONCLUSION

5.1 Introduction

Gabon is a country where the ability to conceive holds strong socio-cultural values. Sub-fertile couples are mostly subject to emotional distress in Gabon, and the inability to find reasons for their childlessness renders the situation more difficult to face. In addition, economic, emotional and psychological consequences have been associated with infertility.

After interacting with seventeen gynaecologists and four sub-fertile couples in Gabon, a crucial need to train health professionals on ART procedures was observed. The current study emphasizes the need to assist sub-fertile couples residing in Gabon, and to provide an approach to make ART treatment accessible in developing countries where there is limited availability of ART units as indicated in Addendum B. Although the outcome of the study will not be favourable to all sub-fertile couples, the efforts highlight the possibility to introduce low-cost ART in Gabon.

5.2 Development of the SW-10 method

Although a number of studies have demonstrated the efficiency of different sperm preparation techniques, there is limited indication to recommend any particular sperm preparation method, with no differences reported in pregnancy rate between the swim-up and the density gradient centrifugation techniques[176,175]. Optimizing sperm quality through sperm processing methods that are equally simple, affordable and effective is essential in developing countries (such as Gabon). The primary section of this project consists of testing a modified swim-up method (SW-10) using minimal equipment.

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5.2.1 Sperm swim-up method using three syringes (5, 10 and 20 ml)

The recommended sperm preparation technique for IUI by the World Health Organization is the swim-up method[181]. This section of the research has been designed to optimize a swim-up method by using syringes of different volumes, allowing various surface areas for the separation of spermatozoa from the seminal plasma, consequently affecting the population of sperm harvested.

The mean post-processed sperm concentration, progressive motility and velocity parameters were significantly higher in the SW-10 group compared to the SW-5 and SW-20. Consequently, significant higher TMC was found in harvested sperm samples treated using the SW-10 method. These results confirmed previous studies by Keel *et al*. (1990) who reported an increase in sperm TMC due to a larger surface area of sperm migration during the swim-up method[239]. In addition, a meta-analysis by Van der Weert *et al*. (2004) indicated a post-wash TMC between 0.8-5 $x10^6$ of sperm as a good predictor of a successful IUI[240]. The mean total sperm motility was significantly higher in post-wash sperm samples obtained using the SW-5 when compared to that of the SW-20 method. However, the mean sperm concentration was found to be statistically higher in the SW-20 group. The implications of sperm motility characteristics for successful *in vitro* fertilization were highlighted in a study by Hirano *et al*. (2001) who found a significant correlation between sperm motility parameters obtained by CASA and fertilization rates[241]. Therefore, the SW-5 technique can be considered as the second option after the SW-10 method. Particular difficulties were experienced in the course of the experimentation. Aspiration of semen was most difficult using the 20 ml syringe. However the 10 ml volume syringe was the easiest and most practical to use during experimentation. This could be due to the difference in surface areas of the syringes whereby the 20 ml syringe having a larger surface area (diameter).

The results of this experiment indicated that the TMC after SW-10 technique (6.62 $x10^6$) is significantly higher when compared to SW-5 and SW-20 processing. Numerous sperm parameters including total motile count (TMC) and morphology can have a

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significant impact on IUI outcome[202]. Ombelet *et al*. (2003) reported that a minimum inseminating motile sperm count (IMC) of 1x10^6 with >5% normal spermatozoa could predict successful IUI result[18]. A study by Nikbakht *et al.* and Kim *et al.* indicated a >5% normal morphology spermatozoa and a TMC of between 5-10 x10^6, could influence IUI success rate[202,204]. Based on literature the SW-10 technique was selected and compared to the commercial available SEP-D kit in the following experiment.

5.2.2 Comparison of the SW-10 method and SEP-D kit

The SEP-D kit is a device consisting of five syringes pre-filled with a commercial available medium. Processing a single semen sample by means of SW-10 costs R229 (€15.40[E]), which is less expensive than using the SEP-D kit (R347.47; €23.36[D]).[F] Gentis *et al*. (2012) reported no significant differences between the SEP-D kit and DGC in respect of sperm motility and concentration[187]. However, the authors highlighted the need to compare the SEP-D kit with other sperm preparation methods[187].

For all sperm parameters evaluated in the current study, not including morphology, statistically significant higher results were obtained in the SW-10 group compared to the SEP-D kit ($p<0.001$). These results are similar to the ones obtained by Gentis *et al*. (2012) who indicated no significant difference between the SEP-D kit and the swim-up method in respect of normal sperm morphology[187]. Sperm motility and morphology have been reported to have a significant effect on IUI success[242,243]. A study performed by Sun *et al*. (2012) showed an increase in pregnancy rates following IUI in couples with male partners presenting with a high percentage of morphologically normal spermatozoa[244]. The latter study indicated pregnancy rates of 7.60% for patients with <5% normal spermatozoa and 13.62% in semen samples with 10-14% morphologically normal sperm[244].

[E]All costs were obtained in ZAR, with an exchange rate of € 1 to ZAR 14, 87 (02/09/2015). All calculations include 14% Value Added Tax (VAT).
[F]The costs were obtained from a South African supplier of SEP-D kit, disposable syringes and media.

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Sperm plasma membrane integrity (sperm vitality) is an important determinant for successful fertilization, since it plays a role in sperm capacitation, the acrosome reaction and assisting in the binding of the sperm cell to the oocyte membrane[245]. Vitality tests allow for the distinction between vital and non-vital cells in an immotile population of spermatozoa[246]. The hypo-osmotic swelling (HOS) and the eosin-nigrosin tests were used in this study to evaluate sperm viability after being processed using the SW-10 and the SEP-D kit. A study by Tartagni *et al*. (2002) revealed an increased in miscarriage rate where male patients presented with less than 50% live sperm as determined by the HOS test[247]. The HOS test results obtained from semen processed using the SW-10 (79.47%) were statistically higher than those obtained using the SEP-D kit (70.05%) ($p<0.001$). Although the eosin-nigrosin test was first introduced in the 1950s, the test was used only in mammalian cells[246], until developed for use in washed human sperm cells in 1990[248]. The difference in eosin-nigrosin results between the two sperm washing methods was statistically significant ($p<0.001$), with 82.31% viable sperm when using the SW-10 method and 72.00% for the SEP-D kit method.

A prospective cohort study by Duran *et al.* (2002) highlighted the impact of DNA quality on IUI outcome[249]. An increase in sperm DNA damage levels has been evident mostly in infertile males[250]. The integrity of DNA in sperm was assessed using the Halosperm G2® assay. This method has been reported to have a simple protocol[251]. However the cost of Halosperm G2® kit varies depending on location, being more expensive (R7,536; €506[G]) in South Africa than in Europe (R4,332; €291.32), as per cost received from a South African supplier of Halosperm G2®kit. A significantly higher percentage of spermatozoa with fragmented DNA were recovered from the SEP-D kit method (23.20%), compared to that of the SW-10 method (13.70%) ($p<0.001$). The negative correlation between motility parameters and percentage of sperm with DNA fragmentation obtained in this study confirmed previous research by Tandara *et al*. (2013)[252]. In the latter study, the authors assessed sperm DNA fragmentation using

[G]All costs were obtained in ZAR, with an exchange rate of € 1 to ZAR 14, 87 (02/09/2015). All calculations include 14% Value Added Tax (VAT).

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Halosperm G2® kit, and reported that a high DNA fragmentation index had a negative impact on motility parameters, as well as possibly embryo quality[252].

Although the SW-10 method did not significantly produce a larger number of morphologically normal spermatozoa in comparison to the SEPD kit, the method yielded spermatozoa with better DNA conformation and plasma membrane integrity.

5.2.3 Density gradient centrifugation

The difference between the SLC and the DGC processing methods with regard to sperm motility and concentration was evaluated in the study. Results obtained showed a significantly higher TMC in the SLC group when compared to that of the DGC group ($p<0.001$), and also a significantly higher mean harvested sperm concentration was evident in the SLC group. However the mean sperm motility was significantly higher in the DGC group ($p<0.05$). Similar results were reported by Turhan *et al.* (2011) superior post-wash progressive motility was achieved by DGC, and higher concentration by means of SLC[205]. Although the SLC provided better harvested sperm concentration, the literature indicates the DGC as the most effective and efficient method for IUI[253]. To avoid post-processed sperm contamination, some laboratories use DGC combined with the ProInsert*TM* (Nidacon International) which permits access to the sperm pellet without exposure to contaminants in the upper gradient layers during semen preparation[177,254].

Results from this section indicated that both the SW-10 and the SLC methods have proven to be appropriate methods for sperm preparations for IUI, depending on semen samples characteristics. However, the media in the SLC (PureSperm® 80) is more expensive than the PureSperm® Wash media used for the SW-10, the difference being R 1,840 (€123.73)[H] per 100 ml. Furthermore, the SW-10 requires less equipment and is less time-consuming and simpler to perform compared to the SLC. Consequently the SW-10 can be fundamental for the establishment of a low-cost IUI programme in

[H]All costs were obtained in ZAR, with an exchange rate € 1 to ZAR 14, 87 (02/09/2015). All calculations include 14% Value Added Tax (VAT).

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developing countries such as Gabon, where a survey to evaluate the level of infertility assistance was conducted among gynaecologists practising in Libreville.

5.3 Feasibility of establishing an IUI programme in Libreville (Gabon)

In 2011, the society of medical health care providers in Gabon had 545 members[255]. In the same year, a national census estimated the total Gabonese population as 1,594,000, which translates into a ratio of 1 medical practitioner to 2,924 citizens (0.34:1,000). In other countries, such as South Africa this ratio indicated 0.77 doctors to 1 000 people[256]. A cut-off value of 1 doctor per 1 000 has been approved and is recommended by the World Health Organization (WHO, 2008)[257]. Gynaecologists represented 8% of all specialities however only 3% of all the health professionals in Gabon **(See Addendum G)**[255]**.**

The current study showed that infertility related consultations represented 45% of all gynaecological consultations in Libreville. Similar results were obtained by Meye *et al*. (2007) during a study performed at four hospitals located in Libreville[6]. The authors reported 40% infertility related consultations in Libreville, with 75.6 % of sub-fertile women (32±6 years of age) and 88.6% of sub-fertile men (38.6±6 years of age) presenting with secondary infertility[6]. Couples attending infertility related consultations together represented only 15% of couples, with 85% of females seeking assistance on their own[6].

The present survey indicated that gynaecologists generally refer male patients to four pathology laboratories (2 public and 2 private) in Libreville for basic semen evaluations. The spermiogrammes are then interpreted and results provided to the patients. As semen analyses obtained from these laboratories do not provide all the functional and pathological semen evaluations such as some microscopic evaluations (i.e. round cell concentration) and microbiological tests, patients are referred to clinics/hospitals outside the country for more detailed semen assessments. Franken (2013), highlighted the

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importance to improve semen analyses in developing countries, and recommend the setting-up of a quality assurance programme for the laboratory technicians[258].

Sima *et al.* (2013) estimated a secondary infertility rate of 82.3% in Gabon, with tubal factor infertility being the predominant cause (66.4%)[259]. *Chlamydia trachomatis* was the leading sexually transmitted infection (74.7%) in Gabon[259]. The current survey highlighted the absence of primary and secondary health facilities to diagnose and treat couples who wish to have their own genetic children. For this reason most gynaecologists were obliged to refer female patients for infertility treatment abroad. Patients were predominantly referred to Cameroon (65%), a neighbouring country, where two infertility clinics are available, and Ghana (35%).

The average income per month in Gabon amounts to US$ 839 per citizen (in 2012)[260], and the cost estimation for a couple to receive ART treatment in South Africa, as provided in **Addendum F (Case D1)**, amounts to R110,000 (US$8,333)[I],hence only couples with the financial resources can afford transport, accommodation, diagnostic and therapeutic infertility treatment costs. This Addendum highlights the endogenous difficulties encountered by Gabonese citizens in order to find reasons for their childlessness. These couples or individuals, described in **Addendum F**, are facing the stigma attached to being childless in Africa in general and particularly Gabon. In the Gabonese society having a family lineage is one of the basic priorities for all couples. The value of children in an African context was explored by Dyer. Children offer social security, fulfil emotional desires, provide social status, secure conjugal ties, secure rights of property and assist with labour[261].

Assisting these couples by providing a proper understanding of the reason for not having a child and the treatments available could be an important approach in overcoming the stigma of infertility in Gabon. While ART treatments in developed countries evolved quickly, the situation in developing countries is completely divergent,

[I]All costs were obtained in ZAR, with an exchange rate of US$ 1 to ZAR 13, 20 (02/09/2015). All calculations include 14% Value Added Tax (VAT).

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where financial barriers are one of the main challenges[262]. Added to this, the burden of the West African Ebola virus impacts on the already restrained public health system[263].

Politically stable and with four large public hospitals (General Hospital of Libreville; Agondje Hospital; Military Hospital; Josephine Bongo Hospital) in Libreville, Gabon has the potential to support both basic medical and advanced ART procedures[21]. Participant gynaecologists estimated that more than 25 patients for all practices could be referred for IUI treatment per month; with the possibility of more than 50 male patients to be clinically evaluated for possible male factor infertility. The establishment of an IUI programme in Gabon, was supported by all gynaecologists, who also expressed their willingness to collaborate in such a venture.

Approaches to provide affordable diagnostic and accessible therapeutic infertility treatment are discussed in the literature[15,201,262], leading to technological development such as alternative sperm preparation methods, low-cost laboratory supplies, and a simplified embryo culture system[264]. Pilot studies and clinical trials to validate these alternative methods are ongoing, therefore possible implementation of these procedures worldwide could be considered. Even though the problem of childlessness is generally not considered a priority health issue, initiatives for low-cost infertility treatment in developed and developing countries (with limited resources) are evident in the efforts of the European Society of Human Reproduction and Embryology (Task Force in Developing Countries and Infertility)[265] and The Walking Egg Project[266]. These initiatives encourage research and advance service delivery, advocacy and networking to achieve global access to infertility care[266].

In culmination, suggestions can be made to optimize low-cost sperm preparations and improve accessibility to infertility diagnosis and possibly treatments in Libreville, Gabon:

- Office-based sperm tests to simplify the inclusion of sub-fertile couples in an IUI programme should be encouraged, as part of a research trend to support and promote simplified ART procedures.

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- The simplification of sperm processing for IUI using a syringe could contribute to the tWE's scope and approach towards accessible ART[266].

- The SW-10 syringe method has been found to be simple to perform, and could be an alternative to the SEP-D kit/ SLC or DGC processing methods.

- The use of the SW-10 sperm processing method is patient profile dependent, based on sperm motility and concentration. The method however is not suitable for patients with seminal infections and/or blood-borne viruses or a sub-standard semen profile.

- The development/analysis of low-cost single step sperm tests e.g. performing the HOS test with water[267]should be promoted as part of basic sperm analyses.

- Since semen evaluation can be seen as the foundation of male infertility assessment, the performance and interpretation of spermiogrammes is therefore of the utmost importance. Results from this thesis could be beneficial to the Gabonese Society of Obstetricians Gynaecologists and Reproduction, to promote semenology training programmes in Gabon.

- Current basic spermiogrammes performed at four different pathology laboratories in Libreville could be extended to include microbiological testing, standardization and continuous semenology training programs [268].

- The establishment of an IUI programme at a public service health facility in Libreville could initiate and build local ART knowledge, and limit expenditure on foreign health services.

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With a combination of efforts including advocacy, training of gynaecologists practising in Gabon by specialists in reproductive care, the establishment of an IUI programme in Libreville, using SW-10 method is feasible. This research project can be seen as an initiative to raise awareness of the limited access to infertility treatment in developing countries, specifically in Gabon, and the possibility to establish cost-affordable ART in those countries.

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CHAPTER 6: ADDENDA

- **A: Use of contraceptives and fertility rates in northern and southern Africa**
- **B: This addendum provides information on fertility clinics in Africa. In addenda B2, B3 and B4, countries are listed according to the number of ART units existing in each one**
- **B1: Tunisia, Morocco, Sudan, Algeria, Cameroon, Uganda, Tanzania, Rwanda, Kenya, Togo, Mali, Burkina Faso, Ivory-coast, Benin, Senegal, Ghana, Namibia, Mauritius and Malawi**
    - **B2: Nigeria**
    - **B3: Egypt**
    - **B4: South Africa**
- **C: Solutions used during experiments**
- **D: Questionnaires completed by gynaecologists practising in Gabon**
- **E: Pre-processed semen sample parameters of donor and patient used in the study**
- **F: Interactions with Gabonese citizens seeking information on fertility clinics in South Africa**
- **G: Diagram depicting the comparison between general practitioners and specialists registered as members of the Health Professional Association in Gabon**

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ADDENDUM A: Use of contraceptives and fertility rates in northern and southern Africa

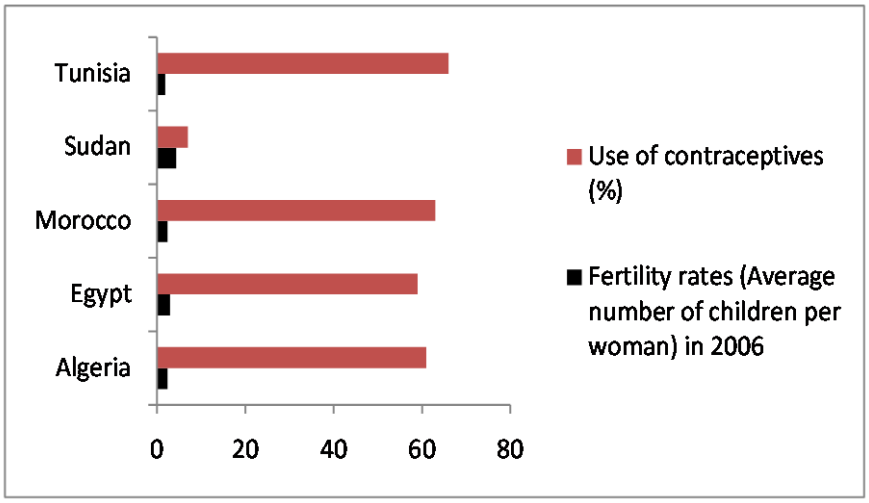

Figure 6.1: Bar graph illustrating the comparison between the use of contraceptives and fertility rates in northern Africa (Adapted from the United Nations Children's Fund, 2008)[269]

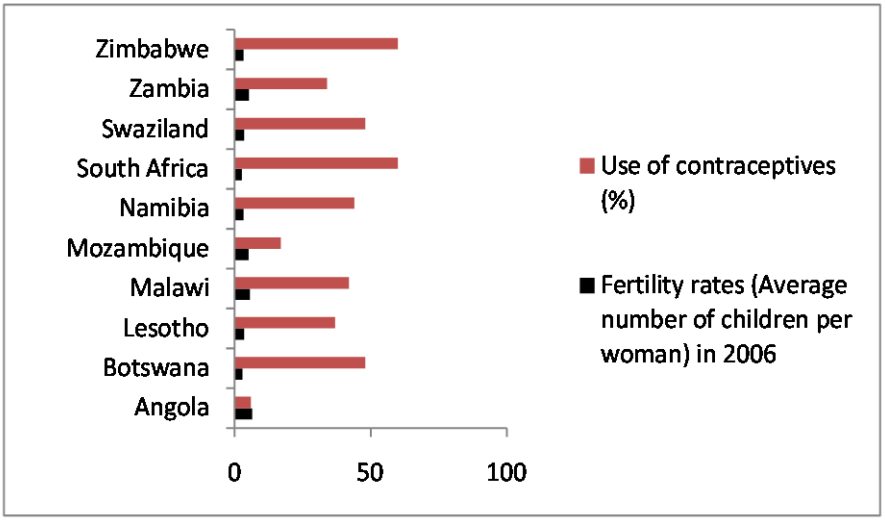

Figure 6.2: Impact of contraceptives on fertility rates in southern Africa (Adapted from the United Nations Children's Fund, 2008)[269]

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ADDENDUM B: Fertility clinics in Africa

This addendum provides names of fertility clinics and contact numbers/websites in various African countries. The number of fertility units in the leading countries, i.e. Nigeria, Egypt and South Africa, are listed separately.

Addendum B is subdivided as follows:

B1: Northern Africa (Tunisia, Morocco, Sudan, Algeria); Central Africa (Cameroon); Eastern Africa (Uganda, Tanzania, Rwanda, Kenya); Western Africa (Togo, Mali, Burkina Faso, Ivory coast, Benin, Senegal); and Southern Africa (Namibia, Mauritius and Malawi)

B2: Nigeria

B3: Egypt

B4: South Africa

In Addenda B2, B3 and B4, countries are listed according to the number of units in each one.

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ADDENDUM B1: Fertility units in Africa excluding Nigeria, Egypt and South Africa

Country	Unit	Telephone number/Website
Northern Africa		
Tunisia	• Clinique Alyssa • Cinique de l'Espoir • Clinique El Farabi • Clinique Les Jasmins • Clinique Les Oliviers • Pole PMA SUD Clinique Megrine	• www.cliniquealyssa.com • www.clinique-espoir-tunisie.com • contact@cilinique-alfarabi.com • www.clinique-les-jasmins.com • http://www.lesoliviers.maghreb • http://polepmasud.com
Morocco	• Centre de Fertilité Al Amal de Fès • Centre de Fertilité Al Boustane • Centre de Fertilité Al Oumouma • Centre de Fertilité Anfa • Centre de Fertilité Argana • Centre de Fertilité Casablanca • Centre de Fertilité Dar El Baroud • Centre de Fertilité de la Capitale • Centre de Fertilité de l'Hermitage	• hart.doc@menara.ma • 00 212 5 37 73 80 00 • 00 212 5 22 82 06 06 • 00 212 5 22 23 52 25 • climea@iam.net.ma • 00 212 5 22 22 19 28 • fbayane@iam.net.ma • 00 212 5 37 76 72 7 • www.labobiomer.com

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	• Centre de Fertilité des Orangers	• docteurzahimohamed@hotmail.com
	• Centre de Fertilité EL AMAL	• 00 212 5 48 82 89 85
	• Centre de Fertilité FIVMA	• 00 212 5 22 36 74 05
	• Centre de Fertilité IRIFIV	• 00 212 5 22 39 25 30
	• Centre de Fertilité Maârif	• 00 212 5 22 23 40 14
	• Centre de Fertilité Riviera	• 00 212 5 22 98 46 28
	• Centre de Fertilité INJAB	• injab@iam.net.ma
Sudan	• Dr Elsir Abuelhassan Fertility Centre	• contact@drelsirfertility.com
	• Royal Care International Hospital IVF	• www.fertilitysudan.com
	• Royal Care International Hospital IVF	• http://www.royalcare-sd.com
Algeria	• Centre Hospitalo-Universitaire (CHU) Nafissa-Hamoud d'Hussein Dey	• 021 49 56 56/021 49 56 04
	• TIZIRI Private IVF Centre	• 00213772664757
Central Africa		
Cameroon	• Clinique de L' Aeroport	• http://www.cliniquedelaeroport.com
	• Clinique Odyssee	• http://www.clique odyssee.com

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Uganda	• Bethany Women's Hospital	• info@bethanywomenshospital.org
	• Kampala Gynaecology and Fertility Clinic	• (256) 772-520-248
	• Lifesure Fertility and Gynaecology Centre	• http://www.lfsure.com
	• Nordica Fertility Centre	• info@nordica.org
	• Paragon Fertility Centre	• +256 75 335 3534
	• Women's Hospital International and Fertility Centre	• www.womens-hospital.net/
Tanzania	• Bugando Medical Centre (BMC)	• www.bugandomedicalcentre.go.tz
	• Dar IVF and Fertility Clinic	• http://www.darivf.com
	• Malpani Infertility Clinic	• info@drmalpani.com
	• Sanitas Medical Centre	• www.sanitasmedics.com
	• The Aga Khan Hospital, Dar es Salaam	• www.agakhanhospitals.org/dar/
Rwanda	• Kigali Fertility and Diagnostic Centre	• +250 789 184 444
Kenya	• The Karen Hospital	• www.karenhospital.org
	• LifeBridge Baby Center	• lifebridgeclinic2013@yahoo.co.uk
	• Nairobi IVF Centre Ltd	• noreh@africaonline.co.ke

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Western Africa		
Togo	• Biasa Clinic	• http://www.cliniquebiasa.org
Mali	• Clinic Kabala	• http://www.cliniquekabala.com
Burkina Faso	• Clinique de la Grace Marie	• lagracemarie03@yahoo.fr
Ivory Coast	• Clinique Médicale Fatima • Clinique Procréa	• +225 22 52 38 40 • http://www.Cliniqueprocrea.com
Benin	• Clinique OVO	• www.cliniqueovo.com/
Senegal	• Clinique de la Madeleine • Clinique du Cap	• Cmd@Cliniquedelamadeleine.com • www.cliniqueducap.com
Ghana	• Upscale Angels Fertility • Pro Vita Specialist Hospital • Baby Dust • Finney Hospital • Ruma Hospital • Sinel Specialist Hospital • Lister Hospital • Lighthouse Mission Hospital & Fertility Center • Jubail Specialist Hospital • Lapaz Community Hospital Fertility Centre	• www.upscalefertilitygh.com • www.provitaspecialisthospital.com • www.whatclinic.com/fertility/ghana • www.finneyhospital.com/ • www.rumaivf.com/ • www.sinelhospital.com/ • www.thelisterhospital.com/ • www.lighthousemissionhospital.org/ • www.jubailhospital.com/ • www.lapazcommunityhospital.org/
Southern Africa		
Namibia	• Rhino Park Hospital	• www.hospital.com.na

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Mauritius	• Medicare Clinics • Nouvelle Clinique du Bon Pasteur Ltée	• www.medicare-mauritius.com • www.dcdmconsulting.com
Malawi	• Embangweni Hospital	• www.embangweni.com/hospital

ADDENDUM B2: Nigeria

Unit	Telephone number	Website
Bridge Clinic	(+61 8) 8539 3232	www.thebridgeclinic.com
DIFF Hospital	+234-09-290-8426	info@diffhospital.com
Eko Hospital Fertility Centre	+234-01-4960159	www.ekohospitals.com
George's Memorial Medical Centre	+234-12-715-320	iketubosin@georgesmedical.com
Hope Valley Fertility Clinic	+234 1 461 8989	www.thehopevalleyclinic.com
Medical Art Centre	+234 1 342-9031	www.medicalartcentre.com
M and M Hospital	+234-82-227-798	www.mmfertilityhospital.com
National Hospital Abuja	+234-80-975-200	www.nationalhospitalabuja.net
Nisa Premier Hospital	+234-0703 417 9895	www.nisa.com.ng
Nordica Fertility Centre	+234-80-743-434	www.nordicalagos.com
Omni Advanced Fertility Centre	+234 1 762 4327	www.ivflagos.org
Roding Medical Centre	+234-1-2716057	wecare@therodingmedicalcentreltd.com
St. Ives Specialist Hospital : IVF and Fertility Unit	+234-0803 949 4531	www.stivesng.com
George's Memorial Medical Centre	+234-12-715-320	iketubosin@georgesmedical.com

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ADDENDUM B3: Egypt

Unit	Telephone number/website
Adam International Hospital	+202 875503622
AL Safwa Hospital	www.@alsafwahospital.com
Cairo Women's Clinic	www.cairoivf.com
Cairo Fertility Clinic	+202 33366885
Dr Orief's Fertility Centre	+202 23924110
El Amal Ivf Centre	http://elamalivecentre.com
El Nomsrosy Fertility Clinic	www.elnomrosyivf.com
Egyptian IVF-ET Centre	www.egyptianivfcentre.com
Gohar Women's Health Clinic	info@gohar-hospital.com
Hayat Fertility Centre	+202 23596169
Horus IVF Centre	mouselhy@hotmail.com
Kamal Shoair Hospital	osamashaeer@gmail.com
Nozha International Hospital	www.alnozha-hospital.com
NSA Diagnostic Laboratory	www.nsa-lab.com
Sunrise Fertility Centre	www.sunriseivf.net
Queensway Gynaecology Clinic	+202 23374945
Wadi El-Neel Hospital	+202 24523855

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ADDENDUM B4: South Africa

Units	Telephone Number	Website
GAUTENG UNITS		
Johannesburg		
Bio Art Fertility Centre	+27 (0)11 484 4700	www.bioartfertility.co.za
Gynae Care	+27 (0)11 475 3600	https://www.practo.com
Gynomed Clinic	+27 (0)11 796 1100	www.gynomed.co.za
Life Centre Johannesburg	+27 (0)11 788 1100	www.lifecentre.co.za
Medfem Clinic	+27 (0)11 463 2244	www.medfem.co.za
Netcare Park Lane Fertility Centre	+27 (0)11 480 4143	www.parklanefertilitycentre.co.za
Sandton Fertility Clinic	+27 (0)11 884 8172	www.sandtonfertility.com
Vitalab Fertility Clinic	+27 (0)11 911 4700	**www.vitalab.com**
Pretoria		
Nordica Fertility Centre	+27 (0)12 807 1956	www.nordica.org
Pretoria East Fertility Clinic	+27 (0)12 883 8854	https://www.netcare.co.za
Steve Biko Academic Hospital (Public)	+27 (0)12 354 2540	www.pah.org.za
Wilgers clinic	+27 (0)12 807 0232	http://www.lifehealthcare.co.za
Rustenburg		
Sthemba Fertility Centre	+ 27(0)14 59 0210	www.sthembafertility.co.za

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Gauteng Independent Specialists		
Femina Clinic	+27 (012) 323-4011	www.netcare.co.za
Park Lane Clinic	+27 (011) 484-3700	www.parklanefertilitycentre.co.za
Wilgers Clinic	+27 (012) 807-0232	www.wilgersinfertilityclinic.co.za
KWAZULU-NATAL		
Durban		
Centre for Assisted Reproduction and Endocrinology (CARE)	+27 (031) 267 7920	www.careclinic.co.za
Chelmsford Medical Centre	+27 (031) 201-9408/09	www.safindit.co.za
LIFE Centre	+27 (011) 788 4784	www.lifecentre.co.za
Nordica Fertility Clinic	+27 (031) 904-2592	www.nordica.org
Kwazulu-Natal Independent Specialists		
St Augustines Hospital	+27 (031) 202-7563	www.netcare.co.za
FREE STATE		
Bloemfontein		
Femspes Clinic	+27 (051) 436 8956	www.femspes.co.za
University of the Free State, Dept Obstetrics, IVF	+27 (051) 405-3385	www.health.ufs.ac.za

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WESTERN CAPE		
Cape Fertility Clinic	+27 (082) 774 8494	www.capefertility-clinic.co.za
Groote Schuur Fertility Unit (Public)	+27 (021) 404-6027	www.ifaasa.co.za
Pan Lab Fertility Clinic	+27 (721) 930 4433	fertilitydoctor.co.za
Tygerberg Infertility Clinic (Public)	+27 (021) 938 5487	www.aevitas.co.za
Wijnland Fertility	+27 (021) 882 9666	www.wijnlandfertility.co.za
Western Cape Independent Specialists		
Christiaan Barnard Chambers	+27 (21) 480 6111	www.fertilityspecialist.co.za
Port Elizabeth		
Fertility and Wellness Centre	+27 (041) 392 6295	www.fertilityunit.com

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ADDENDUM C: Solutions used during experiments

Experiment title	Chemicals	Suppliers	Cat no
Experiment (i)	PureSperm® Wash	Nidacon International	PSW100
Experiment (ii)	VitalScreen	FertiPro	FP12VI02
	d-Fructose	SIGMA	F2543
	Sodium Citrate	SIGMA	S-4641
	Haematoxylin	Merck Serono	109253200
	Ethanol	SBAH	4164080LC
	Denaturant Agent (DA)	Halotech	G2 1502
	Lysis solution (LS)	Halotech	G2 1502
	Staining solution A (SSA)	Halotech	G2 1502
	Staining solution B (SSB)	Halotech	G2 1502
Experiment (iii)	PureSperm® 40	Nidacon International	PS40-100
	PureSperm® 80	Nidacon International	PS80-100

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ADDENDUM D: Questionnaire completed by gynaecologists practising in Gabon

Questionnaire: As part of an MSc project entitled:
Evaluating the feasibility of low cost sperm preparation methods within a prospective intra-uterine insemination programme in Gabon

Mr Lionel Wildy Moungala, Department Obstetrics and Gynaecology, University of Pretoria, RSA

This questionnaire should be completed by gynaecologists practicing in Gabon.

Please mark **(X)** the relevant block where appropriate for each question. Comment where necessary.

All information obtained during this study is strictly confidential. Results will be reported in a scientific article as well as in a dissertation.

Your participation in the study will be appreciated; Bear in mind that you are not obliged to answer questions that you may feel uncomfortable with.

For official use only

Date: _____

1. Practice information

1.1 General information

Initials & Surname:……..Name of practice: ……

Province: ……Town: ……..

E-mail:…….Fax no:……..

Location: Hospital Clinic Residence Other

Years in practice: Less than 1year 1 to 5 years 6 to 10 years
More than 10 years

1.2 How many patients do you consult with on a *monthly basis*?

Fewer than 5	Between 5-25	Between 26-50	More than 50

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1.3 On average, how many couples do you consult with per month regarding childlessness/infertility?

Fewer than 5	Between 5-25	Between 26-50	More than 50

2. Male patients

2.1 Are you able to diagnose, treat and/or refer male patients for infertility testing? (e.g. spermatology analyses), If yes please mark (X)

Diagnose ☐ Treat ☐ Refer ☐

If you do *refer* male patients, please complete questions 3.2 and 3.3

2.2 Referral laboratory information:

Name of Laboratory: ……

Town: ……

Country: ……

2.3 What semen parameters are evaluated?

Macroscopic evaluation of semen samples

Volume ☐ pH ☐ Viscosity ☐ General appearance ☐

Microscopic evaluation

Morphology ☐ Motility ☐ Concentration ☐ Round Cell Concentration ☐

MC&S ☐ Peroxidase test for activated seminal white blood cells ☐

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3. Female patients

3.1 Do you have direct access to ultrasound devices in your practice?

Yes	No

If yes, specify manufacturer type: …….

Specify probe: Abdominal Vaginal

3.2 Do you test the female patients for the following: anovulation, endometriosis, tubal occlusion and endocrine factors? If yes please estimate as a percentage)

	Tested	Not tested	%
Anovulation:	☐	☐	
Endometriosis:	☐	☐	
Tubal occlusion:	☐	☐	
Endocrine factors:	☐	☐	

Other (specify):……

3.3 Do you refer the female patients for further fertility treatment?

Yes	No

If yes, please answer question 3.4

3.4 Referral unit information

Name of unit: ……

Town: …….

Country……

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4. Personal opinion

4.1 Would an Assisted Reproductive Technology unit benefit patients attending your practice if the following services were offered?

Diagnostic:

Screening for sexual transmitted diseases	Yes	No
Semen diagnostic evaluation	Yes	No
Hysteroscopy	Yes	No
Endoscopy	Yes	No

Therapeutic:

Intrauterine insemination	Yes	No
In vitro fertilization	Yes	No
Intra-cytoplasmic sperm injection	Yes	No

4.2 How many patients would be referred to a diagnostic spermatology laboratory on a monthly basis?

Fewer than 5	Between 5-25	Between 26-50	More than 50

4.3 How many patients with patent fallopian tubes would be referred for IUI on a monthly basis?

Less than 5	Between 5-25	Between 26-50	More than 50

4.4 Would you be interested to attend a training programme on how to perform basic infertility diagnoses/ treatments?

Yes	No

4.5 Would you be interested in participating in the setting up of an ART unit in Libreville?

Yes	No

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4.6 Indicate whether you would like to be informed of the outcome of the present study (2014/15) via e-mail

Yes	No

Thank you for your time.

Correspond to:

Mr Lionel Wildy Moungala,

Reproductive Biology Laboratory

Department Obstetrics and Gynaecology

University of Pretoria, RSA

Tel: +27 (0) 12 354 2062/2208

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ADDENDUM E: Pre-processed semen sample parameters of donors and patients used in the study

Summaries of semen parameters of donors and patients used in the following studies:

- development of a simplified sperm swim-up method to obtain motile sperm with minimal equipment;
- comparison between the simplified sperm swim-up method and the commercially available SEP-D kit; and
- comparison between the single-layer and density-gradient centrifugation, based on post-processed sperm parameters

In all the graphics, the World Health Organization's (2010)[J] reference values for normal sperm parameters are indicated with dotted lines.

[J] World Health Organization. 2010. Laboratory manual examination and processing of human semen. Cambridge , UK:Cambridge University Press, 5th Ed.

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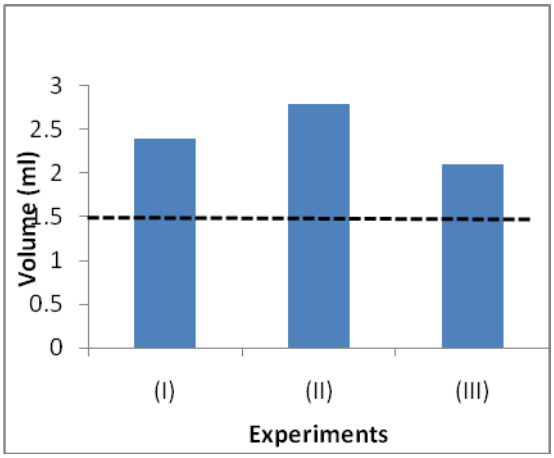

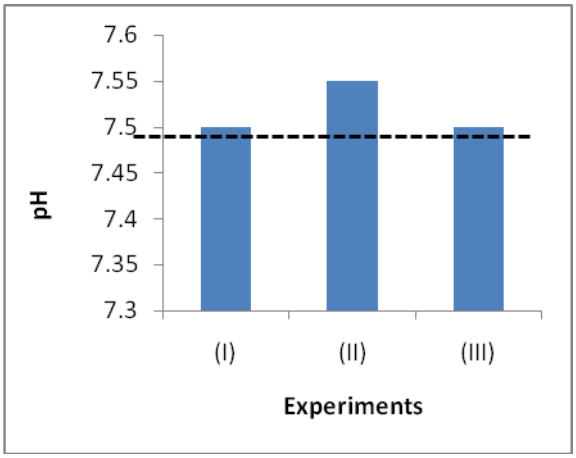

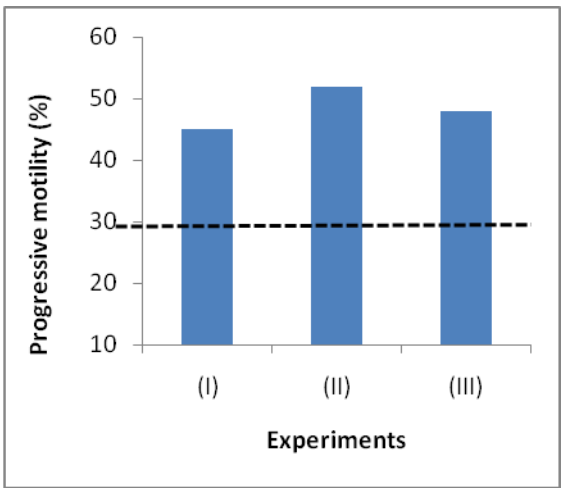

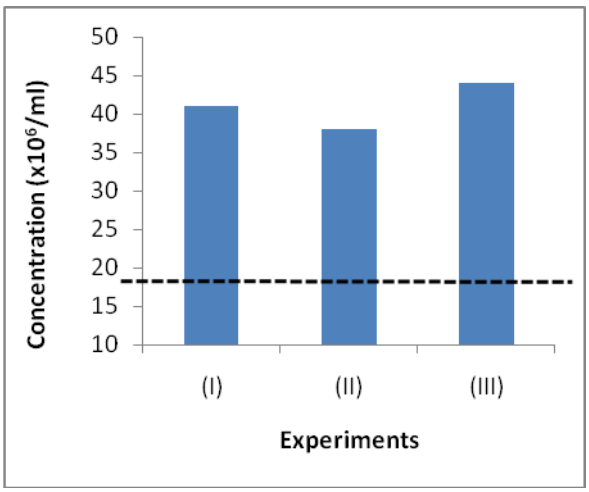

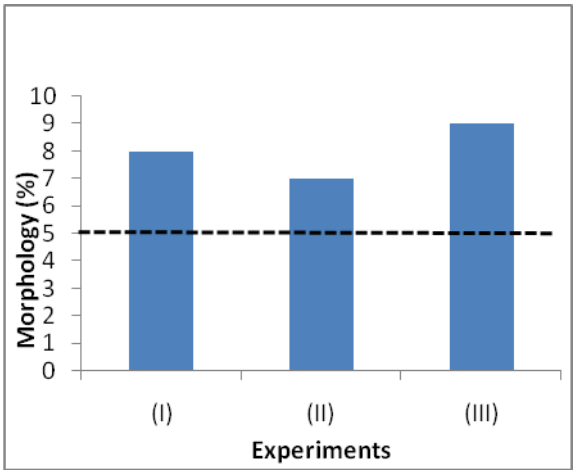

Figure 6.3: Sperm parameters determined for donors who participated in the experiments

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ADDENDUM F: Interactions with Gabonese citizens seeking information regarding fertility clinics in South Africa

Introduction

Case A

On 25 July 2014 while in Gabon, Mrs A (35 years old) telephonically contacted the student and told him that she had obtained his cell number from a gynaecologist who practicised at the General Hospital in Libreville. She requested to see him since she was looking for information on possibly travelling to South Africa for *in vitro* fertilization (IVF) treatment. An arrangement was made to meet at a coffee shop in Mbolo shopping centre in Libreville. Personal communication between the student and Mrs A followed, on 26 July 2014 in Mbolo from 15:00-17:00.

At the meeting Mrs A expressed her thanks and appreciation for the opportunity to explain what she was experiencing. “It is not easy to find people who can listen to you and understand you. My gynaecologist asked me to contact you in order to have additional information regarding infertility treatment”. Mrs A went on to explain that she had been married for 6 years, she and her husband had been trying to have a child for 4 years. She told the student that she had consulted a gynaecologist at the Hospital Centre in Libreville. After the test, the gynaecologist explained to her that her uterus appeared to be “fine”, but that only one of her “trompe[K]” (Fallopian tube) was blocked. Despite this, she could fall pregnant. The gynaecologist then requested to meet her husband at the next visit. Mrs A explained that she was totally confused because her husband’s family always blamed her for their childlessness and not her husband. She said: “In January 2014 his mother gave me 1 year to fall pregnant. If not, she will organize a second wife for her son. During the past 2 years, I have practised traditional rituals and ceremonies in Lambarene (a town located 75 kilometres south of the equator), consulting a “Nganga[L]” who told me that I was suffering from a spiritual disease and needed a spiritual bath. In February 2014, after consulting with the

[K] Trompe(s)= Fallopian tube(s)
[L] Nganga= Traditional healer who is able to treat infertility

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gynaecologist I asked my husband to go with me for another consultation, he refused. I started questioning myself, on why is he scared to go with me to the doctor."

Mrs A continued: "On the 22nd of May while cleaning the house, I found a spermiogramme done in Cameroon for my husband. At first I didn't understand. I went back to the gynaecologist with the spermiogramme, and he told me that my husband has a problem with his sperm". Mrs A said that she had confronted her husband who agreed that the test was his but he indicated that nothing was wrong; therefore he did not want any help. Mrs A added that because she respected her husband, she had promised him to keep everything secret. She suggested that they should go for IVF treatment in Cameroon or Ghana, but he did not want to go. She then requested information on infertility clinics in South Africa, relating to costs and success rates. The student forwarded the requested information via e-mail to Mrs A. No further communication followed.

Case B

On 29 July 2014, Ms B (32 years old) a family friend, called and said she wanted to share her experiences with the student so that he could provide information that would guide her. They met at the student's at home in Libreville, between 11:00-12:00. Ms B informed the student that her boyfriend was 37 years old. In 2009, due to gynaecological complications, her left "trompe" (Fallopian tube) was removed in Canada. She had tried to fall pregnant since 2011, but so far had been unsuccessful. The student enquired whether she had consulted a gynaecologist since then. Ms B confirmed that she had seen a gynaecologist in 2012 who informed her that her right "trompe" was blocked. Ms B said that she was supposed to get married in June 2013, but her partner's family totally refused to give consent because they wanted her to have a child before marriage. She continued: "My companion belongs to the "Fang tribe[M]" and you know how the ability to have children, is very sacred. My boyfriend really loves me. He is patient, and understands me. I have heard so many stories coming from my boyfriend's family: implying that I am not spiritually clean and I use traditional medicine.

[M] Fang= Traditional tribe where the bride price increases with the number of children the woman produces

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According to my boyfriend's family these are the reasons why I cannot conceive. The latest story is saying that I am now HIV positive. My partner is constantly fighting with his sisters because of what they are saying about me".

The student then enquired whether her family had supported her, and how the negative comments had impacted on her. Ms B said that her father had died in 2006 and her mother in 2010. The only family member, who supported her, was her younger sister who was 28 years old. She said that she had always dreamt of having her own children. In January 2014, her boyfriend's friend tried to convince him to go for the "Iboga ritual[N]", in order to find out whether her sterility was due to a spiritual problem.

The student then asked for an explanation of an Iboga ritual. Ms B explained that the Iboga is a traditional, hallucinogenic root which facilitates communications with the ancestors. According to Ms B: "The Iboga root would have helped me to ask my ancestor to unblock me and let me have a child." She then confessed that she was really scared. She verbalised her belief system as follows: "Since I was born I have never been to or performed any traditional ceremony. My family is Catholic, we only believe in God, not in roots. I believe that there is medical possibility to treat my problem. I really need your help." Ms B enquired about clinics in South Africa and mentioned that she had heard of a clinic in Cameroon. She said that she was unsure because the latter mentioned clinic was very expensive and the treatment seemed no better than that offered in South Africa. Cost is however not an issue for them, as she is of opinion that they can afford the treatment. The student then provided her with contact details of an assisted reproduction unit at Pretoria East Hospital. No further information was obtained from Ms B.

Case C

On 27 July 2014, Mr C (29 years old) telephonically contacted the student and asked to meet with the student at Okala (suburb of Libreville). The meeting took place in the afternoon. During the interaction Mr C explained that he had dated his ex-girlfriend for 6

[N] Iboga= Hallucinogenic plant medicine used in several rituals in Gabon

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years (from 2006 to 2012). He indicated that their relationship had deteriorated from the time she said that she wanted to have a child before marriage. His girlfriend was the only daughter in the family and her mother enquired constantly when they would have a child. During this period together they tried to have a baby. Mr C indicated that they had consulted a gynaecologist in 2009. The doctor was of opinion that there was nothing wrong with his girlfriend, but he advised the man to go for a spermiogramme test. In 2011 during a work trip to Senegal, he visited a male clinic in Dakar. After his consultation with a specialist, he obtained the results of the spermiogramme, which disclosed poor sperm quality. The doctor told him that the condition could be due to the use of steroids. Mr C said that he wanted to keep the information secret and he indicated that "my ex-girlfriend didn't know anything about me going to the male clinic."

The student then asked Mr C for how long and why he using steroids. Mr C said that he had been using steroids since 2000, when he started going to gym. He added: "I can say that I was abusing steroids". He specified that he stopped using steroids in 2011. In 2012 because he was unable to deal with the pressure to produce a child, he decided to break up with his girlfriend. Six months after the break-up he met his current wife and married her in 2013. His family was against the union because they didn't know why he had broken up with his ex-girlfriend. Mr C said: "Personally I took this decision of marrying her because of my past. I still love my ex-girlfriend but there is nothing I can do." Mr C said that his wife showed more understanding of his problem, therefore he was willing to obtain medical advice. Mr C asked the student for information on fertility clinics in Pretoria or Johannesburg in South Africa, which the student provided. A consultation was booked for Mr C and his wife for April 2015 at the fertility clinic in Pretoria. Not being able to fall pregnant after the procedure, Mr C and his wife then decided to travel to a clinic in Canada where ART treatments are less expensive than in South Africa.

Case D1

Mrs D1, a 34 year old Gabonese nurse, and her husband obtained the student's cell number from a mutual friend, and asked to meet the student. They met at the Libreville

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International Airport, when the student was waiting for a flight to South Africa. During this brief meeting Mrs D1 expressed her difficulty to have a baby and requested further contact information, which the student provided.

Two days after his arrival in Pretoria, the student received an e-mail from Mrs D1 saying that she had contacted fertility clinic X and R45,000 (€ 3,032)[O] was required to pay for ART. As she was very concerned about the cost, the logistics and the facilities, she sought a second opinion. The student advised Mrs D1 visits another fertility clinic, where the cost of IVF was more affordable (R35,000; € 2,353). In February 2015 Mrs D1 came to South Africa with her husband for infertility treatment. The student assisted her with transport and accommodation.

Mrs D1 told the student that in 1998 she had had her first ectopic pregnancy. She was admitted to hospital, and given medication. Days later she was operated on. What she did remember was the doctor telling her that she could still have kids. In 1999, she had a beautiful baby boy. In 2003, she met the man who was to become her husband in 2010. Meanwhile, in 2005, they had a boy (the first one with him). After getting married in 2010 they tried to have another baby but with no success.

In 2011 she had 2 miscarriages. According to her doctor, there was nothing wrong. In 2013, she got pregnant. Days later, she started feeling that something was not right. She did a pregnancy test which turned out to be negative. She did not feel as if she was pregnant. Her husband was really shocked. When the doctor told them that it was another ectopic pregnancy, she wanted to end her life. “My faith, everything that made me who I am, was taken away from me.” The doctor gave her an injection as treatment for the ectopic pregnancy.

A couple of days later she was rushed to hospital because one of her Fallopian tubes had ruptured. The doctor reassured her that she could have children with only one tube.

[O]All costs were obtained in ZAR, with an exchange rate of €1 to ZAR 14, 87 (02/09/2015). All calculations include 14% Value Added Tax (VAT).

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Mrs D1 added: “I think at one point I didn’t want more children. But I knew my husband wanted this more than anything. So, I couldn’t give up.” In 2014, when she went to see a new gynaecologist in Libreville, she was told about the IVF treatment.

Mrs D1 said: “Four years of emotional and physical pain. If it was not for my husband I would have given up, but he supported me. At one point I told him to go find another woman that could give him babies. He got very mad at me because of what I was saying. It is frustrating when people ask us how come we don’t have other kids yet. They don’t realize the everyday struggle we are going through. Not a day passes we don’t think about it. I thank God for having a supportive and understanding husband. We couldn’t tell anyone about going through this procedure. I didn’t want them to look at me differently, seeing me as the woman who could not give children to their son. I believe that experience like this either breaks or makes a couple.”

In addition, Mrs D1 provided an estimate of the total ART attempt expenditure incurred for the IVF treatment during this time. Including the IVF procedure cost, travelling expenses, accommodation, and general amenities, the couple used R110 000 from their savings to cover costs. The procedure was successful and Mrs D1 is currently expecting a baby boy.

Case D2

Mr D2 (38 years old) and his wife (31 years old), contacted the student on 28 of July 2014. The couple obtained the student’s cell number from a gynaecologist in Gabon. During the telephonic conversation Mr D2 and his wife explained that although they were already in contact with a fertility clinic in Cameroon, they would like to know about other clinics in South Africa. The student provided them with the details of the cost of IVF in two fertility clinics in Pretoria. There was no further communication between the student and the couple.

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ADDENDUM G: Registered Health Professionals in Gabon

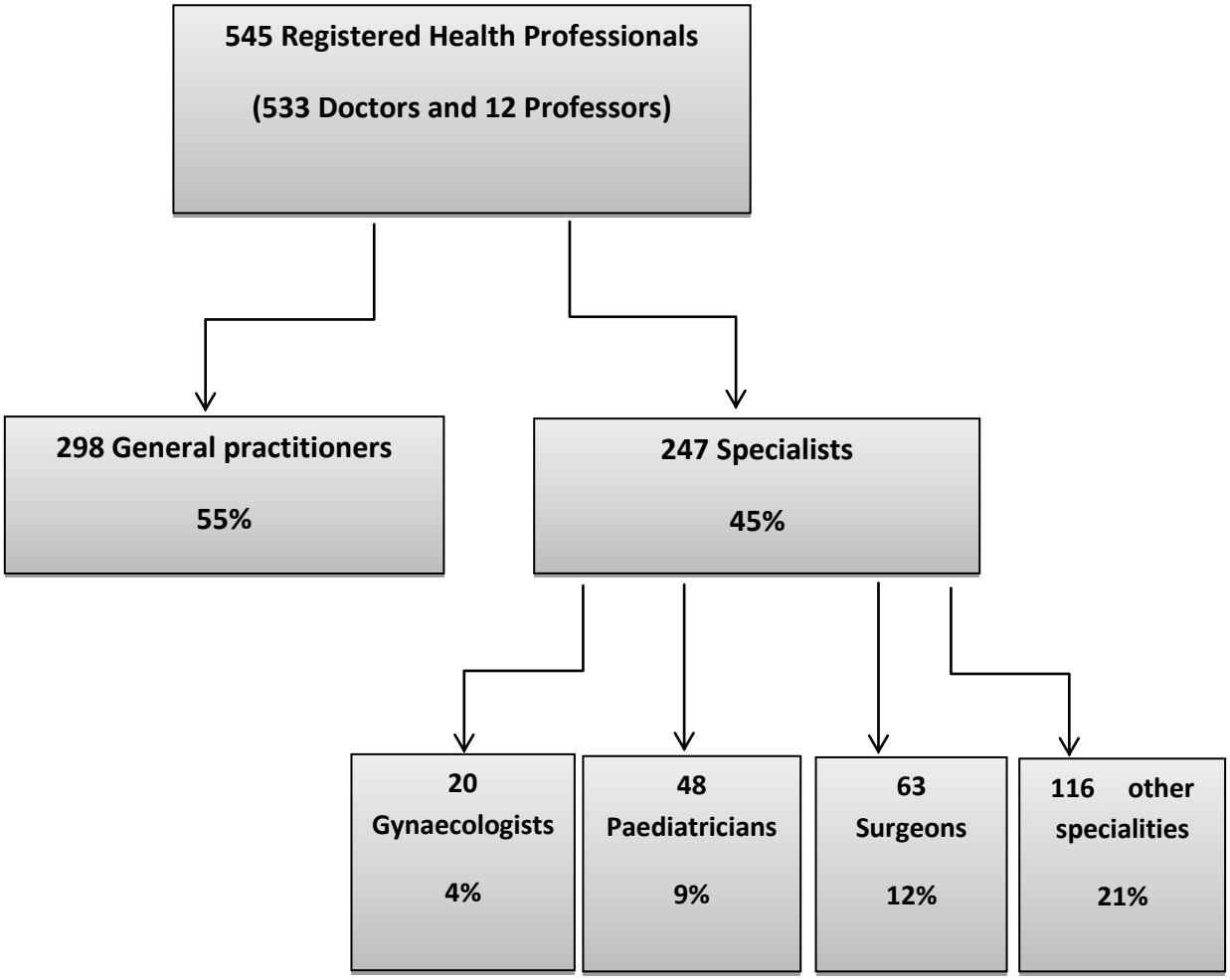

Figure 6.4: Diagram depicting the number of general practitioners and specialists registered as members of the Health Professional Association in Gabon[255]

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ADDENDUM H: Research outputs

In preparation

- Moungala L, Huyser C. Infertility in Gabon: A survey to determine diagnostics and medical support to patients. *International Perspectives on Sexual and Reproductive Health*. (Submitted)- Attached
- Moungala L, Boyd L, Huyser C. Sperm processing: a simplified swim-up method. *Andrologia.* (In preparation)

Oral presentation

- Moungala L, Huyser C. Infertility in Gabon: A survey to determine diagnostics and medical support to patients. South African Society of Reproductive Medicine and Gynaecological Endoscopy (SASREG) National Congress 2015 (30 October-01 November), Johannesburg, South Africa.- (Abstract attached)

Poster presentation

- Moungala L, Boyd L, Huyser C. Sperm processing: a simplified swim-up method. South African Society of Reproductive Medicine and Gynaecological Endoscopy (SASREG) National Congress 2015 (30 October-01 November), Johannesburg, South Africa. (Abstract attached & poster)

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PLEASE INDICATE PREVERANCE WITH X	**ORAL presentation**	**x**
	POSTER presentation	

TITLE:
Infertility in Gabon: A survey to determine diagnostics and medical support to patients

AUTHORS:
L Moungala & C Huyser

AFFILIATION:
Reproductive Biology Laboratory, Department of Obstetrics and Gynaecology,
University of Pretoria
Steve Biko Academic Hospital

INTRODUCTION:
Africa has been reported to have the highest incidence of infertility globally, with Gabon experiencing one of the lowest birth rates in central Africa. Access to fertility clinics in Africa is limited, and treatment is mostly restricted to private settings. Since no assisted reproduction technology (ART) services are available in Gabon, sub-fertile couples with access to financial means have to travel abroad for diagnostic and therapeutic ART treatment.

AIM:
To conduct a survey among gynaecologists practicing in Libreville (Gabon) to determine the level of infertility assistance available and the feasibility of establishing an intrauterine insemination (IUI) programme in Gabon.

MATERIALS AND METHODS:
The Gabonese Society of Obstetricians Gynaecologists and Reproduction (SGORR) assisted the investigator to identify the 20 practicing gynaecologists in Gabon. The participants were visited twice to handout the questionnaires personally with concurrent interviews/interpretations and then collections of the questionnaires. This descriptive study was conducted in private and public hospitals in Libreville, between July and December 2014.

RESULTS:
All participants reside in Libreville, the political and administrative capital of Gabon. Approximately one third of nationals live in Libreville which is a coastal city controlling export of timber and crude oil. Seventeen (85%) surveys were completed, with three gynaecologists not available during the study. Between 25-50 patients visit gynaecological practices per month and nearly half (45%) of these consultations were infertility related. Male patients were referred to 4 different pathology laboratories in Libreville, where basic semen analyses were performed without any microbiology testing. Respondents (65%) referred female patients for further infertility workup and treatments in Cameroon and Ghana. Approximately one-third of all couples are unable to access further medical assistance.

CONCLUSION:
Participants were in agreement that basic infertility diagnosis/treatment and training programmes including the establishment of ART services are desperately needed in Libreville. Capacity development through basic infertility diagnostic training courses, combined with standardization of spermatological evaluations in the private and public health sectors should be the focal points in the roll-out of fertility treatment in Gabon. Since tubal obstructions are a primary aetiology factor in females in Gabon, a stand-alone IUI program will be ineffective. A reproductive health program is needed to prevent infertility associated pathologies and also a range of ART procedures to assist infertile couples. Gabon, i.e. Libreville has the infrastructure, gynaecological expertise with patient demand and capability to serve as an appropriate base for the advancement of reproductive treatment facilities, in collaboration with ART initiatives from developed countries.

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PLEASE INDICATE PREVERANCE WITH X	**ORAL presentation**	
	POSTER presentation	**x**

TITLE:
Sperm processing: a simplified swim-up method using a 10ml syringe

AUTHORS:
L Moungala, L Boyd, C Huyser

AFFILIATION:
Reproductive Biology Laboratory,
University of Pretoria,
Steve Biko Academic Hospital.

INTRODUCTION:
The simplicity and cost-effectiveness of intrauterine insemination (IUI) validates the procedure as a first-line treatment for infertility. Several sperm washing techniques are used for IUI, depending on sperm parameters. The challenge lies in the development of an efficient, but affordable and simple sperm purification method, with minimal equipment and procedural steps.

AIM:
This study investigated a simplified sperm swim-up (SW) method and consisted of two sections: i.e. comparing three volume syringes (5ml - SW-5; 10ml - SW-10 and 20ml - SW-20) combined with the standard swim-up procedures. Subsequent experimentation evaluated thereafter the procedure that resulted in the best harvested sperm sample versus processing using the commercial SEP-D kit.

MATERIALS AND METHODS:
Semen samples (n=25 for baseline & n=20 for subsequent experimentation) were obtained from patients and donors participating in the Reproductive Biology Laboratory's donor registry programme. Only samples from HIV-1 sero-negative males with a sperm morphology ≥ 4%, >15x 10^6 sperm/ml, progressive motility >40% and 3 ml volume were included in the study:

(I) Each semen sample was divided into three equal aliquots, and prepared using the SW-5, SW-10 and SW-20. A volume of 1 ml PureSperm™ Wash was aspirated in the syringe followed by 1 ml of semen, placed at 45° angle and incubated for 60 minutes at 37°C.

(II) Semen samples were split into two equal aliquots and processed using the SW-10 (10 ml syringe) procedure compared to the commercial SEP-D kit.

Post-processed semen analyses included: sperm concentration and motility for both experiments while sperm morphology, vitality, plasma membrane integrity and DNA fragmentation (Halosperm® G2) were also evaluated in the second experiment.

RESULTS:
The results indicated that semen processed using the SW-10 method resulted in a significantly higher sperm concentration (28.47 x10^6/ml) and progressive motility (77.54%), when compared to the SW-5 (15.97 x10^6/ml and 50.62%) and SW-20 (19.16 x10^6/ml and 46.52%) procedures ($p<0.001$). When comparing the post-processed sperm samples obtained through the SW-10 procedure compared to the commercial SEP-D method, a significantly increase ($p<0.005$) in sperm motility (87.05% vs 75.35%) and concentrations (17.10 x10^6/ml vs 14.35 x10^6/ml) were observed. The SW-10 method yielded statistically higher percentage of spermatozoa with non-damaged plasma membrane (82.31% vs 72.00%, for Eosin and Nigrosin test and 79.47% vs 70.05% for the Hypo-osmotic swelling test) ($p<0.0001$), as well as less DNA fragmentation (13.70% vs 23.20%) ($p<0.0001$). However, no statistically differences were observed with regards to normal morphology ($p<0.479$).

CONCLUSION:
The SW-10 method yielded higher quality spermatozoa in comparison with the commercial SEP-D kit. This method is simplistic, with no centrifugation steps and few disposables needed. The method can be easily implemented in applicable patients in developing countries with limited resources. This research can be considered as an office-based procedure or part of a series of low-cost ART procedures designed for use in resource-constrained settings.

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SPERM PROCESSING:
A simplified swim-up method using a syringe

INTRODUCTION

- Intrauterine insemination (IUI) is generally used as the first line treatment for infertility, due to the simplicity and affordability of the procedure[1]
- Optimizing sperm quality through sperm processing methods that are equivalently simple, affordable and effective, are essential in developing countries, where basic ART procedures are infrequently accessible
- According to current literature, 1x10^6 inseminating motile count (IMC) and sperm morphology are the most critical parameters contributing to IUI success[2]

AIM

To develop a simplified swim-up method comparing generally available latex-free syringes of specific volumes for sperm processing, also against the commercially available SEP-D kit (SureLife).

MATERIALS AND METHODS

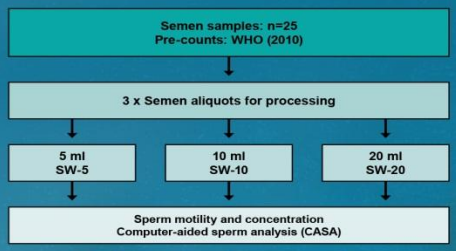

Figure 1: Diagram of the simple sperm swim-up method using syringes of different volumes

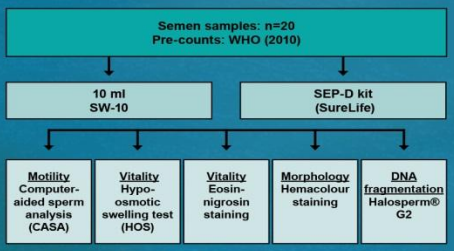

Figure 2: Flow diagram of sperm processing and evaluation methodology comparing the SW-10 technique and the SEP-D kit

RESULTS

Table 1: Comparison of CASA (motility and concentration) outcome obtained after the SW-5, -10 and -20 methods

Parameters	SW-5 (a)	SW-10 (b)	SW-20 (c)	p-value (95% CI)		
	Mean (Standard deviation)			(a vs b)	(a vs c)	(b vs c)
Concentration (10^6/ml)	15.97 (8.04)	28.47 (8.83)	19.16 (9.12)	<0.001 (0.173;0.312)	0.012 (0.022;0.165)	<0.001 (0.105;0.157)
Rapid progressive motility (%)	50.62 (10.58)	77.54 (9.02)	46.52 (10.27)	<0.001 (0.151;0.351)	0.014 (0.0261;0.043)	<0.001 (0.243;0.441)
Total motility (%)	74.17 (12.40)	83.12 (10.43)	67.03 (12.87)	0.014 (0.016;0.143)	0.018 (-0.146;-0.012)	<0.001 (0.092;0.131)
TMC[3] ($x10^6$)	2.42 (14.05)	6.62 (12.46)	2.67 (14.88)	<0.001 (0.244;0.401)	0.479 (-0.049;0.106)	<0.001 (0.115;0.171)

Figure 3: Disposable Promex™ (5 and 20 ml) and 10 ml from Kendall monoject™ syringes used for the current study

Table 2: Summary of sperm parameters (motility, concentration, morphology, plasma membrane integrity and DNA fragmentation) using the simplified swim-up method and commercial SEP-D kit

Parameters	Motility (%)	Concentration (10^6/ml)	Plasma membrane tests (%)		Morphology (%)	DNA fragmentation (%)
			HOS	Eosin-Nigrosin		
Simplified swim-up Mean (SD)	87.05 (4.18)	17.10 (5.95)	79.47 (6.31)	82.31 (5.15)	9.75 (2.83)	13.70 (3.85)
SEP-D Mean (SD)	75.35 (4.86)	14.35 (4.35)	70.05 (9.98)	72.00 (8.56)	8.10 (1.66)	23.2 (6.77)
p-value (95% CI)	p<0.0001 (0.111;0.163)	p<0.0001 (0.123;0.177)	p<0.0001 (0.114;0.172)	p<0.0001 (0.125;0.176)	p=0.42 (0.116;0.164)	p<0.0001 (0.098;0.162)

Figure 4: Commercially available SEP-D kit

DISCUSSION

- The SW-10 method yielded significantly higher sperm motility and concentration overall (p>0.05)
- The SW-10 method provides optimal semen to media interface where the PureSperm® Wash media support sperm harvesting superbly
- Without requiring sophisticated disposables or equipment, the SW-10 method is easy to perform and can therefore be implemented in developing countries, providing access to more affordable ART procedures

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