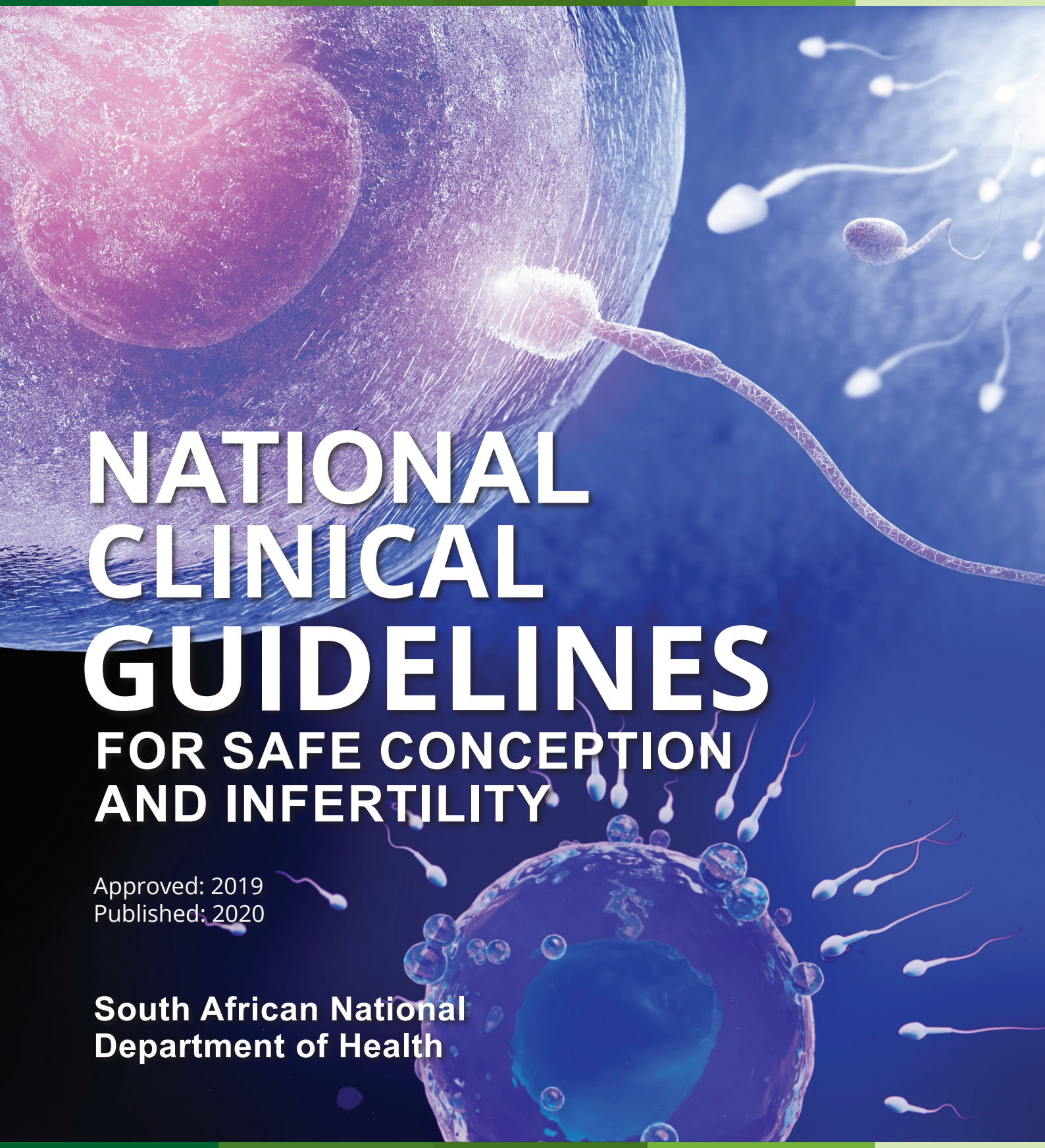

NATIONAL CLINICAL GUIDELINES

FOR SAFE CONCEPTION AND INFERTILITY

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for Safe Conception and Infertility

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Contents

Tables

Figures

Foreword

The clinical guideline on safe conception and infertility is responding to the National Health Insurance (NHI) principle of universal health access for all. It aims to promote, protect, and ensure the full and equal enjoyment of all human rights and fundamental freedoms by all, including persons with disabilities, and to promote respect for their inherent dignity. The guideline is also addressing the needs of lesbian, gay, bisexual transgendered and intersex, (LGBTQI+) population in the context of their reproductive rights that has been lacking. Currently, the demand for services to help these communities to build a family is on the rise. This requires an adequate and rights-based response from the country and the health care providers as a whole.

The guideline is intended to present an approach to prevention, management and psychosocial care of people seeking infertility treatment, and seeks to fill the clinical and policy gap in South Africa, as outlined in the 2019 Integrated Sexual and Reproductive Health Policy document. In South Africa, one in six couples experience some form of infertility [(1)]. The estimates of prevalence are not very accurate because some cases are not reported.

These guidelines provide guidance to all fertility clinic staff - doctors, nurses, midwives, counsellors, social workers, psychologists, embryologists, and administrative personnel -, who have contact with patients and make decisions regarding their care, and can deliver routine fertility care and/or make referrals to specialist care. It also allows the advocacy groups and other concerned government departments to create a social and medical cohesion to address various myths and misconceptions around the infertility challenge.

Services for infertility should be accessible, and include information, diagnosis, assessment, and comprehensive fertility services. These guidelines will offer best practice and evidence-based management and care of people with infertility in South Africa. It will provide recommendations for holistic care for infertility, including prevention, evaluation, treatment, and psychological care.

Dr A Pillay
Acting Director-General: Health
November 2019

Acronyms

AFC	Antral follicle count
AMH	Anti-Mullerian Hormone
ART	Assisted reproductive technologies
ARV	Antiretroviral
BBV	Blood-borne viruses
BMI	Body mass index
CASA	Computer-Assisted Sperm Analyzer
ESGE	European Society of Gynecological Endoscopy
ESHRE	European Society of Human Reproduction and Embryology
ET	Embryo transfer
FSH	Follicle-stimulating hormone
GIFT	Gamete intrafallopian transfer
HAART	Highly active antiretroviral therapy
HCG	Human chorionic gonadotropin
HEPA	High-efficiency particulate air
HIV	Human immunodeficiency virus
HSG	Hysterosalpingography
ICMART	International Committee for Monitoring Assisted Reproductive Technologies
ICSI	Intracytoplasmic sperm injection
IUI	Intrauterine insemination
IVF	In vitro fertilisation
LGBTQI+	Lesbian, gay, bisexual, transgender, queer and intersex
LH	Luteinizing hormone
NTD	Neural tube defects
OHSS	Ovarian hyperstimulation syndrome
PCOS	polycystic ovary syndrome
PGT	Preimplantation genetic testing
PID	Pelvic inflammatory disease
PMTCT	Prevention of mother-to-child transmission
SAHIVCS	Southern African HIV Clinicians Society
STI	Sexually transmitted infection
TB	Tuberculosis
TESA/TESE	Testicular sperm aspiration/ extraction
TSH	Thyroid-stimulating hormone
UAE	Uterine Artery Embolisation
VL	Viral load
WHO	World Health Organization

Definitions of Terms

An internationally accepted glossary of terms is adopted to ensure consistency and universal communication, as recommended by the International Committee for Monitoring Assisted Reproductive Technologies (ICMART) [2].

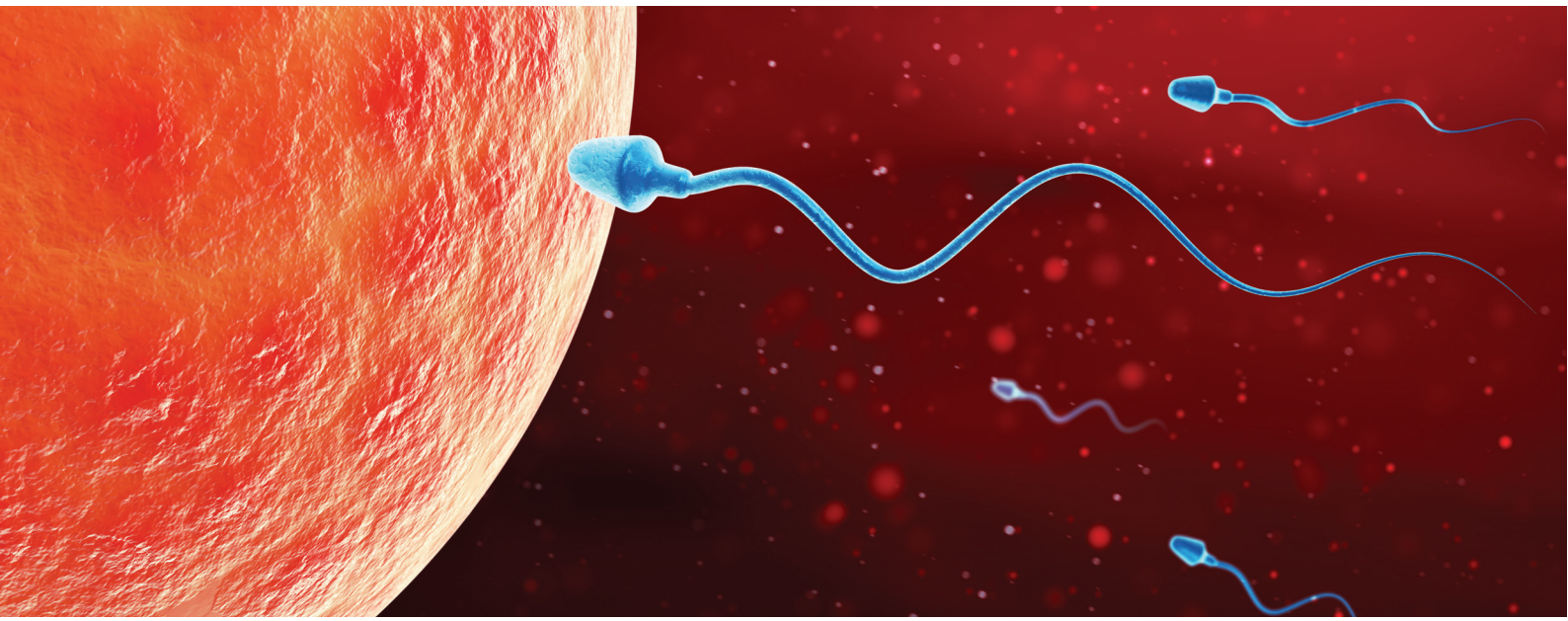
Term	Definition
Aliquot	A sample with a mass or volume that is a known fraction of the whole.
Aneuploidy	An abnormal number of chromosomes in a cell. The majority of embryos with aneuploidies are not compatible with life.
Assisted Reproductive Technology (ART)	All interventions that include the in vitro handling of both human oocytes and sperm, or of embryos, for the purpose of reproduction. This includes, but is not limited to, in vitro fertilisation (IVF), embryo transfer (ET), intracytoplasmic sperm injection (ICSI), embryo biopsy, pre-implantation genetic testing (PGT), assisted hatching, gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer, gamete, and embryo cryopreservation, semen, oocyte, and embryo donation, and gestational carrier cycles. Reduced percentages of motile and morphologically normal sperm in the ejaculate below the lower reference limit. The age by which a child is defined differs according to the the context for which the child is seeking services. For example, definitions may vary for a case of sexual assault, or a girl seeking access to TOP, HIV testing, contraception or other services. The appropriate definition should be determined on a case-by-case basis depending the individual's care needs Reduced percentage of motile sperm in the ejaculate below the lower reference limit.
Asthenoteratozoospermia	Reduced percentages of motile and morphologically normal sperm in the ejaculate below the lower reference limit.
Asthenozoospermia	Reduced percentage of motile sperm in the ejaculate below the lower reference limit.
Azoospermia	The absence of spermatozoa in the ejaculate.
Cryopreservation	The process of slow freezing or vitrification to preserve biological material (e.g., gametes, zygotes, cleavage-stage embryos, blastocysts, or gonadal tissue) at extremely low temperatures.
Dewar	An insulated container used to store liquefied gases, having a double wall with a vacuum between the walls and silvered surfaces facing the vacuum.
Fecundability	The probability of pregnancy, during a single menstrual cycle in a woman with adequate exposure to sperm and no contraception, culminating in a live birth. In population-based studies, fecundability is frequently measured as the monthly probability.
Fecundity	Clinically defined as the capacity to have a live birth.

Hypogonadotropic hypogonadism	Gonadal failure associated with reduced gametogenesis and reduced gonadal steroid production due to reduced gonadotropin production or action.
Infertility	A disease characterised by the failure to establish a clinical pregnancy after 12 months of regular, unprotected sexual intercourse or due to an impairment of a person's capacity to reproduce either as an individual or with his/her partner. Fertility interventions may be initiated in less than one year based on medical, sexual and reproductive history, age, physical findings, and diagnostic testing. Infertility is a disease, which generates disability as an impairment of function.
Intracytoplasmic sperm injection (ICSI)	A procedure in which a single spermatozoon is injected into the oocyte cytoplasm.
Obstructive azoospermia	Absence of spermatozoa in the ejaculate due to occlusion of the ductal system.
Oligospermia	A term for low semen volume now replaced by hypospermia to avoid confusion with oligozoospermia.
Oligozoospermia	Low concentration of spermatozoa in the ejaculate below the lower reference limit.
Oocyte	The female gamete (egg).
Ovarian hyperstimulation syndrome (OHSS)	An exaggerated systemic response to ovarian stimulation. It may be classified as mild, moderate, or severe according to the degree of abdominal distension, ovarian enlargement, and respiratory, hemodynamic, and metabolic complications.
Ovarian reserve	A term generally used to indicate the number and/or quality of oocytes, reflecting the ability to reproduce.
Ovulation	The natural process of expulsion of a mature egg from its ovarian follicle.
Primary childlessness (Primary infertility)	A condition in which a person has never delivered a live child, or has never been a legal or societally-recognised parent to a child.
Primary female infertility	A woman who has never been diagnosed with a clinical pregnancy and meets the criteria of being classified as having infertility.
Primary male infertility	A man who has never initiated a clinical pregnancy and meets the criteria of being classified as infertile.
Secondary involuntary childlessness (Secondary infertility)	A condition in a person with a child wish, who has previously delivered a live child, or is or has been a legal or societally-recognised parent to a child. A major cause of secondary involuntary childlessness is infertility.
Secondary female infertility	A woman unable to establish a clinical pregnancy but who has previously been diagnosed with clinical pregnancy.

Secondary male infertility	A man who is unable to initiate a clinical pregnancy, but who had previously initiated a clinical pregnancy.
Semen analysis	A description of the ejaculate to assess the function of the male reproductive tract. Characteristic parameters include volume, pH, concentration, motility, vitality, the morphology of spermatozoa, and the presence of other cells.
Subfertility	A term that should be used interchangeably with infertility.
Testicular sperm aspiration/extraction (TESA/TESE)	A surgical procedure involving one or more testicular biopsies or needle aspirations to obtain sperm for use in IVF and/or ICSI.
Unexplained infertility	Infertility in couples with apparently normal ovarian function, fallopian tubes, uterus, cervix, and pelvis and with adequate coital frequency; and normal testicular function, genito-urinary anatomy, and a normal ejaculate. The potential for this diagnosis is dependent upon the methodologies used and/or those methodologies available.
Varicocoele	A venous enlargement in the testicular pampiniform plexus.

Preamble

The problem of infertility has long been identified as a priority by clinicians in South Africa. To date, it has not been defined as a disease by the policymakers in South Africa in accordance with the World Health Organisation (WHO) definition. This has resulted in services that are inaccessible, scarcely available, and at prohibitive costs. At the time of going to press, there are only two public sector hospitals that offer the full range of fertility treatment, with two additional public hospitals offering services in a public-private partnership agreement with private clinics. These guidelines seek to fill the clinical and policy gap in South Africa, where the policy component is outlined in 2019 Integrated Sexual and Reproductive Health Policy for South Africa.



Section 1. Introduction

What is infertility, and how does it affect people?

The WHO has defined infertility as a “disease of the reproductive system" resulting in a disability [3]. Infertility is the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected intercourse. It is classified as primary or secondary depending on whether there has been a previous pregnancy.
Infertility is a global problem that transcends race, religion, culture, class, and economic status. It has an impact on the couple’s psychological, financial, medical, and social wellbeing. People affected by this disease have higher rates of depression, anxiety, suicide, divorces, intimate partner violence, and societal stigma. It should be classified as a disability and should be protected under the United Nations Convention on the Rights of Persons with Disabilities, adopted in 2006, whose purpose was to “promote, protect and ensure the full and equal enjoyment of all human rights and fundamental freedoms by all persons with disabilities, and to promote respect for their inherent dignity” [4].

Burden of disease

The burden of the disease worldwide is high. It is estimated that 34 million women, predominantly from developing countries, are infertile, and infertility in women is ranked the fifth highest serious global disability [3]. In South Africa, one in six couples experience some form of infertility [1]. The estimates of prevalence are not very accurate because some cases are not reported.

LGBTQI+ communities

The needs of the lesbian, gay, bisexual, transgender, and intersex (LGBTQI+) population in the context of their reproductive rights, has been lacking. Currently, the demand for services to help this community to build a family is on the rise. This requires an adequate and rights-based response from the country and the healthcare providers as a whole.

Assisted reproduction technologies (ART)

ART has been developed to assist infertile couples in achieving a pregnancy and in becoming parents [5, 6]. Through the widespread global usage of ART, an estimated eight million pregnancies have been achieved worldwide [7]. However, access to these services remains a challenge, largely due to a lack of awareness, availability of treatment, and prohibitive costs.

Stigma

The social stigma associated with the problem of infertility is an added barrier for couples, preventing them from seeking help. Compounded by various myths and misconceptions, the infertility challenge is both epidemiological and social in nature. Fertility services should be accessible, and include information, diagnosis, assessment, and comprehensive fertility services.

1.1 Purpose and scope

Why this guidance has been produced

These guidelines present an approach to prevention, management, and psychosocial care of people seeking infertility treatment. The purpose is to provide uniformity and standardisation of care of an infertile patient to ensure highly effective and cost-effective treatment.

Target users

These guidelines provide guidance to all fertility clinic staff - doctors, nurses, midwives, counsellors, social workers, psychologists, embryologists, and administrative personnel - who have contact with patients and make decisions regarding their care, and can deliver routine fertility care and/or make referrals to specialist care. They should offer a basis for advocacy by the advocacy groups and other concerned government departments to create social and medical cohesion amongst all the stakeholders.

Scope of the guidelines

These guidelines will offer best practice and evidence-based management and care of people with infertility in South Africa. They provide recommendations for holistic care for infertility, including prevention, evaluation, treatment, and psychological care.

Section 2. Safe conception care

Safe conception care recognises the importance of preserving fertility as well as providing preconception care. As illustrated in Figure 1. below, family planning should take a holistic reproductive life-span approach, rather than focusing only on contraception. Patients should be counselled not only about prevention of pregnancy but also how to prevent infertility. This will reduce the interventions needed to correct the preventable causes of infertility. The healthcare providers should use every opportunity to identify the women at risk of infertility and manage that risk appropriately.

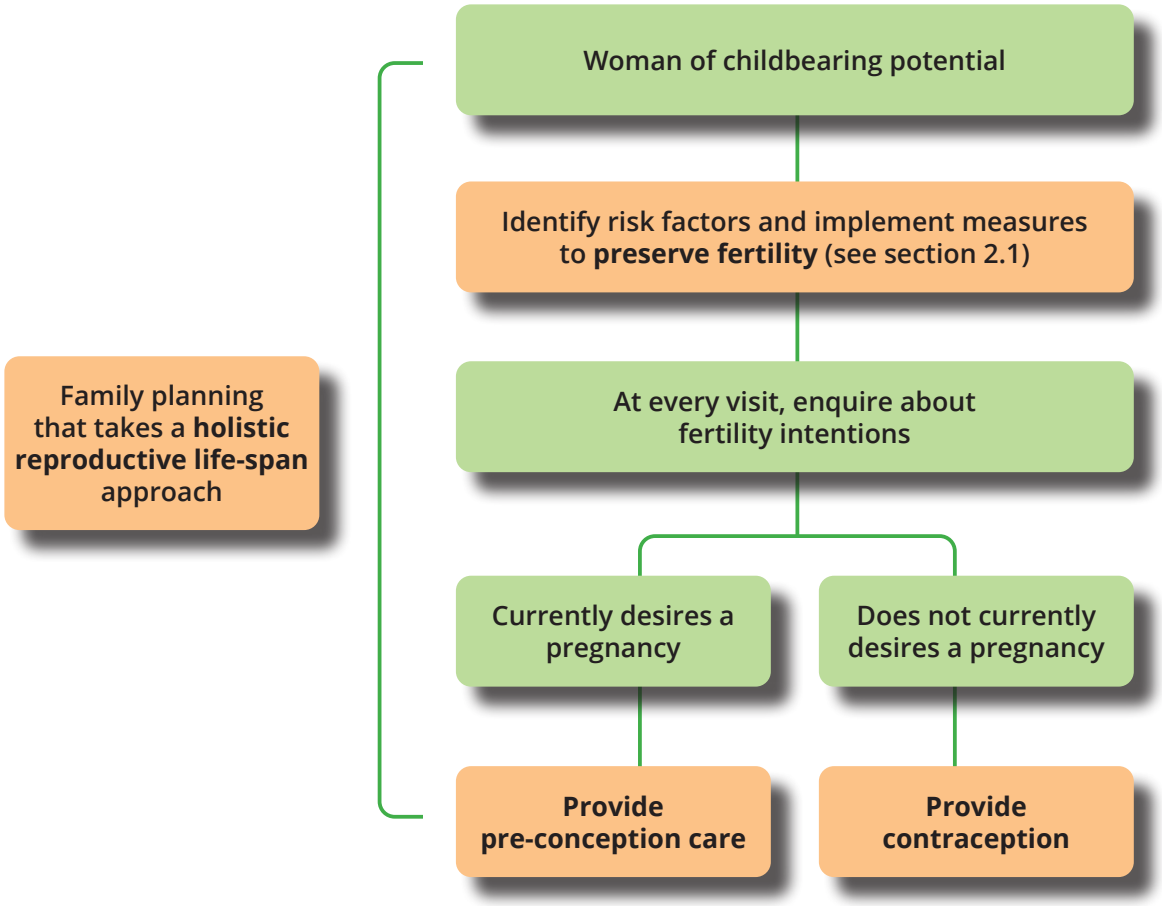


Figure 1. The holistic reproductive lifespan approach to family planning

2.1 Prevention of infertility

Part of the history taking for all women should include their **future fertility plans** and to **screen for lifestyle factors that may play a role in infertility**. The contribution of lifestyle factors in negatively impacting on fertility is shown in Table 1[8]. Counselling should include health-promoting practices and lifestyle modification. The following are evidence-based prevention strategies for infertility:

- Safe sexual practices and prevention of sexually transmitted infection (STI) with screening for STI at every visit
- Maintaining a healthy weight. There is evidence that both female and male fertility are decreased by being either overweight with a body mass index (BMI) of > 25 kg/m^2 or underweight with a BMI < 20 kg/m^2 [9].
- Clients should be advised that fertility declines with age. Options to preserve fertility can be discussed with the healthcare provider.

- Avoid exposure to tobacco products, illicit drugs, and excessive alcohol
- Avoid recreational anabolic steroids in men as these may affect semen parameters
- Take precautionary measures when dealing with certain environmental toxins, pesticides and other chemicals
- Access safe termination of pregnancy within the healthcare services

Table 1. Evidence-based lifestyle factors that affect fertility

Factors that impact on fertility	
Obesity	Time to conception increased 2-fold (if BMI > 35)
Underweight	Time to conception increased 4-fold (if BMI < 19)
Smoking	Relative Risk (RR) of Infertility increased by 60%
Alcohol	RR of Infertility increased by 60% (> two drinks/day)
Caffeine	Fecundability decreased 45% (> 250mg/d)
Illicit drugs	RR of Infertility increased by 70%
Toxins, solvents	RR of Infertility increased by 40%

2.2 Preconception Care

Preconception counselling is important to all women seeking fertility treatment to optimise pregnancy outcomes. This should be in accordance with the WHO preconception care package of preconception care interventions [(10)].

- **Maternal age:** Women over 35 years of age, who are requesting fertility treatment, should be advised of the risks of pregnancy with advanced maternal age.
- **Folic Acid supplementation:** The peri-conception period is an important time to intervene to reduce adverse pregnancy outcomes. Folic acid supplementation, before conception (at least two months) and up to 12 weeks gestation, reduces the risk of neural tube defects (NTD) in the baby [(9)]. Figure 2. shows evidence-based recommendations for folic acid supplementation.

All women, from the moment they begin trying to conceive until 12 weeks of gestation, should take a folic acid supplement.

Women who do not have risk factors for NTDs should take 400µg (or 0.4mg) folic acid daily

Women at high risk of NTD's should be identified and include women who:

- have had a previous pregnancy with neural tube defect
- have a family history of neural tube defects
- take antiepileptic drugs
- take folate antagonists (e.g. methotrexate, sulphonamides, dolutegravir)
- have malabsorption disorders (e.g., inflammatory bowel disease)
- have obesity with BMI > 35 kg/m^2
- have diabetes

Women at high risk should:

- be offered **high-dose supplementation (5 mg folic acid daily)**
- receive information on the risk of recurrence if they had a previous pregnancy with NTD
- be advised on the protective effect of peri-conceptional folic acid supplementation
- be advised to increase their food intake of folic acid

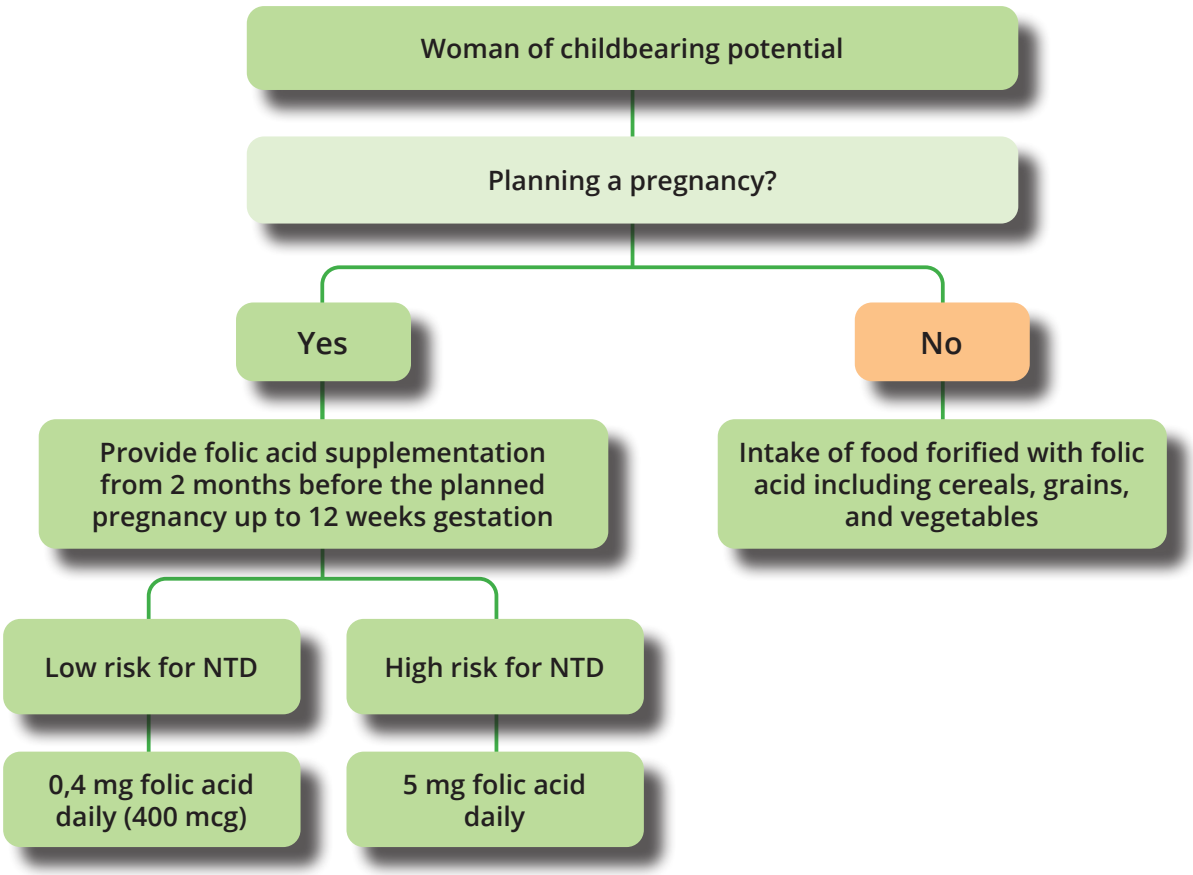


Figure 2. WHO recommendations for folic acid supplementation

2.3 Safe conception for people living with HIV: Risk reduction

For HIV affected couples who have presumed normal fertility, providers have a responsibility to support the couple to conceive safely, **while minimising HIV-related risks**, to ensure optimal maternal and perinatal outcomes. When couples are seroconcordant (both partners living with HIV), the aim is to assist with conception without the risk of superinfection, while simultaneously aiming for unaffected offspring. When the couple is serodifferent/serodiscordant (only one partner living with HIV), the priority is to prevent infection of the uninfected partner while assisting with safe conception and simultaneously aiming for unaffected offspring. Attaining these aims may be facilitated by:

- regularly discussing issues of childbearing and contraception to understand current fertility desires, their concerns about transmission risk and their health care needs
- engaging both partners using a couples-based approach to preconception counselling, as the health and co-operation of both partners is important for safe conception or contraception.

Risk reduction for the couple affected by HIV includes the following components:

During preconception care:

- **folate supplementation** as outlined in section 2.2
- adequate counselling regarding **safer sex practices**:
  - use condoms reliably
  - both partners should avoid having new or additional sexual relationships outside of the current relationship
  - avoid alcohol abuse
- **optimisation of HIV treatment** in the partner living with HIV (serodiscordant couple), or in both partners living with HIV (seroconcordant couple). The following steps are recommended:
  - Provide HIV testing services as applicable and document the HIV status of both partners
  - Identify and manage co-morbidities, including syphilis and other STIs
  - Initiate/continue ART and support good adherence
  - Maintain an undetectable VL, ideally for 4-6 months before conception
  - Consider PrEP for the uninfected partner if applicable
  - Continue to use condoms

During conception

The fertile period should be adequately explained, and safer conception options explored if infertility is not an issue. These options include:

- timed, limited, peri-ovulatory, condomless sex,
- intravaginal insemination, and
- male circumcision.

Should they continue to have concerns about HIV risk, then referral to a reproductive medicine specialist unit for further management may be considered. The fertility status of both partners and their concerns about transmission risk will govern the options available for safer conception (see section 6.4)

Key areas:

1. Safe conception involves preserving fertility as well as providing preconception care
2. Life-style advice concerning extremes of bodyweight, tobacco products, excessive alcohol, and illicit drug use should be part of the counselling, as this may impact fertility
3. Information on age-related infertility should be given and options discussed
4. Folic acid supplementation should be commenced pre-pregnancy
5. Safe sexual practices are key to preventing STI's
6. Risk reduction measures should be taken by people living with HIV

Section 3. Diagnosis and evaluation

3.1 Who should provide infertility services?

To improve the effectiveness and efficiency of treatment and satisfaction of clients, **infertility care should be coordinated and mainly provided by a gynaecologist**, who will refer to an infertility specialist when assisted reproductive technology is required. However, nurses, general practitioners (GP), and urologists are often the first point of contact for a couple seeking infertility treatment.

3.2 Initial assessment before referral to a gynaecologist or infertility specialist

The initial assessment at level 1 should include a relevant history, physical examination, and limited special investigations, as indicated in **Table 4**. Important points to note in the medical and fertility history are:

- duration of infertility
- timing of intercourse
- regularity cycles
- presence of alcohol, smoking, or illicit drug use
- folic acid supplementation
- presence of obesity or low BMI

The initial assessment at the first level of care is important as it will determine who needs further evaluation and referral to a gynaecologist.

3.3 When to evaluate for infertility?

Table 2. When to evaluate persons for infertility

Routine Referral	Early Referral	Immediate Referral
After 1 year of unprotected intercourse • Women < 35years • Regular cycles • No known cause of infertility	After **6 months** of unprotected intercourse • Women ≥ 35 years • Regular cycles • No known cause of infertility	No delay • Women ≥ 40 years • Women with menstrual irregularities • Known cause of infertility • Previous pelvic surgery • Exposure to cytotoxic drugs • Strong family history of premature ovarian insufficiency/early menopause • Suspected endometriosis • All patients requesting tubal re-anastomosis (reversal of sterilisation)

3.4 Standard of care for an infertile couple

As a basic standard of care, all infertile persons should:

- Receive information on infertility causes, prevention and treatment options

- Get an appropriate diagnostic workup
- Have relevant treatment options discussed with them.
- Receive appropriate psychological and emotional support

3.5 Levels of care

Depending on each patient's needs, this standard of care will be provided across four different levels of the health system. Table 4 outlines the services available at each level and the indications for referral from one level to the next.

Table 3. Services to be provided according to levels of care

Levels of care		Services provided	Comments and indications for referral to the next level
Level 1	Primary health care clinics, community health care centres, district hospitals, general practitioners (GP)	• Medical and fertility history • Screen and test for STIs on both partners, including syphilis serology • HIV, HBsAg, HCV Ab • Rubella IgG • Cervical cancer screening • TSH and prolactin levels if indicated	Nurses, general practitioners, and urologists may start an initial assessment for infertility and determine eligibility for referral to a gynaecologist. **Offer advice or refer if:** • >1 year of regular unprotected intercourse in women <35 years • >6 months of regular unprotected intercourse in women >35 years • Known cause of infertility • Irregular menstrual cycles
Level 2	Regional hospital (gynaecologist support)	In addition to all level 1 services: • Hormone profile (Day 2/3 FSH, LH, E2) • Ultrasound examination • Semen analysis • Hysterosalpingography (HSG) • Minor surgical procedures	**Refer if:** • Blocked tubes • Abnormal sperm analysis and hormone profile • Specialised surgery is required to improve fertility If patent tubes + normal sperm analysis – advise on the optimal time for sexual intercourse
Level 3	Provincial tertiary hospital (limited reproductive medicine sub-specialist support)	In addition to all level 1 and 2 services: • Major fertility enhancement surgery • Sperm processing • IUI / timed intercourse • Genetic screening	**Refer if:** • IVF/ICSI is required • Specialised infertility surgery is required • Specialised endocrine service are required
Level 4	Specialised hospitals with reproductive medicine sub-specialist support	In addition to all level 1, 2 and 3 services: • Anti-Mullerian hormone (AMH) test • Sperm processing • Sperm washing (PLHIV) • IUI/IVF/ICSI • Specialised reproductive surgery	These are specialised licensed and accredited fertility clinics that have highly specialised and accredited laboratories and personnel

3.6 Referral routes

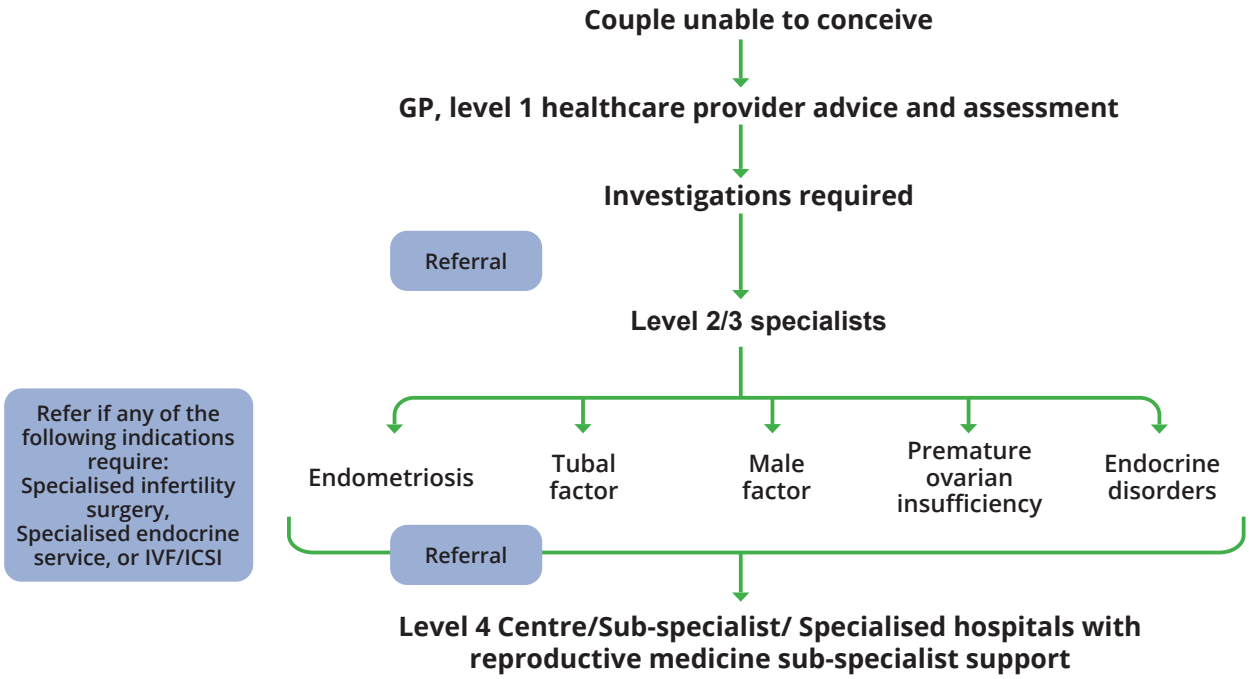


Figure 3. Referral routes for different levels of care

3.7 Evaluation and investigations of an infertile female

Couples who experience problems in conceiving should be evaluated together. The importance of a couple-focused approach is ensuring that no one person bears the burden or responsibility of the problem of infertility. The emphasis is placed on gender equality and collective responsibility and engagement with the health system. This inclusivity has a positive impact on the whole experience of fertility treatment. Special consideration is given to single individuals, who may be seen alone. Evaluation should be done cost-effectively with a systematic approach.

Basic fertility workup

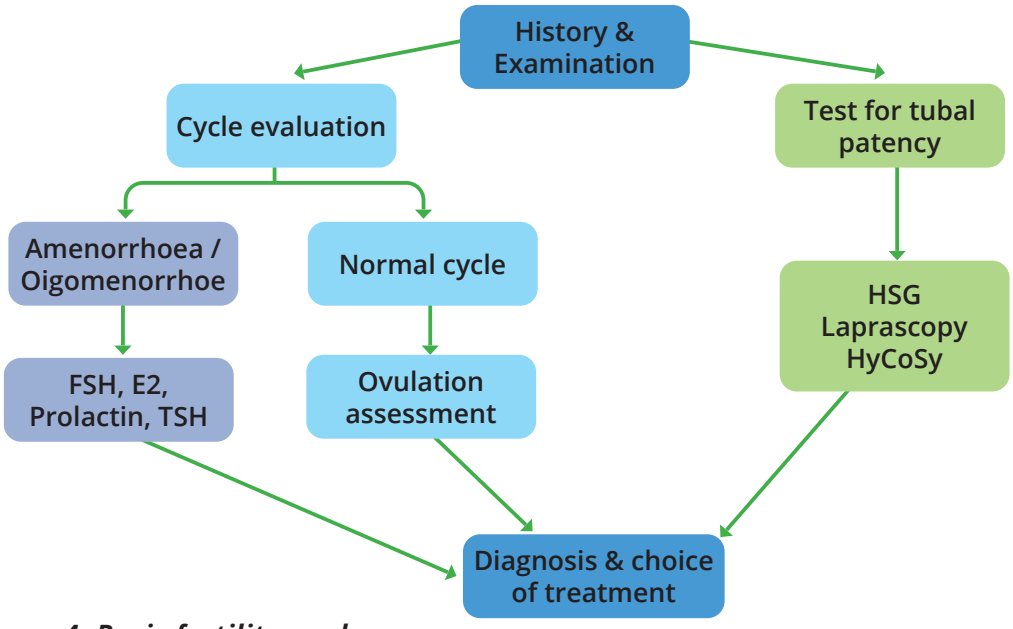


Figure 4. Basic fertility workup

Cycle evaluation

Test for ovarian reserve

Ovarian reserve denotes the reproductive potential of a woman as evidenced by oocyte number and quality. The tests for ovarian reserve may provide information on the likelihood to have a successful pregnancy. The tests do not diagnose decreased ovarian reserve but rather a possible response to ovarian stimulation.

Anti-Mullerian hormone (AMH) levels

Women who are older than 35 years of age should have AMH levels tested. AMH levels are not cycle-dependent and can be done on any day of the cycle. AMH levels of > 1.1ng/ml are associated with a good ovarian reserve, while an AMH of < 0.5ng/mL is associated with a reduced ovarian reserve. AMH levels between 1.0 ng/mL - 3.5ng/mL are associated with a good response to ovarian stimulation. AMH levels above 3.5 ng/mL predict a high response [11, 12]. In this group of patients, caution should be taken, as there is a risk of ovarian hyperstimulation syndrome. Laboratories may vary, and therefore there may be variation in the AMH levels from one laboratory to another. A healthcare provider should know the reference range of the laboratory they are using.

Antral Follicle Count (AFC)

This is the total number of follicles in both ovaries observed with transvaginal ultrasound in the early follicular phase measuring 2-10mm [13]. AFC of less than six is associated with a poor ovarian reserve. AFC is increased in women with polycystic ovary syndrome (PCOS).

FSH, estradiol

Normal follicle-stimulating hormone (FSH) levels should be less than 10 IU/L [13]. A high serum FSH level, greater than 30 to 40 IU/L with a low estradiol level, is suggestive of ovarian failure/insufficiency and is associated with poor ovarian stimulation and failure to conceive. Low or normal FSH levels, with low estradiol levels, denote hypothalamic-pituitary failure and requires further evaluation. Women with abnormal FSH and estradiol levels must be referred for further evaluation.

Prolactin measurement

This test should only be offered to women who have an ovulatory disorder, suspected by irregular menstrual cycle, galactorrhoea or symptoms and signs of a pituitary tumour. It is not to be offered routinely.

Thyroid function tests

Routine measurement of thyroid function should not be offered. Estimation of thyroid function should be confined to women with symptoms of thyroid disease and/or irregular menstrual cycle.

Investigations for tubal patency

Hysterosalpingography (HSG)

This test assesses tubal patency and the uterine cavity. It is the first-line investigation for evaluation of tubal patency.

Hysterosalpingo-contrast sonography (HyCoSy or Foam test)

HyCoSy is a trans-cervical injection of echogenic contrast media and the use of ultrasound to view cavity and tubes.

Laparoscopy and chromopertubation

This investigation is a second-line approach because it is invasive. It is indicated when another pathology is suspected, e.g. endometriosis. Laparoscopy is not solely to assess the tube but to treat any pathology found. This should not be done routinely and not done at lower levels of care.

Investigation to evaluate the uterus

Hysteroscopy

This is used to evaluate the uterine cavity and has the advantage of treating any pathology at the time of diagnosis. Hysteroscopy is not routinely recommended.

Ultrasound

Ultrasound plays an important role in the workup, monitoring, and treatment of infertility. It is an indispensable tool in the management of infertility. It is readily available, non-invasive, relatively less time consuming, and easily repeatable.

Other tests

Cervical cancer screening

Cervical screening should be offered in accordance with the national cervical cancers screening programme guidelines [14].

Tests for infections

See section 3.9

3.8 Investigations to evaluate an infertile male

Semen Analysis

Semen analysis should be performed and interpreted according to the 2010 WHO semen criteria as in Table 5 below [15].

In case of a normal semen analysis, no-repeat semen analysis should be done, and no andrological tests should be performed. In case of abnormal semen analysis, a repeat semen analysis should be done, and further andrological tests must be performed at the discretion of the treating physician.

Table 4. WHO reference values for semen characteristics

Parameter	Lower reference limit
Semen volume (ml)	1.5 (1.4 – 1.7)
Total sperm number (10^6 per ejaculate)	39 (33 – 46)
Sperm concentration (10^6 per ml)	15 (12 – 16)
Total motility (PR + NP, %)	40 (38 – 42)
Progressive motility (PR, %)	32 (31 – 34)
Vitality (live spermatozoa, %)	58 (55 – 63)
Sperm morphology (normal forms, %)	4 (3.0 – 4.0)
Other consensus threshold values	
pH	≥ 7.2
Peroxidase-positive leukocytes (10^6 per ml)	< 1.0
MAR test (motile spermatozoa with bound particles, %)	< 50
Immunobead test (motile spermatozoa with bound beads, %)	< 50
Seminal zinc (µmol/ejaculate)	≥ 2.4
Seminal fructose (µmol/ejaculate)	≥ 13
Seminal neutral glucosidase (mU/ejaculate)	≥ 20

Lower Reference limits (5th centiles and their 95% confidence intervals) for semen characteristics

Adapted from WHO laboratory manual for the examination and processing of human semen, fifth edition

Special investigations

Endocrine Evaluation

Initial hormonal evaluation should include measurement of serum FSH, luteinising hormone (LH), prolactin, and total testosterone (T) concentrations in cases of azoospermia or severe oligospermia. Should the initial tests be abnormal, then more extensive evaluation and referral to an endocrinologist must be considered.

Ultrasonography

Scrotal ultrasound can help as an addition to physical examination and to exclude testicular tumours. Varicoceles that are not palpable clinically and only diagnosed with ultrasound are not clinically relevant. A urologist should do this evaluation.

Post-ejaculatory urinalysis

Low ejaculatory volume or absent ejaculate may indicate retrograde ejaculation. To exclude retrograde ejaculation, a post-ejaculatory urinalysis should be performed in men having an ejaculate volume is less than 1ml [16]. The urine is alkalised and post-masturbation, urine is centrifuged and examined under high magnification. The presence of sperm in the post-ejaculatory urinalysis suggests retrograde ejaculation, but there is no consensus about the minimum number of sperm that need to be present.

Tests for anti-sperm antibodies

Tests for anti-sperm antibodies are controversial, and routine testing is not recommended.

Testis biopsy or aspiration

Testis biopsy should be considered in men with azoospermia, and if spermatozoa are present, it should be frozen for the use in assisted reproduction.

3.9 Investigation for infections

HIV & Hepatitis

- People undergoing IVF treatment should be offered to test for HIV, hepatitis B, and hepatitis C.
- If HIV positive, a CD4 count and an HIV viral load test must be done.
- Align with STI guidelines and PMTCT guidelines [17].

Rubella IgG

- Testing should be offered.
- Women who are susceptible to rubella should be offered vaccination and advised not to become pregnant for at least one month following vaccination.

Chlamydia screening

- Where possible, STI screening and prevention should become routine and integrated into all health visits [17].
- Chlamydia antibody test to detect the antibodies to *Chlamydia Trachomatis* should not be done as its clinical utility is limited.
- Chlamydia screening should align with the STI Guidelines.

Key areas:

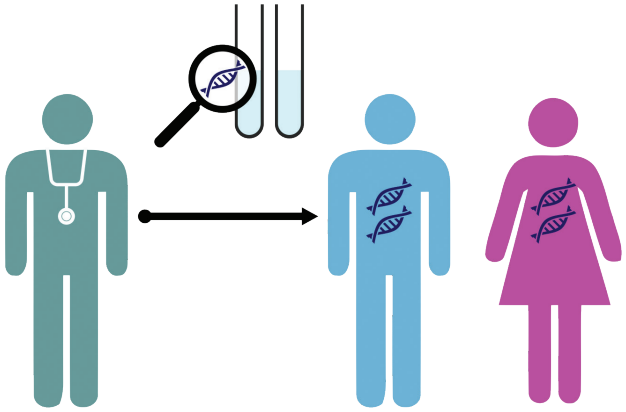
- Standard of care of infertile persons involves providing information on infertility causes and prevention and have treatment options discussed with them.
- Persons requesting fertility care should be provided with appropriate diagnostic workup and receive appropriate psychological and emotional support as a basic standard of care.
- An infertile female patient should receive a test for ovulation and a test for tubal patency as a minimum package of testing.
- An infertile male should receive a semen analysis as a minimum investigation.

Section 4. Genetic screening

The role of genetic screening in ART is a continuum from identifying the causes of infertility, to identifying and exclusion of carriers of inherited disorders to antenatal testing once pregnancy is achieved.

Genetic screening aims to facilitate

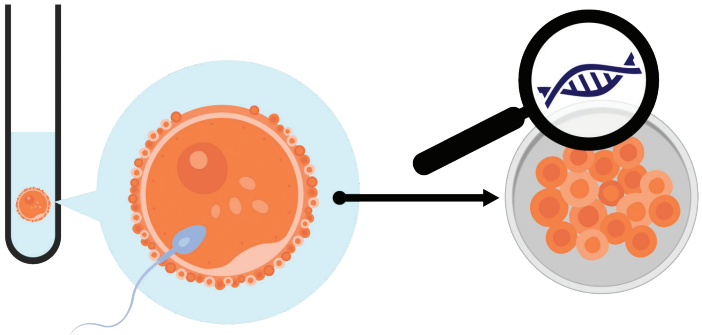
a **IDENTIFICATION OF THE INFERTILITY CAUSES**



Why?

- **genetic causes related to infertility**
- **genetic diseases transmissible to offspring**

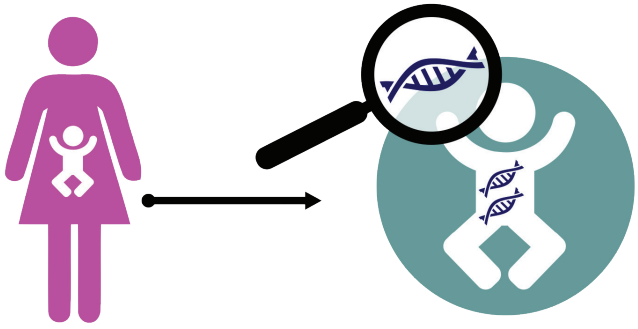
b **IDENTIFICATION OF GENETIC DISEASES TRANSMISSIBLE TO OFFSPRING**



Why?

- **genetic diseases**
- **chromosomal abnormalities**
- **aneuploidy**

c **DIAGNOSING GENETIC ABNORMALITIES DURING PREGNANCY**



Why?

- **genetic diseases**
- **chromosomal screening**

Genetic tests for genetic disorders include the following:

1. **Prenatal screening tests:**
   a) Carrier screening
   b) Prenatal genetic screening
      - 1st and 2nd trimester combined screening (ultrasound and biochemical tests), or
      - Non-invasive prenatal testing (NIPT)

2. **Prenatal diagnostic tests:**
   a) Amniocentesis or
   b) chorionic villus sampling (CVS)

3. **Preimplantation genetic testing**

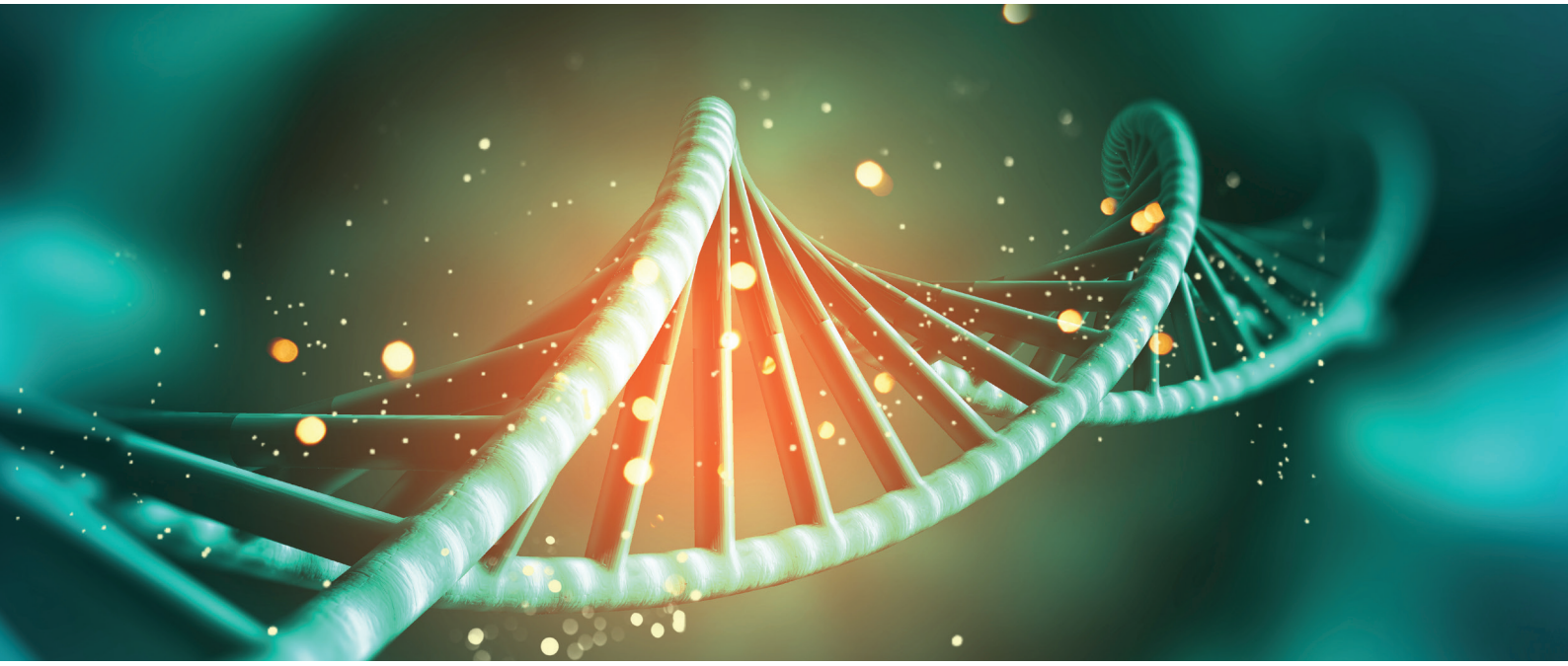
Preimplantation genetic testing (PGT) is the testing of early stage embryos for genetic abnormalities. It comprises a group of genetic assays used to evaluate embryos before transfer to the uterus. One or more cells from each embryo obtained from IVF/ICSI is sent for genetic testing.

4. **Karyotyping: Genetic testing on products of conception**

The role of karyotyping is in investigating the cause of recurrent miscarriages. The test has limitations as it does not covers all the chromosomes. This may cause some chromosomes to be missed.

Men with non-obstructive azoospermia and severe oligozoospermia are at increased risk of having genetic and chromosomal abnormalities [18]. Karyotyping must be considered in these situations. Men with congenital bilateral absence of the vas deference have a strong association with cystic fibrosis carrier status. Testing for the presence of a CFTR gene mutation should be done

The different genetic tests and the time to perform the tests through the stages of reproduction are shown in Figure 4 below.



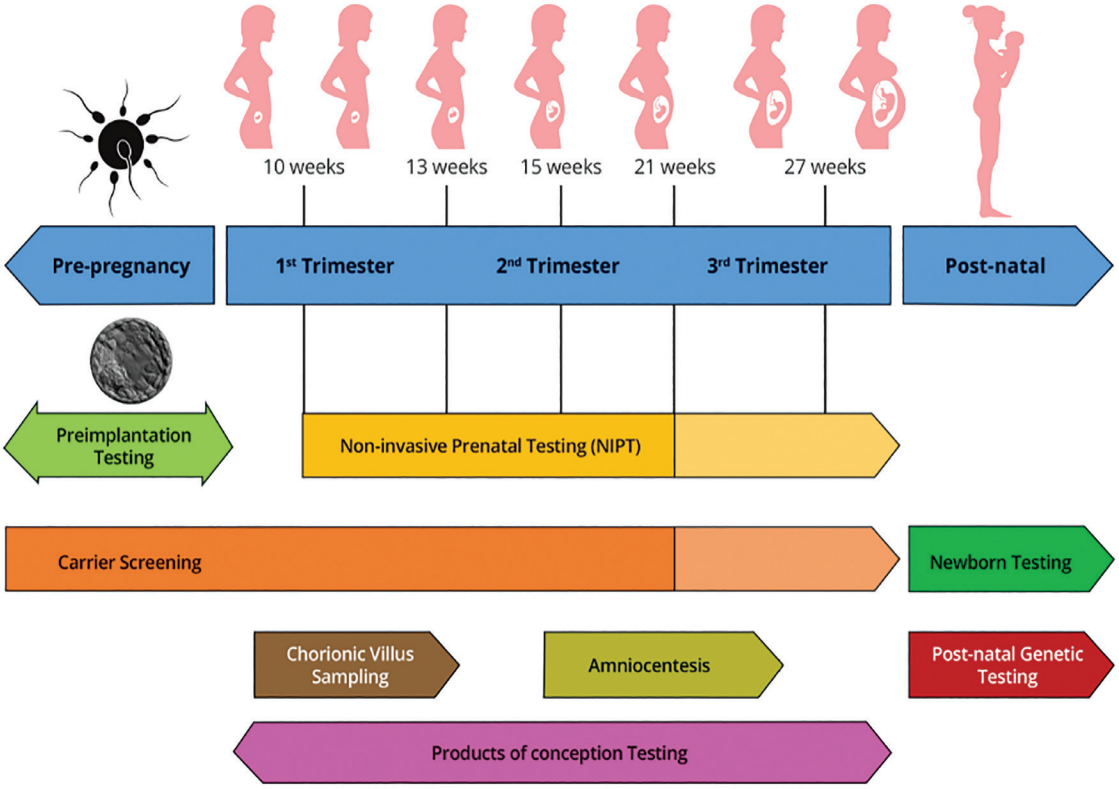


Figure 5. Recommended genetic testing

Key areas:

- Genetic screening should be done in accordance with the relevant guidelines.
- The treating physicians should discuss and offer genetic counselling and appropriate testing.
- Screening results should be used to offer appropriate care, according to risk.
- Routine testing is not recommended.

Section 5. Counselling and psychological support

5.1 The psychological impact of infertility

There is tremendous psychological stress in people who are unable to achieve a pregnancy. In addition to the social stress and stigma, the diagnosis of infertility itself causes distress. The diagnosis and treatment of their condition are often very lengthy and cumbersome. They also have to manage the psychological stress of the knowledge that they may not achieve pregnancy despite the extensive interventions. Some clients discontinue prematurely purely because of the burden of treatment. The stress, financial burden, and the repeated visits and investigations, which are often very invasive, have an impact on the drop-out rate of patients. In those that achieve a pregnancy, they still experience anxiety throughout the pregnancy.

Providing high-quality fertility health care implies not only focusing on medical treatment for patients to achieve a successful pregnancy but also emotionally supporting patients and healthcare providers in managing the psychological effects. The European Society of Human Reproduction and Embryology (ESHRE) Guidelines for Routine Psychosocial care in infertility and medically assisted reproduction – A guide for fertility staff, gives recommendations of psychological management of patients before, during, and after treatment [19].

Key areas:

- Psychological care enables couples, their families, and their healthcare providers to optimize fertility care and manage the psychological and social implications of infertility and its treatment.
- Infertility counselling should be undertaken by qualified and registered counsellor and/or psychologist.
- Patient-centred care is important as it focuses on the patient's experience of illness and health care.
- Psychological management should be provided before, during, and after treatment.
- It is recommended that all staff coming into contact with patients have a basic understanding of the psychosocial aspects.

Section 6. Causes of infertility and treatment options

The causes of infertility and their estimated percentage contribution are depicted in Figure 5 below [20] . However, causes of infertility may vary in the different geographic areas related to the prevalence of the risk factors. Prevalence studies in each region are needed.

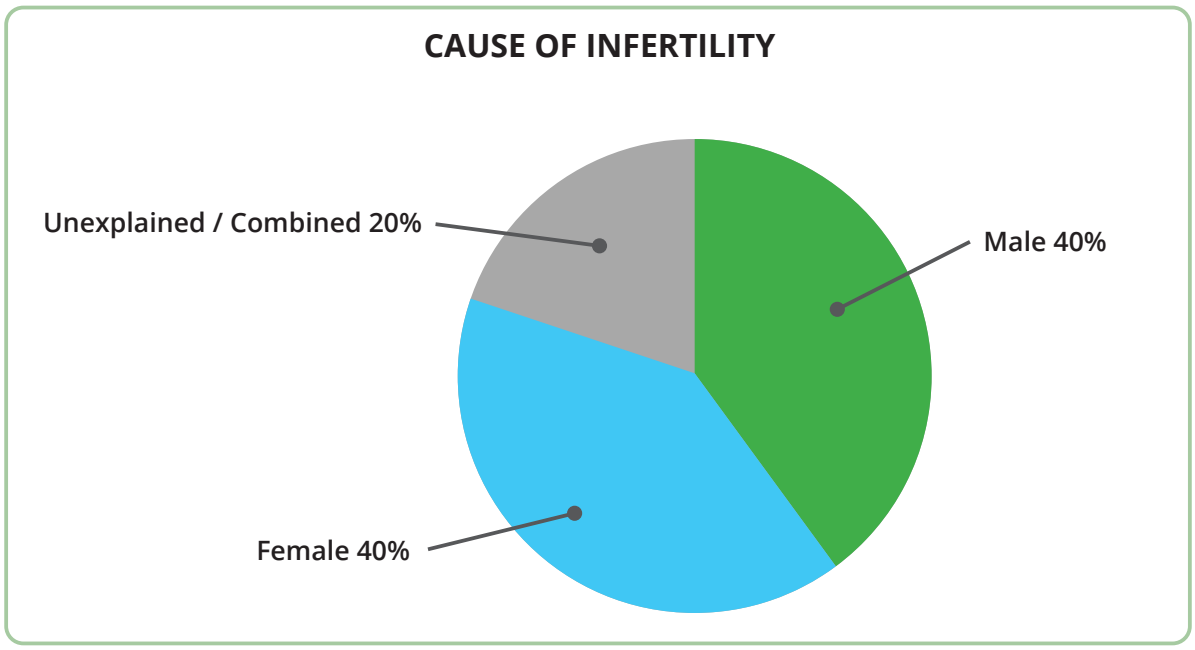


Figure 6. Causes of infertility

6.1 Female factor infertility

Introduction

Depending on geographic location, 40% of all causes of infertility may be solely due to female factors as depicted in figure 5 above [15, 21]. For adequate management, the contributing factors have to be identified before a treatment plan is instituted. For normal reproduction function to occur, the following factors are needed:

- Normal ovarian reserve
- Regular ovulatory cycles
- Patent fallopian tubes with normal mucosal lining (at least one fallopian tube)
- Normal uterus and endometrial function
- Patent outflow tract (uterus, cervix and vagina)
- Normal thyroid, pituitary gland and cerebrum function
- Other factors that have a direct impact on human reproduction include the presence of endometriosis, chronic medical conditions, and adrenal gland dysfunction

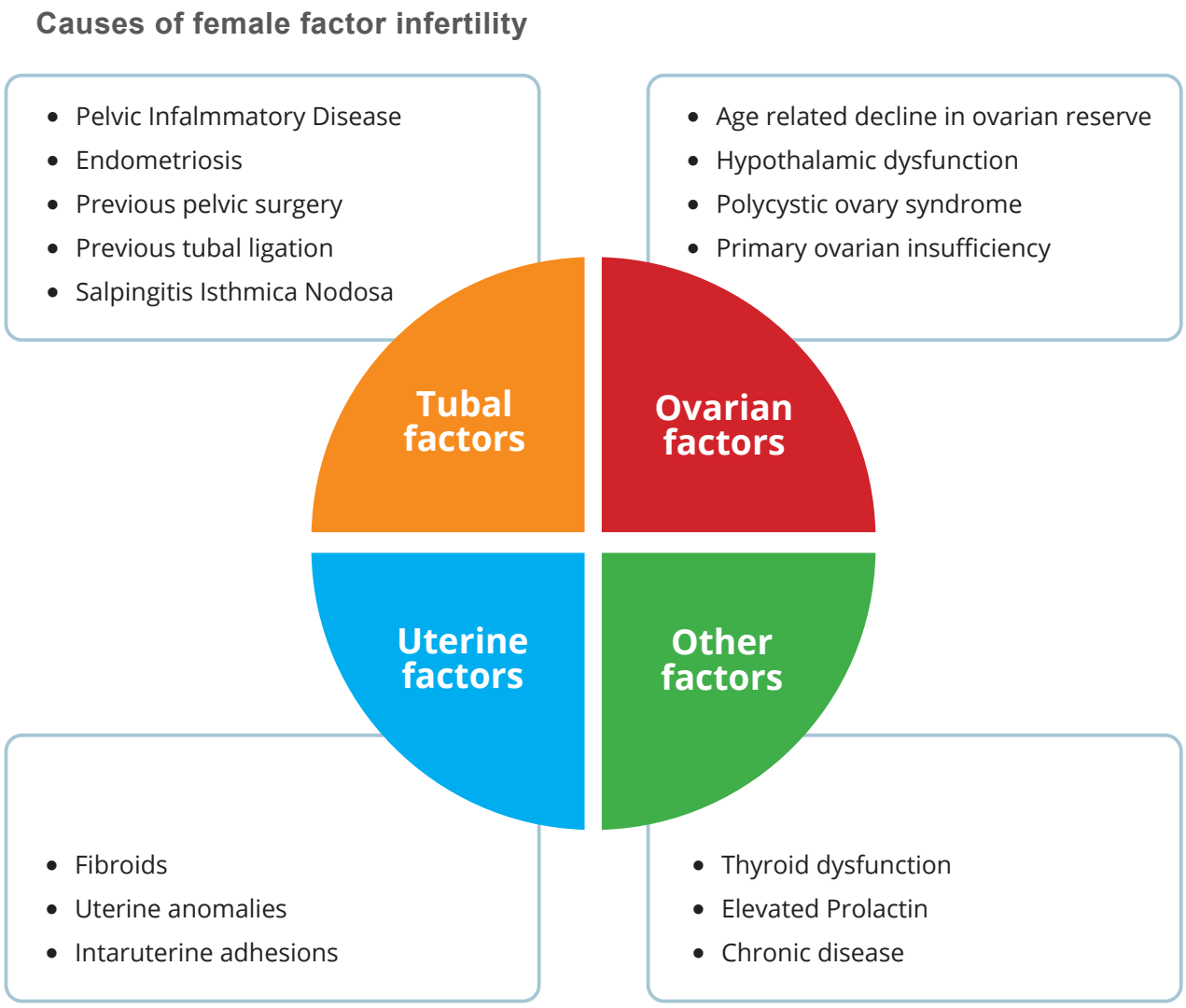


Figure 7. Schematic representation of causes of female factor infertility

Ovarian factors

- *Ovarian reserve:* The quality and quantity of oocytes decline with age, resulting in a progressive decline in fertility and an increase in miscarriage rate. The ideal age to conceive is between the ages of 28 to 32 years [22, 23]. The antral follicle count should be used to measure the ovarian reserve in all patients. The anti-Mullerian hormone (AMH) should be measured in patients with endometriosis, chronic medical conditions, conditions known to affect the ovarian reserve (like Turner's syndrome) and those above the age of 35 years. The figure below provides a schematic representation of the number of primordial follicles present in the ovaries and the chromosomal quality of oocytes in relation to female age and corresponding reproductive events [24]

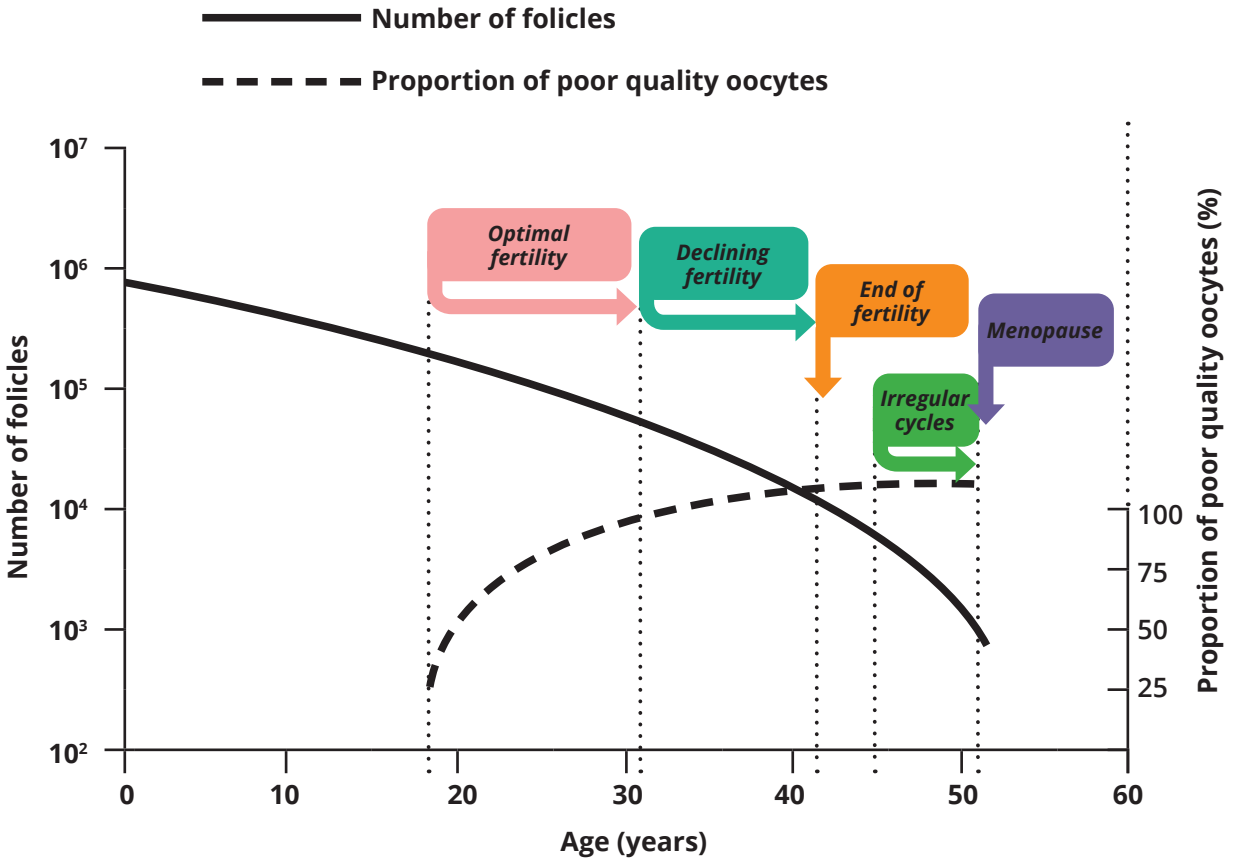


Figure 8. Age related decline in primordial follicle number and quality over time

Patients, whose ovarian reserve is reduced or exceedingly high, should be referred to and managed by reproductive medicine specialist.

- *Ovulatory dysfunction:* Ovulatory dysfunction is a major cause of infertility, affecting up to 33% of patients [25]. When a woman does not ovulate spontaneously, she will not conceive without medical intervention. The WHO classifies anovulatory disorders into three groups: [25, 26].
  - Group 1: Hypothalamic-pituitary failure
    - Hypothalamic-pituitary dysfunction
    - Anorexia nervosa
    - Excessive exercise (top athletes)
  - Group 2: Hypothalamic – pituitary – ovarian (HPO) dysfunction
    - Polycystic ovary syndrome (PCOS)
  - Group 3: Ovarian failure
    - Turner's syndrome
    - Primary ovarian insufficiency

Table 5. WHO classification of ovulatory disorder and management

WHO group 1 (Hypothalamic-pituitary failure)	**WHO group 2** (HPO dysfunction e.g. PCOS)	**WHO group 3** (Ovarian failure)
FSH – low **LH - low** **Estradiol - low**	**FSH – normal** **LH – normal** **Estradiol - normal**	**FSH - High** **LH – High** **Estradiol - low**
• Lifestyle management • Increase BMI >18 • Reduce excessive exercise	• Lifestyle management • Weight reduction of at least 5 %	• IVF using donor eggs
• Psychotherapy	1st line: • Clomiphene citrate 25 to 150 mg daily for five days starting on day 3-6 of cycles • Alternatively, letrozole 2,5 – 7,5 mg daily	• Clomiphene citrate and letrozole contraindicated
• Gonadotropins including FSH and LH	2nd line: • Gonadotropins or laparoscopic ovarian drilling if a person has clomiphene failure or resistance.	• Psychological counselling recommended
• Clomiphene citrate contraindicated	3rd line: • IVF when all other options were unsuccessful	• Psychological counselling recommended

Tubal factor infertility:

Blocked fallopian tubes are the most common cause of female factor infertility in South Africa [27-30]. It is most frequently caused by previous pelvic inflammatory disease (PID), endometriosis, previous pelvic surgery, or previous tubal ligation.

Treatment options include assisted reproduction or tuboplasties, which are only available at certain public sector hospitals. Unfortunately, many patients cannot afford ART, and tubal surgery may be the only available option for them. Tuboplasty should be offered to patients with previous tubal ligation and those with good prognosis hydrosalpinges. An experienced gynaecological surgeon should do the tubal reconstruction surgery. Patients with poor prognosis hydrosalpinges should also be offered tubal surgery to remove the damaged fallopian tubes as the fluid from the hydrosalpinges has been associated with poor pregnancy outcome in ART cycle.

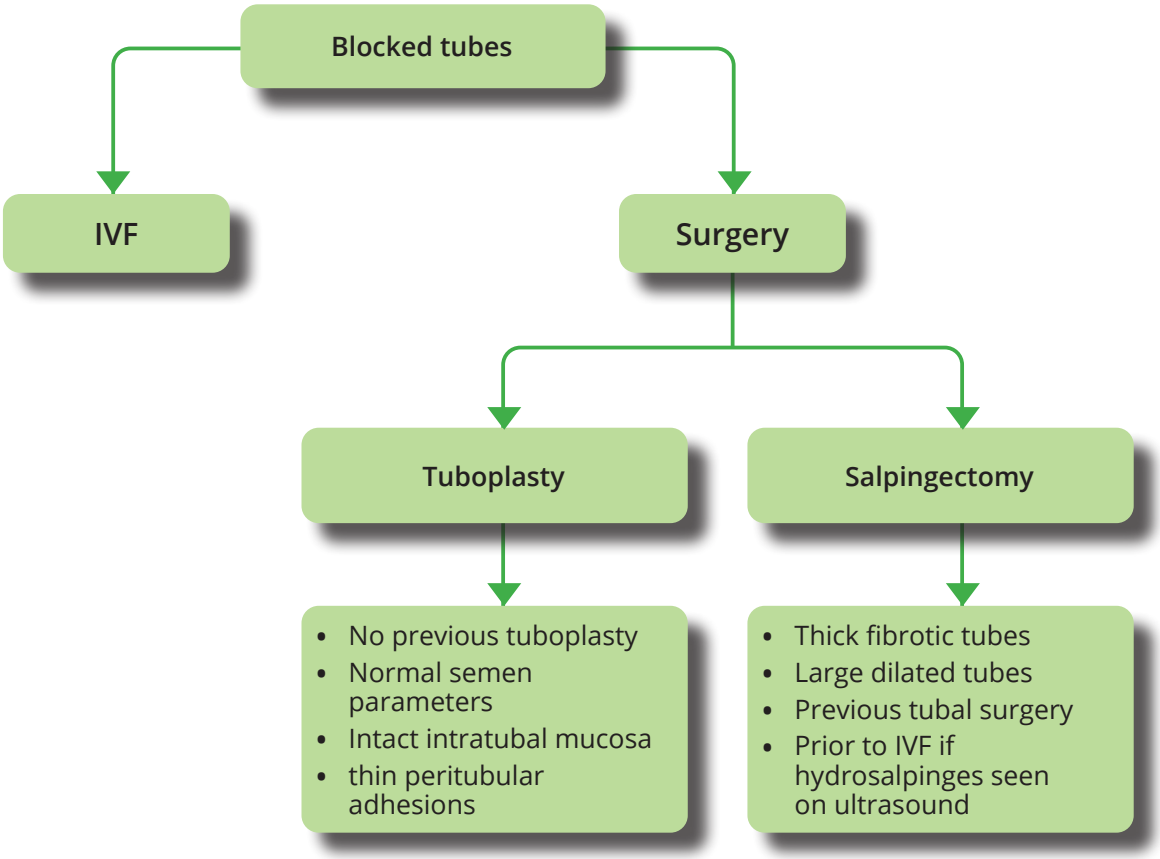


Figure 9. Algorithm for the management of tubal factor infertility

Uterine factors

The uterus is a major organ that can impact on reproductive outcome. Some uterine abnormalities are amenable to surgery which can improve pregnancy outcomes, and some are not.

Fibroids

About 1/3 of women will have fibroids. Most fibroids do not have a negative impact on reproductive outcome and thus require no treatment. Figure 10 below illustrates the European Society of Gynecological Endoscopy (ESGE) classification of submucous fibroids. Submucosal fibroids are mostly symptomatic. They cause heavy menstrual bleeding, prolonged menstrual bleeding, implantation failure, and recurrent pregnancy losses. Submucosal fibroids of ESGE type 0, 1 and 2, should be removed, preferably through hysteroscopy or laparoscopy according to the type [31, 32]. Large intramural fibroids that are 5cm or more in size should also be removed as they have been associated with poor reproductive outcome. Laparoscopic myomectomy is preferred over laparotomy if the doctor's skills are adequate.

Uterine artery embolisation (UAE) is available and sometimes offered to patients instead of myomectomy. There have been several patients who conceived after UAE. However, serious morbidity has been reported. There is a high risk of a morbidly adherent placenta, miscarriage, low birth weight, intrauterine growth restriction, preterm delivery, and post-partum haemorrhage post-UAE [33]. Its effect on future pregnancy and fertility remains unclear. All patients who conceived after UAE require close monitoring.

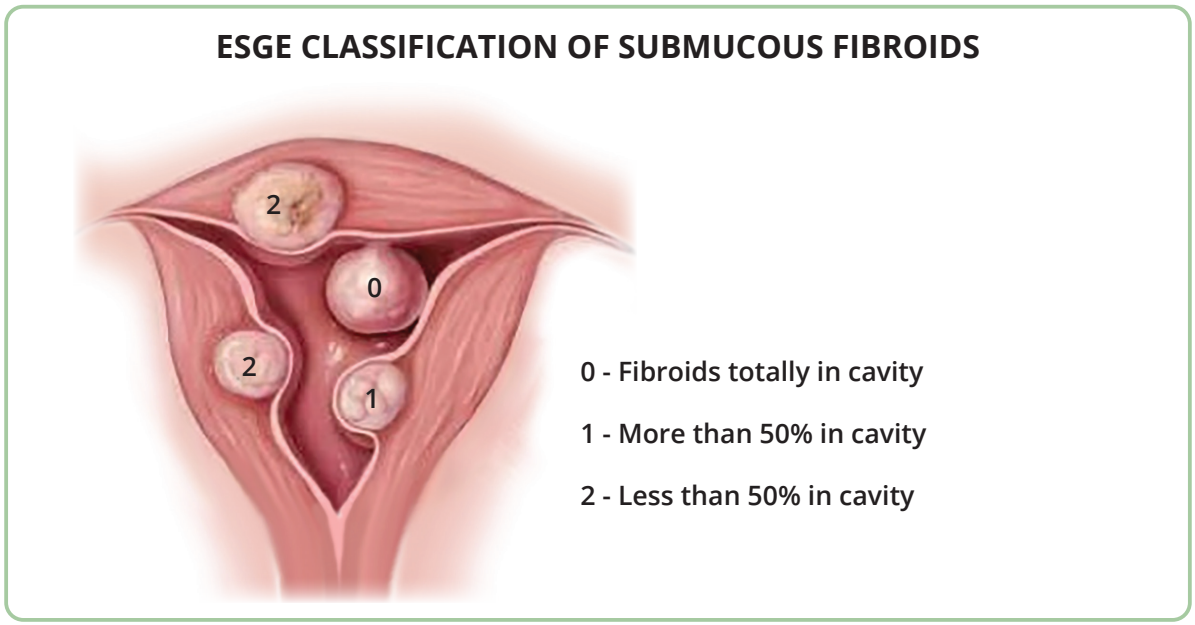


Figure 10. Classification of submucous fibroids

Uterine anomalies

Septate uterus:

The septate uterus may cause reproductive failure and an increased risk of miscarriages. Women with a septate uterus should be referred to a reproductive medicine specialist for evaluation and assessment to determine if hysteroscopic removal will improve fertility outcomes.

Uterus didelphis and unicornuate uterus:

Patients with a double uterus (didelphis) and those with a unicornuate uterus should not be offered any surgery except if there is a non-communicating horn of the unicornuate uterus or functional horn that creates chronic pelvic pain.

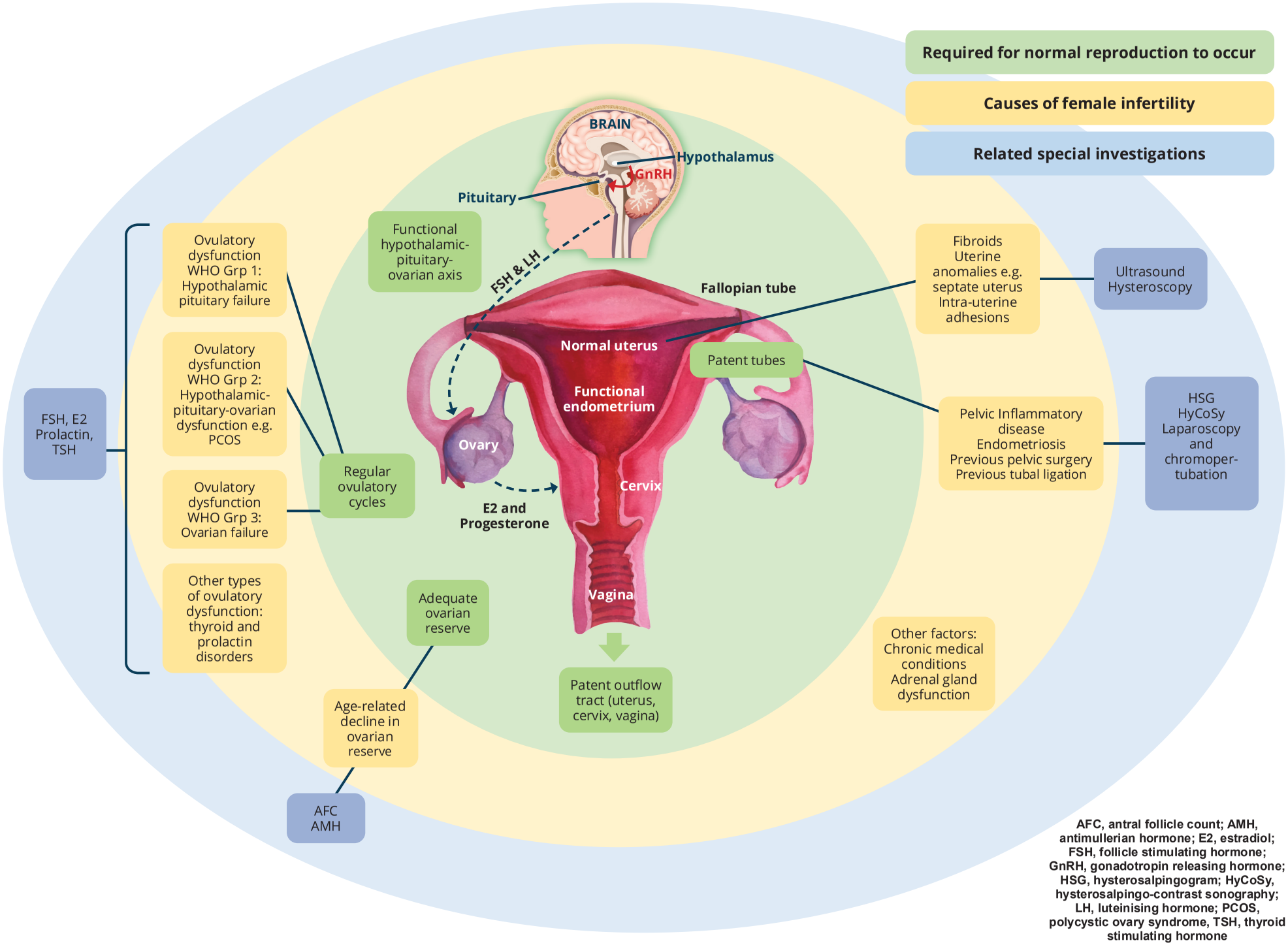
Bicornuate uterus:

The efficacy of doing metroplasty in patients with a bicornuate uterus is still not fully understood. It should not be done as a routine procedure but must be considered in patients with previous reproductive failure. It can be performed via laparoscopy or laparotomy.

Asherman's syndrome

Asherman's syndrome presents as secondary amenorrhea or hypomenorrhea. The major cause of these intrauterine adhesions is intrauterine curettage post-partum or post-abortion. Treatment of severe Asherman's syndrome is extremely difficult. These patients should be referred to an endoscopic surgeon for hysteroscopic resection of the synechiae. In very severe cases, the patient may require the services of a surrogate. In the South African setting, genital tuberculosis (TB) should be investigated as a potential cause of severe Asherman's syndrome.

The figure below gives an overview of the components required for normal reproduction, the causes of female fertility and the related special investigations.



AFC, antral follicle count; AMH, antimullerian hormone; E2, estradiol; FSH, follicle stimulating hormone; GnRH, gonadotropin releasing hormone; HSG, hysterosalpingogram; HyCoSy, hysterosalpingo-contrast sonography; LH, luteinising hormone; PCOS, polycystic ovary syndrome, TSH, thyroid stimulating hormone

Figure 11. The female reproductive system, related pathology, and investigations

The role of dilatation and curettage (D&C) in reproduction

D&C should NEVER be used to improve the reproductive outcome of patients. It should NOT be offered to patients with heavy menstrual bleeding. Rather offer hysteroscopy for diagnostic and treatment purposes.

Table 6. Surgery to improve female fertility and levels of care

Minor surgery Level 2/3/4	• Diagnostic laparoscopy and chromopertubation • Salpingectomy • Hysteroscopic polypectomy • Cervical cerclage
Major surgery Level 3/4	Ovaries • Cystectomy • Drilling Fallopian Tubes • Reversal of sterilisation • Tuboplasty Uterus • Myomectomy (laparoscopy, hysteroscopy, laparotomy) • Asherman's syndrome • Septum resection • Metroplasty Endometriosis

Key areas:

- Counselling is an important part of fertility treatment
- Lifestyle modification can improve infertility in some cases
- Indications for surgery must be aimed at improving fertility
- The male partner must have a semen analysis before surgery is performed on the female partner to exclude the male factor
- There must be access to assisted reproduction technologies

6.2 Male factor infertility

Male factor infertility contributes to 30%-40% of infertility in couples overall[20].

It is imperative that infertility not be seen as either a female or male problem but that the problem is addressed as a couple. Education of the population about male infertility is needed to eliminate the stigma around the condition and to address the reluctance of some men to be tested. The evaluation of the male must always occur in parallel to the evaluation of the female. Invasive procedures like laparoscopy or myomectomy should not be performed without a semen analysis on the male partner. The fact that a man has children in a previous relationship should never be a reason not to test the man.

Reproductive History

A detailed history should include:

- Duration of infertility, coital frequency, and timing, as well as erectile dysfunction
- Pubertal development and childhood illnesses specifically enquiring about undescended testis
- Previous surgery (inguinal hernia repair)
- Medical history such as diabetes mellitus and upper respiratory disease
- Chronic medication
- History of sexually transmitted infections
- Smoking, alcohol, recreational drugs use, and anabolic steroid use
- Exposure to gonadotoxins (e.g. chemotherapy and radiation therapy)

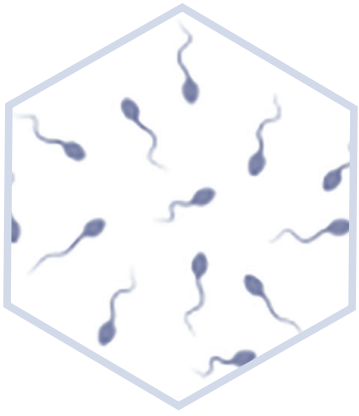
Physical Examination

General physical examination and examination of the genitalia must be performed. Secondary sex characteristics, body habitus, hair distribution, and breast development, should be documented. Attention should be given to penile abnormalities, the testis volume and the presence of a varicocele. In cases of azoospermia, palpation for the presence of the vas deference must be performed.

When the initial semen analysis is normal, no further investigation is necessary. When the semen analysis is abnormal, a second semen analysis is recommended after six weeks. Should the reproductive history be abnormal, or the semen parameters remain abnormal, evaluation by a urologist or reproductive medicine specialist is indicated.

Semen Analysis

Semen analysis is the most important test for the evaluation of male subfertility. A standardised instruction for semen collection is 2-3 days abstinence [34]. Semen can be collected through masturbation into a specimen cup or by intercourse with the use of special semen collection condoms that is not toxic to sperm. The specimen must ideally be collected at the laboratory, but if collected at home, it must be transported at room temperature within an hour to the laboratory [15].



The results of semen analysis conducted as part of an initial assessment should be compared with the World Health Organization 2010 reference values.

General management

- Counselling should be offered before, during, and after investigation and treatment, because fertility problems can cause psychological stress and put pressure on relationships.
- Excessive alcohol consumption may impair semen quality.
- Smoking affects semen quality and may influence the chance of conception, and men and women should be advised to stop smoking.
- Men who have a high BMI are at increased risk of infertility [35].
- Recreational drugs interfere with male and female fertility, and use of anabolic steroids suppresses spermatogenesis.

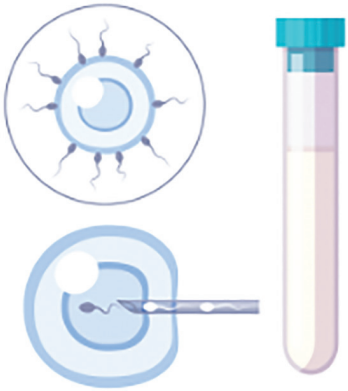
Medical management

- The use of anti-oestrogens, gonadotrophins (HCG and FSH), androgens, bromocriptine, or kinin-enhancing drugs for semen abnormalities are not recommended due to the lack of evidence for their efficacy. Corticosteroids should not be used for anti-sperm antibodies.
- Men with hypogonadotropic hypogonadism should be treated with gonadotrophins.
- The use of antioxidant therapy may be beneficial in idiopathic semen abnormalities.

Surgical management

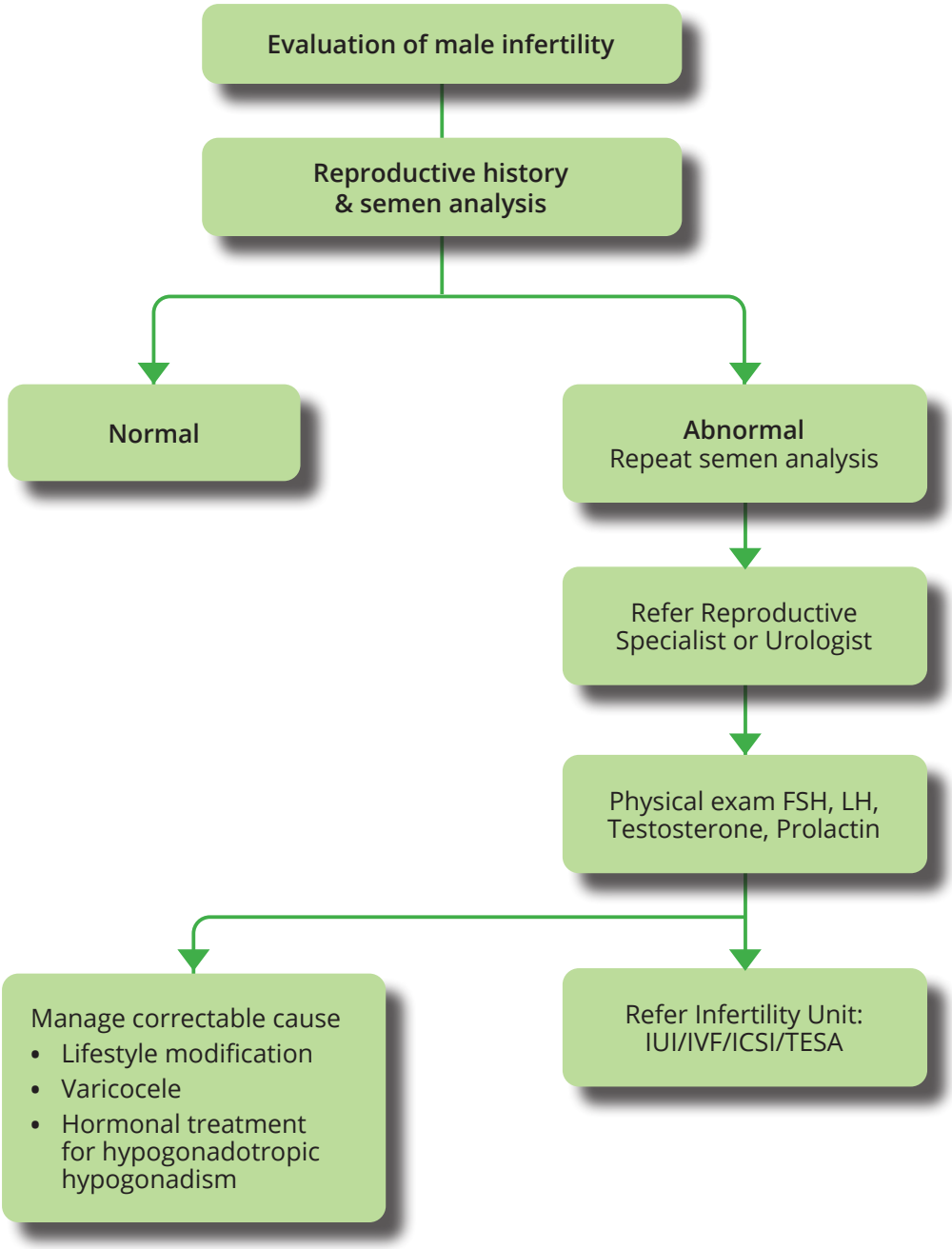
- Surgery for varicoceles may be considered when 1) there is a clinically palpable varicocele, with abnormal semen parameters present, and 2) no female factor for infertility is present [36]. In cases where IVF needs to be performed for female factors, surgery for varicoceles is not indicated.
- Vasectomy reversal should be considered in men with a fertility wish after vasectomy. Female factors must first be excluded. Alternative treatment is testicular sperm extraction and IVF or ICSI. The surgery should be performed by an experienced surgeon using microsurgical technique.

Assisted reproduction



- Intrauterine insemination (IUI) should be considered in cases of mild male factor. Maximum of 3-6 cycles should be done.
- Intracytoplasmic sperm injection (ICSI) is the recommended treatment of choice for infertility due to male factor where appropriate.
- In cases of azoospermia surgical retrieval of sperm with ICSI should be offered.
- Donor sperm is used in cases where ICSI cannot be performed.

Figure 12. Algorithm for the approach to male factor infertility



Key areas:

- Counselling should be offered before, during, and after investigations and treatment
- Lifestyle modification can improve fertility in some cases
- Semen analysis is the most important test for the evaluation of male subfertility
- Andrologic testing and other investigations should be done when a second semen analysis is abnormal
- There should be access to assisted reproductive technologies

6.3 Unexplained infertility

Unexplained infertility is defined as infertility, where the cause remains unknown after extensive and comprehensive investigations. The management of unexplained infertility should be individualised. Lifestyle modification and advice on adequate sexual intercourse and the best timing of intercourse should be discussed. Other treatment modalities can then be offered according to efficacy, cost, ease of use and safety.

Treatment options may include expectant management, oral ovulation induction agents, such as clomifene citrate or letrozole, with intrauterine insemination (IUI), or in vitro fertilisation (IVF). IUI remains a reasonable treatment option for unexplained infertility in the South African setting because of the lower costs and safety. It is less invasive and less cumbersome treatment. A maximum of 3-6 cycles should be done. If there is no successful pregnancy after 3-6 cycles, the high-resource intensive treatment option of IVF should be considered. Patient selection is important, and the patient's wishes should also be taken into consideration.

6.4 Safe conception for people living with HIV

Introduction

Human immunodeficiency virus (HIV) infection affects people in their reproductive years. Up to 40% of those infected are 25-29 years old [37]. Often, they have not yet started their families. As they establish long term relationships, the desire to start a family becomes the logical evolution in the relationship.

HIV affected couples should have the same access to investigations and treatment of infertility as unaffected couples. For HIV affected couples who have presumed normal fertility, providers have a responsibility to support the couple to conceive safely, with minimal HIV-related risks to ensure optimal maternal and perinatal outcomes. When couples are seroconcordant (both partners living with HIV), the aim is to assist with conception without the risk of superinfection, while simultaneously aiming for unaffected offspring. When the couple is serodifferent/ serodiscordant (only one partner living with HIV), the priority is to prevent infection of the uninfected partner while assisting with safe conception and simultaneously aiming for unaffected offspring. Deferring fertility treatments until the risks of HIV transmission have been minimised may be recommended in some situations.

Ethical issues

It is unethical to deny couples affected by HIV their right to reproductive freedom. This has been significantly revolutionised with the introduction of highly active antiretroviral therapy (HAART), with the life expectancy of people affected by HIV reaching that of non-infected people. There are ethical and psychological factors that the treating healthcare provider must consider when providing fertility counselling and treatment for people living with HIV.

- There must be a multidisciplinary approach to HIV management.
- There should be adequate counselling regarding safer sex practices and risk reduction measures.
- The fertility status of both partners and their concerns about transmission risk govern the options available for safer conception.
- The fertile period should be adequately explained, and safer conception options explored if infertility is not an issue.
- Couples should be counselled about the available risk reduction strategies, including antiretroviral therapy and PrEP. Should they continue to have concerns about HIV risk, then referral to a reproductive medicine specialist unit for further management may be considered.
- Healthcare providers at the relevant ARV clinics and day hospitals should receive adequate training.
- There is still the unintended bias toward discouraging individuals who are living with HIV from having children [38].

Why fertility is compromised in people living with HIV

People who are living with HIV often face the following issues that may compromise fertility:

- The consistent use of condoms in itself, while recommended, prevents pregnancy.
- The psychological impact of HIV can have an impact on sexuality and libido.
- In women, there is an increased rate of tubal factor infertility due to high rates of STI's and PID.
    - Higher incidence of menstrual irregularities with protracted anovulation and amenorrhea and an effect on oogenesis [39].
    - HIV-infected women may also have a reduced ovarian reserve [40].
    - The mtDNA content is depleted in oocytes of infertile HIV-infected women under HAART treatment. This mitochondrial defect in oocytes could eventually lead to cell dysfunction and infertility [41].
- In men, the reduced coital frequency because of increased morbidity and poor nutrition may result in low spermatogenesis. There may be progressive damage to sperm morphology, motility, quality, and function as the disease progresses [42].
    - Antiretroviral treatment may cause alteration in sperm characteristics such as a decrease in ejaculate volume and percentage of motile spermatozoa [43].

Safe conception

- In HIV-serodifferent couples, there is a concern of transmission of HIV to the uninfected partner, especially if one partner is not suppressed on treatment.
- In HIV-seroconcordant couples, there is a risk of possible transmission of drug-resistant HIV or superinfection.
- There is a risk of vertical transmission to the child when an HIV-positive female becomes pregnant.
- An HIV-negative woman who seroconverts during conception attempts or pregnancy has a high risk of poor outcomes.

Safe conception in serodifferent couples

Partners can conceive through condomless sex provided the HIV-positive partner is virally suppressed and remains adherent to antiretroviral therapy [44].

- Undetectable viral load is defined as an HIV-1 RNA viral load less than 50 copies/mL [44].
- Sperm washing is also an option for safe reproduction for serodifferent couples where the male is HIV-positive [45].
- HIV is present in semen as a free virus in the seminal plasma and as a cell-associated virus in the non-sperm cells. There is a significant reduction in the risk of viral transmission if spermatozoa are washed free of seminal plasma and non-sperm cells [46].
- This technique, however, is highly specialised and only available in highly specialised laboratories. It also has high-cost implication and should be reserved for high resource settings.

Infertility treatment for people living with HIV

- People living with HIV should also have access to a full range of investigations.
- Antiretroviral (ARV) therapy must be optimised before attempting to conceive.
- Consistent use of ARVs is required, with good adherence to treatment.
- Viral load must be undetectable < 50 copies/ml.

- Monitoring of viral loads every 3-6 months to ensure sustained viral suppression.
- Infertility treatment must be individualised.
- Consider condomless sexual intercourse in those with no infertility diagnosis. Condomless sex should only be undertaken once adherence is confirmed, and the client living with HIV is virally suppressed.
- All clients should be screened for active STIs. If there is evidence of an STI in either or both partners, both partners should be treated.
- Offer assisted reproductive technology when indicated.
- These guidelines are aligned with recommendations of the Southern African HIV Clinicians Society (SAHIVCS) guidelines and 2019 PMTCT guidelines.

Table 7 below outlines the reproductive options available to couples living with HIV.

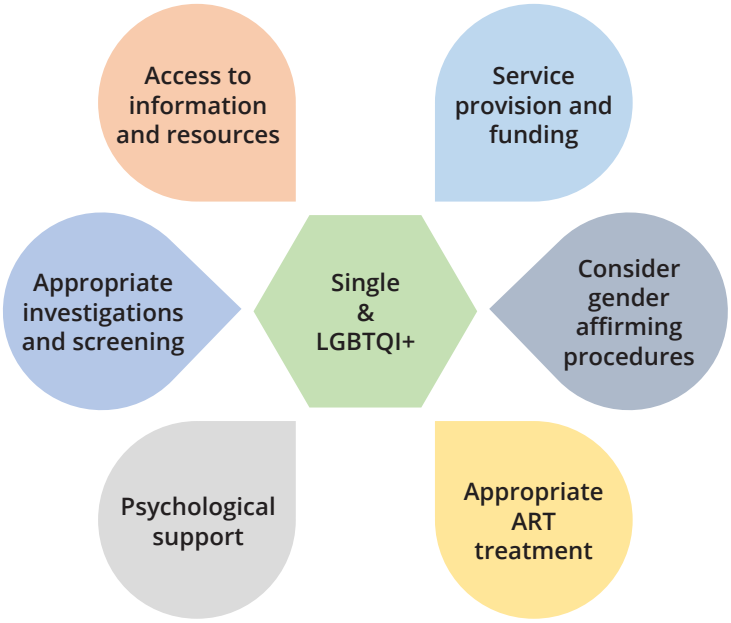
Table 7. Reproductive options for people living with HIV

HIV+ woman HIV- man	HIV+ man HIV- woman	Both HIV+
• Antiretroviral therapy • Undetectable viral load < 50 copies/ml **Fertility not an issue** • Condomless sex • Timed condomless sex* /Self-insemination • Consider PrEP if female not on ARVs for at least six months and viral load ≥ 50 copies/ml **Infertility an issue** • IUI/IVF/ICSI • Adoption	• Antiretroviral therapy • Undetectable viral load < 50 copies/ml **Fertility not an issue** • Condomless sex • Timed condomless sex* • Consider PrEP if male not on ARVs for at least six months and viral load ≥ 50 copies/ml **Infertility an issue** • Sperm washing • IUI/IVF/ICSI • Donor sperm • Adoption	• Antiretroviral therapy • Undetectable viral load < 50 copies/ml **Fertility not an issue** • Condomless sex • Timed condomless sex* **Infertility an issue** • Sperm washing • IUI/IVF/ICSI • Donor sperm • Adoption

* Timed condomless sex -condomless sex acts limited to the peak fertile window, which occurs around the time that the female partner ovulates. Timed condomless sex is recommended if:

- Positive partner(s) not confirmed VL < 50 copies/ml
- Viral load monitoring available
- Client preference
- No STI's

6.5 Fertility treatment for a single person or LGBTQI+



Single persons and the LGBTQI+ community should be free to exercise their sexual and reproductive rights and desire to build a family. Service provision should be non-judgemental, supportive, and understanding. When seeking fertility treatment, they should have access to the full range of investigations and treatment. Treatment options should be discussed with the healthcare provider and should be individualised. Figure 13. depicts the standard of care to be provided.

Figure 13. Standard of care for single and LGBTQI+ persons

6.6 Fertility treatment for the medically complicated person and people with disabilities

Persons with medical conditions and physical disabilities seeking ART should be managed by a multidisciplinary team comprising of a reproductive medicine specialist, physician, feto-maternal specialists, and specialists in the medical condition or disability. Important issues to consider are the effects of pregnancy on the disease/disability, the effects of the disease/disability on the pregnancy, effects of the disease/disability on reproduction, and the effects of ART on the disease/disability. All persons with medical diseases or disabilities should be optimised before initiating ART. Should ART be the treatment of choice, recommendations should be for a single embryo transfer to avoid multiple pregnancy.

The following are a list of disorders that require assessment and optimisation before ART:

- Hypertension
- Cardiac diseases
- Renal failure and renal transplant
- Endocrinopathies; diabetes mellitus, hyperprolactinaemia, and thyroid diseases
- Epilepsy
- Autoimmune disorders
- Thrombo-embolic disorders
- Obesity
- Cancer
- Physical disabilities

Key areas:

- Sexual and reproductive health and rights is a priority
- All persons regardless of their sexual orientation have a right to build a family
- There must be access to individualised treatment
- Preconception care is important to ensure optimisation for pregnancy and good pregnancy outcomes
- Fertility services must be provided in a supportive, non-judgemental, and friendly environment

Section 7. Assisted reproductive technology (ART)

ART procedure is any procedure where eggs are surgically removed from the woman's ovaries and combined with sperm in a laboratory setting and returned to a woman's uterus. The ICMART definition for assisted reproductive techniques refers to all treatments or procedures that include the in vitro handling of both human oocytes and sperm, or embryos, to establish a pregnancy. This includes, but is not limited to, in vitro fertilisation (IVF), embryo transfer (ET), intracytoplasmic sperm injection (ICSI), embryo biopsy, pre-implantation genetic testing (PGT), assisted hatching, gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer, gamete, and embryo cryopreservation, semen, oocyte, and embryo donation, and gestational surrogacy. ART does not include assisted insemination (artificial insemination) using sperm from either a woman's partner or a sperm donor [2]. Artificial insemination, though not included in the definition of ART, forms part of the armament of treatment for infertility. Ovulation induction is required for ART. This is a process whereby exogenous medication is used to assist with ovulation. With IVF and ICSI, controlled ovarian hyperstimulation is used, which is stimulating multiple follicles to develop for surgical removal for ART. Indications and complications of the different ART procedures are shown in Table 8 on the next page.

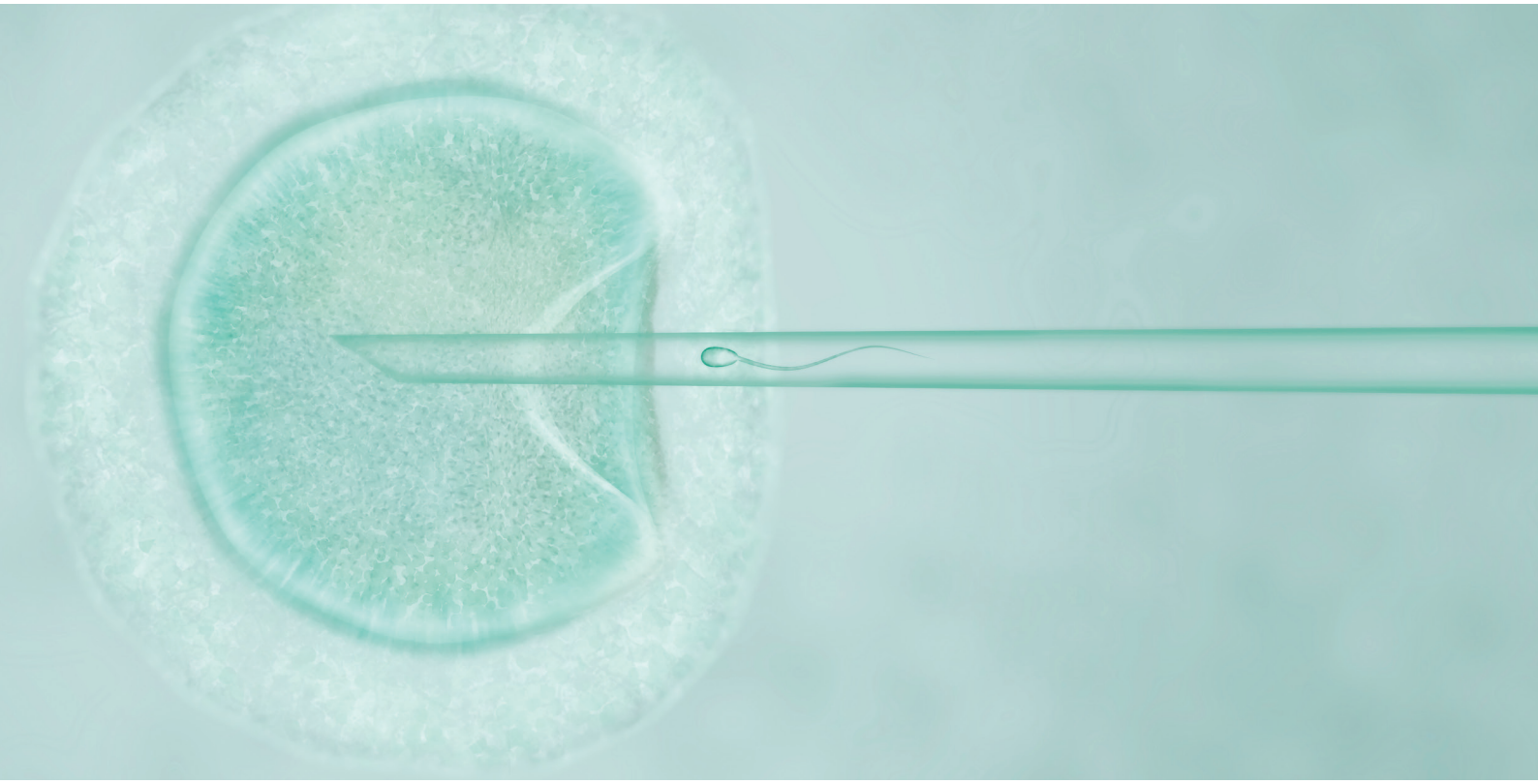


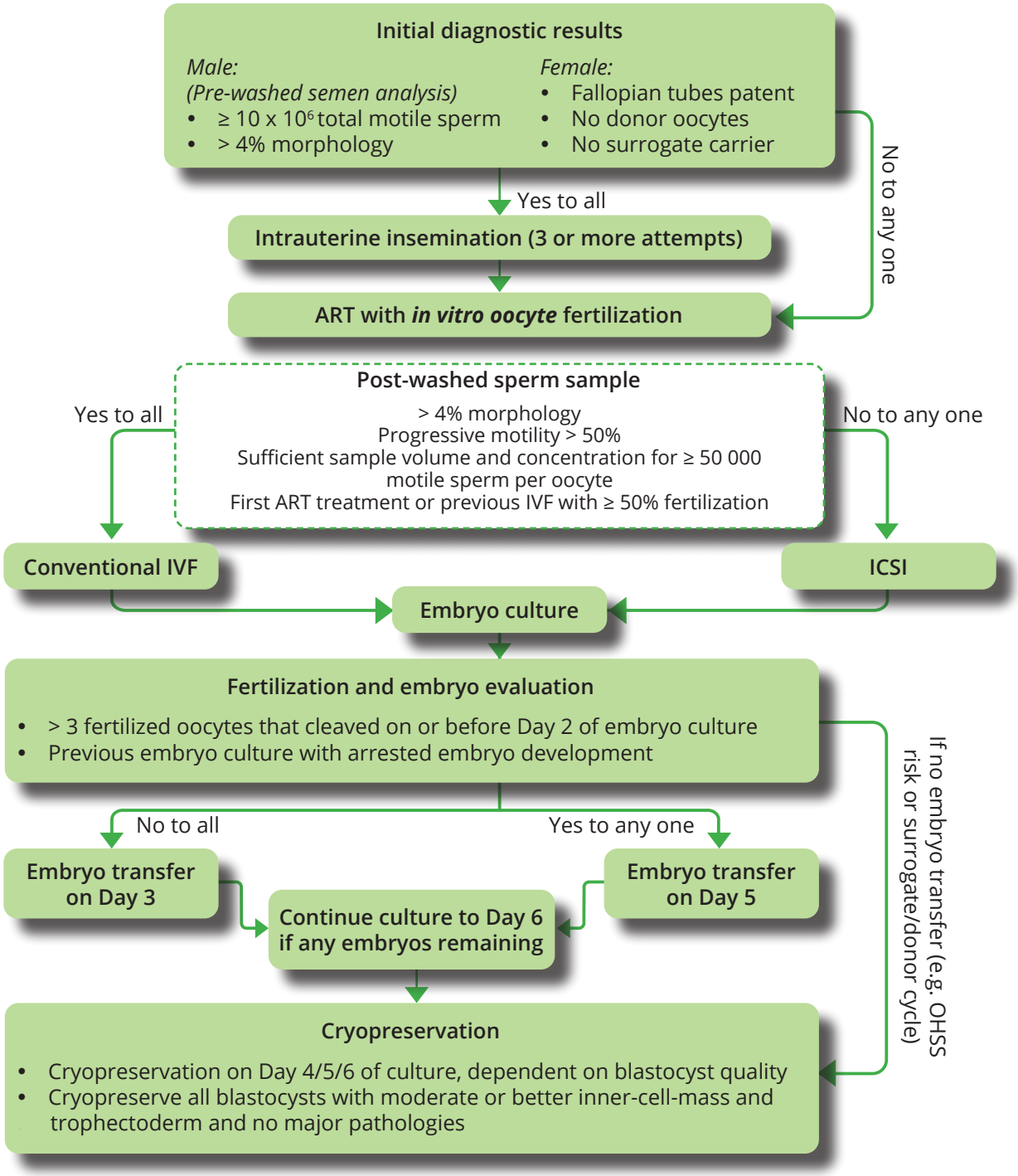
Table 8. ART procedures

Procedure	Indications	Complications
Intrauterine insemination (IUI)	• Unexplained infertility • Mild male factor • Mild endometriosis • Cervical factors • Sexual disorders • Same-sex/single persons	• Multiple pregnancies • OHSS
In vitro fertilisation (IVF)	• Tubal factor • Failed IUI • Severe endometriosis • Diminished ovarian reserve	• OHSS • Multiple pregnancies • Side effects of drugs • Egg retrieval procedure complications • Ectopic pregnancy • Obstetric complications • Cost of treatment
Intracytoplasmic sperm injection (ICSI)	• Male factor • Fertilisation failure with IVF • Fertilisation with epididymal/ testicular sample • Fertilisation with cryopreserved sperm • Fertilisation with immotile sperm	• OHSS • Multiple pregnancies • Side effects of drugs • Egg retrieval procedure complications • Ectopic pregnancy • Obstetric complications • Cost of treatment
Surrogacy	• Absence of a uterus ✓ Hysterectomy ✓ Congenital abnormality • Severe uterine abnormalities ✓ Asherman's syndrome ✓ Endometrial damage • Medical conditions where pregnancy is contraindicated • Chronic reproductive loss • Same-sex male couples/single men	• OHSS • Side effects of drugs • Depression

Section 8. Reproductive laboratory

8.1 Introduction

Laboratory procedures form a fundamental part of assisted reproduction technologies (ART), and selection thereof is crucial. Both the male and female involved should be evaluated to determine the appropriate procedure to follow. Three major factors to consider are whether the female has patent fallopian tubes, if there is male factor subfertility, and history of previous ART treatment cycles [24, 47-49]. The figure below provides an algorithm for the selection of laboratory procedures [15, 47, 48, 50-54].



(OHSS: Ovarian hyperstimulation syndrome; IUI: Intrauterine insemination; ART: Assisted Reproductive Technology; IVF In vitro fertilization; ICSI: Intra cytoplasmic sperm injection)

Figure 14. Algorithm for the selection of laboratory procedures

ART laboratory procedures include, but are not limited to:

- Semen processing (combined with decontamination if necessary)
- Surgical sperm retrieval for obstructive and non-obstructive azoospermia
- Intrauterine insemination (IUI)
- In vitro fertilisation (IVF), with or without the use of intracytoplasmic sperm injection (ICSI), through the use of the patient's own or donor gametes
- Embryo transfer to the patient or the agreed-upon surrogate
- Cryopreservation and storage of gametes and embryos
- Other, e.g. embryo biopsy for pre-implantation genetic screening, time-lapse embryo culture, and sperm functional testing

8.2 Operational requirements

Reproductive laboratory facilities, diagnostic workout of patients, gamete preparation, and embryo culture are an integral part of the ART Unit. The laboratory is an essential part of assisted reproduction, and before performing any procedures, an embryo-safe environment must be guaranteed. The minimum staff and equipment requirements for essential procedures can be seen in Table 9. Access control to the laboratory must be maintained to safeguard this embryo-safe environment. Specific details on equipment used in the ART laboratory can be found in annexure 1. Details on sperm preparation techniques can be found in annexure 2. A summary of critical elements for embryo culture can be found in annexure 3.

Table 9. Minimum staff and equipment requirements for ART procedures

Level of care	Procedures	Staff	Equipment
Diagnostic	Semen analysis	• Embryologist/andrologist **OR** • Trained laboratory technologist (in conjunction with a calibrated Computer Assisted Sperm Analyzer (CASA) and satellite laboratory to confirm results if no embryologist/ andrologist is available)	• Class II Biological Safety Cabinet • Appropriately sized pipettes • Appropriate counting chamber • Upright microscope with: ◦ Phase-contrast ◦ 20x, 40x, 100x magnification • Same as above, with the camera and CASA system attached to the microscope • Internet access to send CASA video
Therapeutic - Basic	Semen processing (IUI)	• Embryologist/andrologist	• Same as for semen analysis • Centrifuge with swing-out rotors
Therapeutic - Advanced	- Embryo culture - ICSI - Embryo transfer - Cryopreservation	• Lab director (embryologist) with: ◦ PhD **OR** ◦ Master's degree and five years of clinical experience **OR** ◦ Ten years of clinical experience • At least one additional embryologist • If more than 150 ART cycles performed per year, an additional embryologist for every 150 ART cycles	• Same as for semen processing • Laboratory with HEPA filtered air supply • Medical fridge • Embryo culture incubator with appropriate gas supply • Laminar flow cabinet • Inverted microscope with: ◦ Modulation contrast ◦ 5/10x and 20/40x magnification ◦ Micromanipulators for ICSI • Liquid nitrogen (LN2) dewars and reliable LN2 supply • Uninterrupted power supply for Embryo culture incubators, fridge and microscopes

Laboratory personnel

Embryologists are specialist laboratory staff who take care and maintain human gametes and embryos in an optimal condition according to good laboratory practices. Embryologists in South Africa fall into two groups: (i) Medical biological scientists (Medical & Dental & Medical Sciences HPCSA board) and (ii) Clinical technologists (Radiography & Clinical Technology HPCSA board). A sufficient number of trained embryologists must be available for appropriate laboratory operations (Table 9) [55-57].

Environment

The location, structure, building materials, fittings and furniture inside the laboratory should be chosen to limit volatile organic contaminants [58]. A "burning-in" period of six weeks is advised for any new equipment before the initiation of ART cycles [58].

Ambient air

Air quality depends on the number and size of particles, and microorganisms/VOCs found in the air and can be maintained by the use [57, 59-61] of (i) high-efficiency particulate air (HEPA) filtration in conjunction with (ii) activated carbon and potassium permanganate filters and (iii) positive air pressure (10-15 Pascal difference) to displace air from the most to the least critical areas in the ART facility [55, 60]. Yearly particulate testing and microbial culture can be done to monitor and validate air quality [62].

Light exposure

Light source and filters can be selected to reduce exposure of gametes and embryos to harmful wavelengths, while direct sunlight in the embryology lab should be avoided [24].

Sterility

Scheduled cleaning and sterilisation of laboratory, workstations, and all equipment must be performed before any procedure [59, 61, 63].

Equipment, quality control, and maintenance schedule

Appropriate equipment must be available for use in the ART laboratory (Table 10), with scheduled maintenance and quality control of the operation thereof (see addendum) [55, 56].

8.3 Spermatology

Semen analysis

The results from a basic semen analysis (with confirmation of abnormal results with a second semen analysis), performed according to the WHO manual for the examination and processing of human semen (15), and as described in "Section 9: Male factor infertility", can be used to determine mild or severe male factor infertility [15, 54, 55].

Mild male infertility can be defined as one or more of the following falling below the WHO lower reference limits:

- seminal volume,
- sperm concentration,
- progressive motility, and morphology, but the total motile sperm count (volume [ml] x concentration [per ml] x progressive motility [%]) in the raw semen sample is above 10 million or the processed sperm sample is above 1 million [47-49].

Sperm processing

For therapeutic procedures, spermatozoa must be removed from the complete semen sample, containing microorganisms, senescent and immature sperm, non-sperm cells, and cell detritus. This can be performed by the following techniques (see addendum for detailed explanation):

- Simple washing
- Direct swim-up
- Density gradient centrifugation (in combination with decontamination if needed)

Sperm processing, in conjunction with decontamination, is advised when seminal pathogens need to be removed before ART treatment [64]. This could include males with blood-borne viruses (BBV) (e.g. HIV, Hepatitis C, etc.) with detectable blood or seminal viral loads [64, 65]. For the ART treatment of patients with BBV, separate facilities or batching of positive patients is advised [64, 65].

Certain males presenting with azoospermia may benefit from surgical retrieval of spermatozoa. Sperm aspiration from the epididymis or testis can be performed. If needed, a biopsy of testicular tissue containing seminiferous tubules can be removed [15, 54-56]. The retrieved tissue and fluids are microscopically evaluated and used for ART on the same day, or after cryopreservation.

8.4 Embryology

Patient and sample identification

Traceability in the ART environment is of utmost importance: identification of samples is mandatory, and double witnessing is recommended at crucial steps [54-56]. Common identifiers used in conjunction are the patient's name and birth date, a unique numerical identifier, and date of the procedure.

Gamete removal and combination

Oocyte removal should be performed in conjunction between the clinician and embryologist. Insemination of oocytes should occur between 38 and 40 hours after human chorionic gonadotropin (HCG) trigger [54, 55]. The selection of insemination procedure (IVF vs ICSI) should be according to the sperm profile, as well as the patient history and previous ART cycles [54, 55]. Standard operational procedural details on performing an ICSI and IVF insemination are described in detail by Thomas Freour in Chapter 11 of the book Standard Operational Procedures in Reproductive Medicine: Laboratory and Clinical Practice (Oxford University Press, 2017), edited by Botros Rizk and Markus Montag [66].

Embryo culture

Embryo culture conditions are influenced by the availability of equipment as well as personal preference (see addendum). Whichever culture method is preferred, an embryo-safe environment must be guaranteed. Evaluation of the oocyte's normal fertilisation (2PN2PB: the presence of two pronuclei and two polar bodies), as well as embryonic development, provides information to be used for embryo selection at the time of embryo transfer or cryopreservation. Figure 12 illustrates the sequence of embryo evaluations [51, 55].

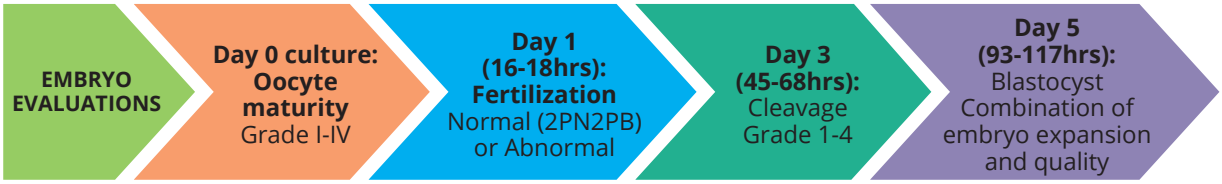


Figure 15. Overview of embryo evaluation timepoints

Embryo Transfer

Each patient is unique, and factors influencing the day of embryo transfer (ET) and the number of embryos to be transferred should be considered before any selections are made. Day 5 of culture is preferred for embryo transfer, but if three or fewer oocytes fertilised normally, or embryo culture cannot be supported for five days, ET can be performed on day 3 of culture [51-53, 55].

Embryo evaluation and recommendation of number for transfer should be discussed with the patients. No more than two embryos should be transferred. For patients younger than 35 years of age and good embryo quality, a single blastocyst transfer on day 5 of culture is advised [52, 54, 55].

Cryopreservation

Cryopreservation is used to preserve and store excess or back-up gametes and embryos. Selection of the cryopreservation method will determine the equipment needed. Internationally, vitrification of oocytes and embryos is preferred.

- Strict criteria for selection of cryopreservable material is essential [51, 54, 55].
- Oocyte vitrification is advised to take place within two hours of oocyte collection, and when warmed, the oocytes should be ICSI's within two hours of warming [49].
- Closed cryopreservation systems and separate storage dewars are advised to be used when working with gametes or embryos from BBV positive patients [64, 65].
- Record keeping of cryopreserved material, as well as upkeep of the liquid nitrogen canisters this material is stored in, should be kept safe, with a scheduled back-up system
- Clear indicator as to where specific samples are stored with easily recognisable identifiers must be used

8.5 Standard operating procedures

Standard operative procedures (SOPs) as part of the documentation system of the ART unit should detail the necessary steps for the operation of equipment, execution of procedures, materials needed, and steps to follow [55, 56, 67]. The use of flow diagrams for ease of understanding procedural steps is recommended. This system forms the backbone of an ART unit to ensure:

- Consistent adherence to procedural standards
- Optimising procedural and environmental conditions
- Safety measures (e.g. double witnessing of samples; difficult ART procedures)
- How to deal with emergencies (e.g. equipment malfunctioning/human errors/power failures etc.)

Policies and laboratory guidelines form part of internationally accepted good laboratory practices and are essential to operate and provide the best clinical outcome for patients [55, 56, 67]. They should include:

- Responsibilities and qualifications of laboratory staff
- Risk reduction measures (with specific reference to infectious agents)
- Aseptic standards of/protective measures in the reproductive laboratory

Key areas:

- Male and female diagnostic workup will identify the correct procedure to follow
- There must be patient and sample identification and traceability
- An embryo-safe culture environment is crucial
- Decisions on appropriate identification of day of transfer and number of embryos to transfer (maximum 2) must be made by both clinician and embryologist
- Facilities must be available for cryopreservation of excess embryos and/or gametes

Section 9. Service delivery platform

This section summarises the minimum staffing and equipment requirements for a service delivery platform to provide fertility care at specialised fertility units (Table 10) and Level 2/3 infertility units (Table 11) .

Table 10. Minimum staffing and equipment requirements at specialised fertility units

	Minimum staffing requirements	Minimum equipment requirement	Minimum service delivery package
Specialised infertility clinic	• Reproductive medicine specialist (clinician with appropriate qualifications) • Specialised fertility nurses • Anaesthetist • Facility for tubal testing, e.g. HSG • NHLS laboratory services • Psychologist • Administrative staff	• Aspiration room • Embryo transfer room • Ultrasound with a transvaginal probe • Theatre equipment • Resuscitation trolley • Consumables • Gynae pack • Filing cabinets	• Counselling • Investigations • ART • Surgery to improve fertility
Reproductive biology procedures	• Lab director (embryologist) with any of the following qualifications: ✓ PhD **OR** ✓ Master's degree and five years of clinical experience **OR** ✓ Ten years of clinical experience • At least one additional embryologist • If more than 150 ART cycles performed per year, an additional embryologist for every 150 ART cycles • Andrologist • Trained laboratory technologist • Administrative staff	• Class II biological safety cabinet • Appropriately sized pipettes • Appropriate counting chamber • Upright microscope with: ◦ Phase-contrast ◦ 20x, 40x, 100x magnification • Centrifuge with swing-out rotors • Laboratory with HEPA filtered air supply • Medical fridge • Embryo culture incubator with appropriate gas supply • Laminar flow cabinet • Inverted microscope with: ◦ Modulation contrast ◦ 5/10x and 20/40x magnification ◦ Micromanipulators for ICSI • Liquid nitrogen (LN2) dewars & reliable LN2 supply • Uninterrupted power supply for embryo culture incubators, fridge and microscopes	• Semen analysis ✓ Diagnostic ✓ Therapeutic • Advanced therapeutic ART

Table 11. Minimum staffing and equipment requirements at level 2/3 infertility units

	Minimum staffing requirements	Minimum equipment requirement	Minimum service delivery package
Level 2/3 infertility clinic	• A clinician with appropriate qualifications • Registered nurses • Facility for tubal testing, e.g. HSG • NHLS laboratory services • Psychologist • Administrative staff	• Procedure room • Ultrasound with a transvaginal probe • Consumables • Gynae pack • Filing cabinets	• Counselling • Investigations • IUI • Minor surgery to improve fertility

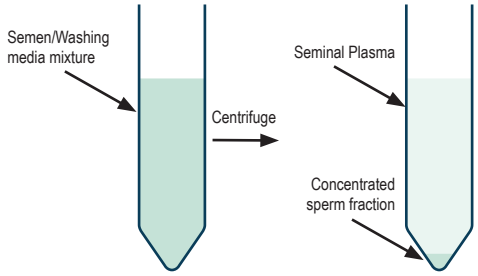
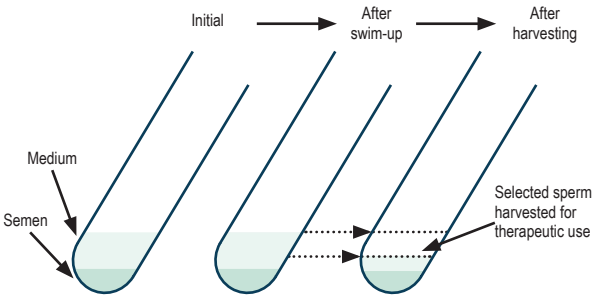
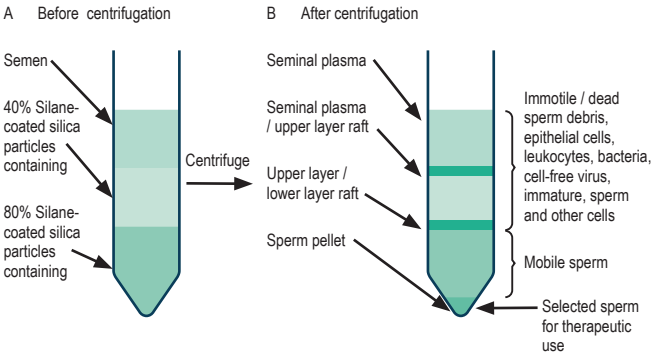
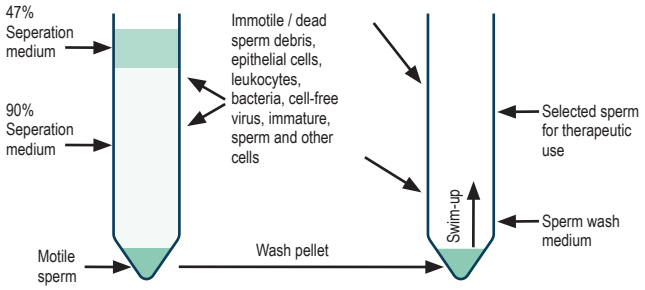
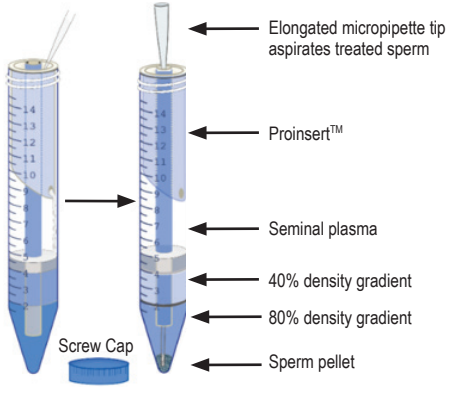
Reproductive biology procedures	• Trained laboratory technologist with a calibrated computer-assisted sperm analyser (CASA) and a satellite laboratory to confirm results (if there is no embryologist/andrologist available)	• Class II biological safety cabinet
		• Appropriately sized pipettes
		• Appropriate counting chamber
		• Upright microscope with:
		  ◦ Phase-contrast
		  ◦ 20x, 40x, 100x magnification
		• CASA system attached to a microscope
		• Internet access to send CASA video
		• Centrifuge with swing-out rotors
			• Semen analysis
			  ✓ Diagnostic
			• Semen processing for IUI

Annexure 1 Equipment used in the ART laboratory

The table below outlines examples of equipment used in an ART laboratory, with proposed maintenance and quality control schedule.

Equipment	Daily	Weekly	Monthly	Yearly
Ambient air HEPA filtration system		• Check positive air pressure difference		• Validation test of filters*
Laminar flow cabinets	• Clean with distilled water	• Check all functions are operational (heated surface, air flow, microscopes)	• Check and calibrate temperature on heated surfaces • Clean with alcohol and distilled water	• Validation test of filters*
Microscopes	• Check normal operations (light, objectives, eye- pieces)	• None	• Kohler illumination • Clean microscopes	• General service*
Tri-gas incubators	• Check gas pressure • Check normal operations (temperature, gas flow rate)	• Check humidification bottle • Perform pH test	• Check in-line gas filters	• General service*
CO_2 Incubators	• Check normal operations (temperature, CO2 level, humidification)	• Replace water baths • Perform pH test	• None	• Sterilise incubator (run self sterilising cycle or by washing) • Replace filters
Bio-flows	• Clean with distilled water	• Clean with alcohol and distilled water	• Check all functions are operational (heated surface, air flow)	• Validation test of filters*
Centrifuges	• None	• Check all operations (rotors, refrigeration unit, seal, lock)	• Treat seal with petroleum jelly	• General service*
Denudation pipettes (mechanical)	• Check normal operations (up-down action, suction)	• None	• Check o-rings	• Replace o-rings (when needed)
Air displacement pipettes	• Check normal operations (up-down action, suction)	• None	• None	• Validate volume of fluid displacement • Calibration if needed*
Medical Fridges	• Check temperature	• None	• None	• General service* (when needed)
Micromanipulators	• Check normal operations (oil chamber full, controllers operational, collet secure)	• None	• None	• General service* (when needed)
				* Service performed by accredited service centre

Annexure 2 Sperm preparation technique

Simple washing	Removal of sperm from the seminal plasma through several washes and centrifugation cycles. Use normal semen samples without any possible pathogens or excess cellular debris for IUI. This can be used in combination with a swim-up step.	
Direct swim-up	Layering of liquified semen under culture medium, or medium over the semen, resulting in a lower sperm yield, but a highly motile sperm fraction. Increasing the surface area of the semen-medium interface and using multiple round bottom tubes or 4-well dishes can improve sperm yield [68].	
Density gradient	Discontinuous density gradient centrifugation offers the best selection of consistently good quality sperm for IUI, IVF and ICSI procedures. The procedure utilises colloidal silica coated with silane and separates cells based upon their density or specific gravity. Mature motile sperm actively migrate into the bottom density gradient layer [68].	
Density gradient centrifugation in combination with sperm swim-up	Discard aspirated supernatants (gradients and cellular debris) from the conical tube, with sperm pellet containing the motile sperm fraction re-suspended in media. Washing of the sperm pellet ensures the removal of gradient particles, and a swim-up follows.	
Semen decontamination	Semen from males who tested positive for the hepatitis C (HCV) or HIV can be decontaminated using density gradient centrifugation with a tube insert for the harvesting of a purified sperm pellet [49]. An aliquot can be frozen for therapeutic ART procedures and a portion submitted for virological evaluation (e.g. HIV-1 DNA & RNA) [69].	

Annexure 3 Summary of critical elements during embryo culture

Culture media type [70]	One-step	• All necessary components present in the media in excess, the embryo uses specific components as needed • Continues culture, especially beneficial when used with time-lapse incubation
	Sequential	• Media composition change according to a developmental stage • Media change necessary on day three of culture
	With oil overlay	• Temperature and osmolarity buffer • Physical barrier • Takes longer for media to gas, but also to out-gas
Oil overlay [71]	Without oil overlay	• Media in direct contact with ambient air • Incubation system must be humidified
Embryo evaluation [72-74]	Time-lapse	• Timing of evaluations are flexible • Embryos remain in culture during an evaluation • More information available per embryo • Better visualisation of morphokinetic events • Automated algorithms to assist in embryo selection • Expensive
	Static	• Single timepoint evaluation of embryos - morphokinetic events can easily be missed • The embryo has to be removed from incubation during an evaluation • Less expensive
Duration of culture [75, 76]	Three days	• Incubation with carbon dioxide in the air • Cleavage stage transfer & cryopreservation
	Five days	• Tri-gas incubation • Blastocyst stage transfer & cryopreservation

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Dr Jeannette Wessels

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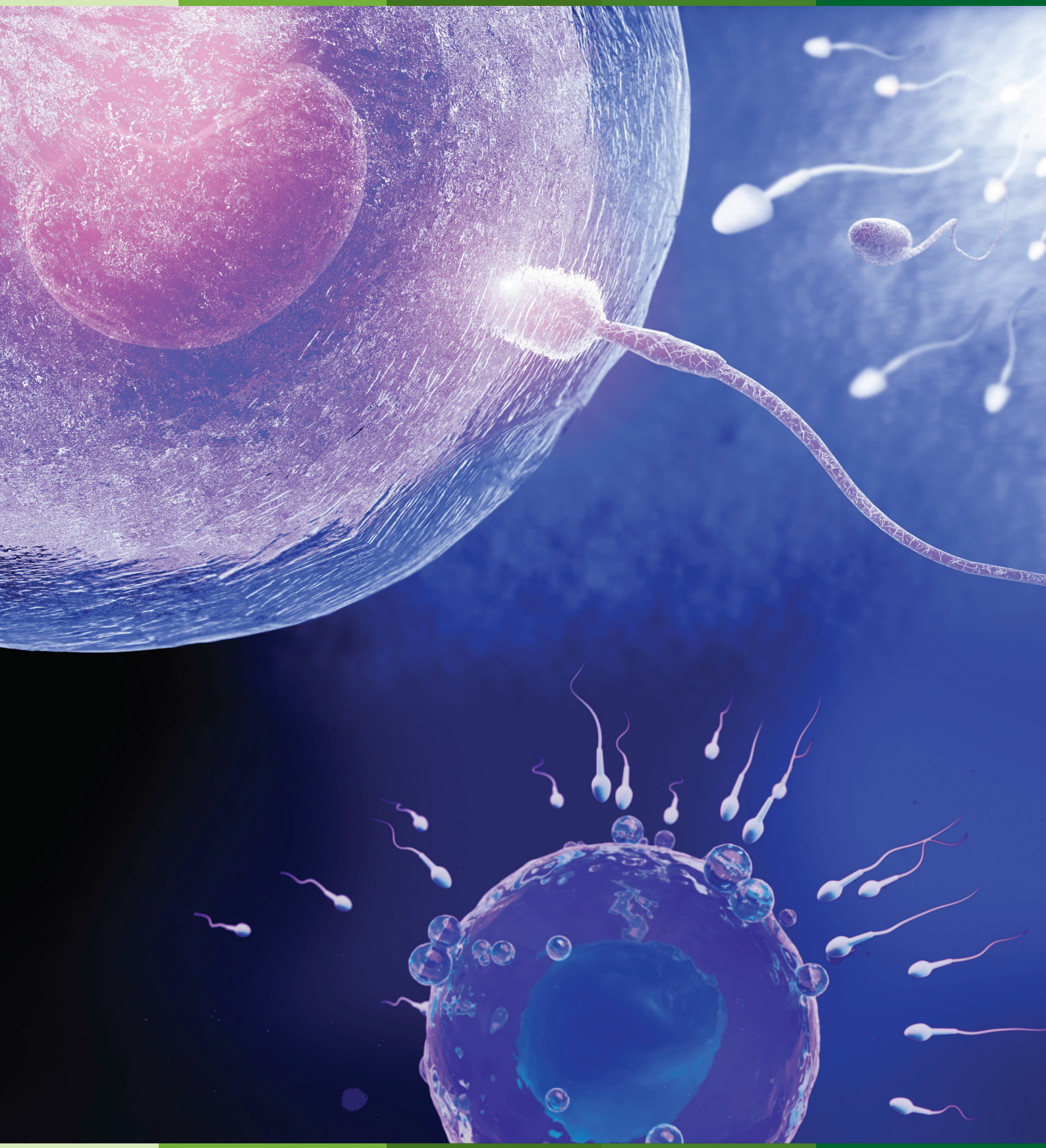

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