

**National Clinical Guidelines for Safe Conception and Infertility**

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National Guidelines for Safe Conception and Fertility

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**Foreword**

The clinical guidelines on safe conception and infertility responds to the National Health Insurance (NHI) principle of universal access, 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 also addresses the needs of the 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 right- based response.

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. In South Africa 1 in 6 couples experience some form of infertility [(Copper D, Morroni C, Orner P, *et al,* 2004)].

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 advocacy groups and other stakeholders to address various myths and misconceptions and the infertility challenge

The Fertility services should be accessible including 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.

Ms MP Matsoso

Director-General: Health

October 2019

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# 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 fertilization |
| 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).1

|  |  |
| --- | --- |
| Term | Definition |
| 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 fertilization (IVF), embryo transfer (ET), intracytoplasmic sperm injection (ICSI), embryo biopsy, preimplantation 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. |
| 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 extreme low temperature. |
| Fecundability | The probability of a 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 | Disease characterized 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 1 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-recognized 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-recognized 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 a 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 function of the male reproductive tract. Characteristic parameters include volume, pH, concentration, motility, vitality, morphology of spermatozoa, and 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 apparently 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 policy makers 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 the 2019 Integrated Sexual and Reproductive Health Policy for South Africa.

# Introduction

The WHO has defined infertility as a “disease of the reproductive system”, resulting in a disability.2

It is a global problem which 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”.3

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.4 In South Africa 1 in 6 couples experience some form of infertility [REF]. The estimates of prevalence are not very accurate due to the fact that some cases are not reported.

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 a rights-based response from the country and the healthcare providers as a whole.

Assisted reproduction technologies (ART) have been developed to assist infertile couples to achieve a pregnancy and to become parents.5, 6 Through the widespread global usage of ART, an estimated eight million pregnancies have been achieved worldwide [REF].However, access to these services still remains a challenge, largely due to a lack of awareness, availability of treatment, and prohibitive costs. 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 of epidemiological and social nature. Fertility services should be accessible including information, diagnosis, assessment, and comprehensive fertility services.

## 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 provide 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 in an effort to create a 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.

# Safe conception care

Safe conception care recognises the importance of preserving fertility as well as providing preconception care. 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 and manage that risk appropriately.

## Preconception Care

Preconception counselling is important to all women seeking fertility treatment in an effort to optimise the pregnancy outcomes. This should be in accordance with the WHO preconception care package of preconception care interventions.7

* **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.8 Figure 1 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 (400μg or 0.4mg folic acid daily).**

**Women at high risk should:**

* 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 offered high-dose supplementation (5 mg folic acid daily)
* be advised to increase their food intake of folic acid
* As applied by the congenital defects guideline

**Identifying women at high risk of NTD’s:**

* previous pregnancy with neural tube defect
* family history of neural tube defects
* women who take antiepileptic drugs
* women who take folate antagonists (e.g. methotrexate, sulphonamides, dolutegravir)
* malabsorption disorders (e.g, inflammatory bowel disease)
* obesity with BMI > 35 kg/m2
* diabetes



Figure 1. WHO recommendations for folic acid supplementation

## Preconception care for people living with HIV: Risk reduction

* Prevent unintended pregnancy
* Prevent new HIV infections in women of reproductive age
* Optimize maternal and paternal health with antiretroviral therapy and ensure that they are adherent to treatment and the viral load is undetectable
* Prevent perinatal HIV transmission (PMTCT)

## 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. 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/m2 or underweight with a BMI < 20 kg/m2.10
* 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 this may affect semen parameters
* [Take precautionary measures when dealing with certain environmental toxins](http://attainfertility.com/article/environmental-toxins), pesticides and other chemicals
* Access safe termination of pregnancy within the healthcare services

Table 1. Evidence based lifestyle factors that affect fertility9

|  |  |
| --- | --- |
| 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 60% |
| Alcohol | RR of Infertility increased 60% (˃ 2 drinks/day) |
| Caffeine | Fecundability decreased 45% (˃ 250mg/d) |
| Illicit drugs | RR of Infertility increased 70% |
| Toxins, solvents | RR of Infertility increased 40% |

**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

# Diagnosis and evaluation

**Standard of care for infertile couple**

All infertile persons should receive information on infertility causes and prevention and have treatment options discussed with them. They should be provided with appropriate diagnostic workup and receive appropriate psychological and emotional support as a basic standard of care.

## When to evaluate?

Table 2. When to evaluate persons for infertility

|  |  |  |
| --- | --- | --- |
| Routine Referral | Early Referral | Immediate Referral |
| After 1 Year of unprotected intercourse | After **6 months** of unprotected intercourse | No delay |
| * Women < 35years * Regular cycles * No known cause of infertility | * Women ≥ 35 years * Regular cycles * No known cause of infertility | * 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) |

## Who should provide fertility services

To improve the effectiveness and efficiency of treatment and satisfaction of clients, fertility care should be coordinated and mainly provided by:

* Gynaecologists, who will refer to fertility specialists when ART is required
* Fertility Specialists

Role of Nurses, General practitioners and Urologists:

* Start an initial assessment for Infertility
* Assess patients prior to referral to a gynaecologist

Role of Gynaecology departments in all clinics/hospitals that deliver primary fertility care:

* Initial assessment for Infertility

Specialised licensed and accredited fertility clinics:

* Performing a complete IVF or ICSI treatment
* Must have highly specialized and accredited laboratories and personnel

## Levels of care

Table 3. Package of services according to the levels of care

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Assessment | Component | Service level check list | | | |
|  |  | Level 1 | Level 2 | Level 3 | Level 4 |
| History | Take medical history |  |  |  |  |
| Take fertility history |  |  |  |  |
| Physical Examination |  |  |  |  |  |
| Hormonal testing | Day 2/3 FSH,LH E2**(e)** |  |  |  |  |
| AMH**(e)** |  |  |  |  |
| Other tests | Prolactin**(r)** |  |  |  |  |
| TSH**(r)** |  |  |  |  |
| HIV**(e)** |  |  |  |  |
| Hepatitis**(e)** |  |  |  |  |
| Rubella IgG**(e)** |  |  |  |  |
| Syphilis serology**(e)** |  |  |  |  |
| Cervical cancer screening**(e)** |  |  |  |  |
| Fertility enhancement surgery  (see section 8) | Minor  Non-specialised |  |  |  |  |
| Major/specialised |  |  |  |  |
| Semen analysis |  |  |  |  |  |
| Hystero-salpingography (HSG) |  |  |  |  |  |
| Intrauterine insemination (IUI) |  |  |  |  |  |
|  |  |  |  |  |
| ART |  |  |  |  |  |
| (r)= recommended (e) = essential  Level 1 = Primary health care clinic, community health care centres, District hospitals, General practitioner (GP)  Level 2 = Regional hospital (Gynaecologist support)  Level 3 = Provincial Tertiary hospital (limited Reproductive Medicine Sub-specialist support)  Level 4 = Specialised hospitals (Reproductive Medicine Sub-specialist support) | | | | | |

Table 4. Services to be provided according to levels of care

|  |  |  |
| --- | --- | --- |
| Levels of care | Service provided | Action |
| Level 1 | Determine:   * Duration of infertility * Timing of intercourse * Regular cycles * Alcohol / smoking / drug use * Obesity / low BMI * Folic Acid supplementation * Tests for STIs on both partners (as per STI Guidelines) HIV, Hep B S Ag, Hep C Ab | Offer advice or refer if:   * >1 year of regular unprotected intercourse in women <35 years * >6 months regular unprotected intercourse in women >35 years * Known cause of infertility * Irregular menstrual cycles |
| Level 2 | * Baseline hormone profile * Ultrasound examination * HSG * Minor surgical procedures * Semen analysis * Anti-Mullerian Hormone (AMH) test | Offer Specialist services  Refer if:   * Blocked tubes * Abnormal sperm analysis and Hormone profile * Specialised surgery to improve fertility   If patent tubes + normal sperm analysis – advise on optimal time for sexual intercourse |
| Level 3 | * Major surgery * Sperm processing * IUI / timed intercourse * Genetic screening | Offer specialist services  Refer if:   * IVF/ICSI required |
| Level 4 | * Sperm processing * Sperm washing * IUI/IVF/ICSI * Specialised reproductive surgery | Offer sub-specialist services |

## Referral routes



Figure 2. Referral routes for different levels of care

## 

## Evaluation and investigations of an infertile female

Couples who experience problems in conceiving should be evaluated together. The importance of a couple’s 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 in a cost-effective manner with a systematic approach.

**Basic fertility workup**



Figure 3. Basic fertility workup

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.

FSH, Estradiol

Normal follicle stimulating hormone (FSH) levels should be less than 10 IU/L.10 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 a low estradiol levels denote hypothalamic pituitary failure and requires further evaluation. Women with abnormal FSH and estradiol levels must be referred for further evaluation.

Anti-mullerian hormone (AMH) levels

Women who are older than 35 years of age should have AMH levels tested. AMH levels are not cycle dependant 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 a transvaginal ultrasound in the early follicular phase measuring 2-10mm.10 AFC of less than 6 is associated with a poor ovarian reserve. AFC is increased in women with polycystic ovary syndrome (PCOS).

**Other tests**

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.

Cervical cancer screening

Cervical screening should be offered in accordance with the national cervical cancers screening programme guidelines.13

**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 second line because it is invasive. It is indicated when other 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 an advantage of treating any pathology at time of diagnosis. (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.

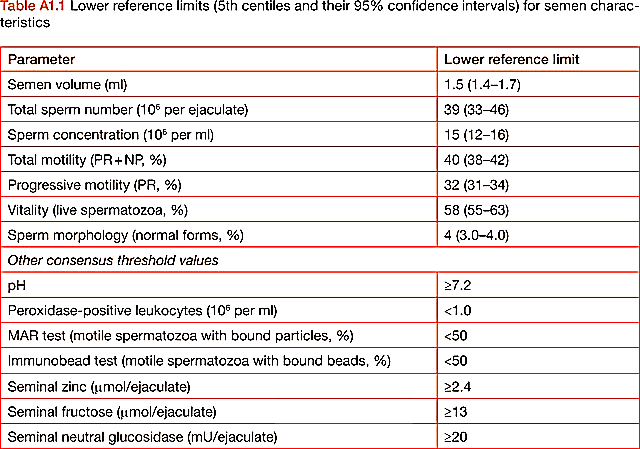
## Investigations to evaluate an infertile male

**Semen Analysis**

Semen analysis should be performed and interpreted according to the 2010 WHO semen criteria as in Table 5 below.14

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 5. WHO reference values for semen characteristics



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, luteinizing 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 helpful 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. This evaluation should be done by an urologist.

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.15 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.

## Investigation for infections

**HIV & Hepatitis**

* People undergoing IVF treatment should be offered testing for HIV, hepatitis B, and hepatitis C.
* If HIV positive, a CD4 count and a HIV viral load test must be done.
* Align with STI guidelines and PMTCT guidelines.16

**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 1 month following vaccination.

**Chlamydia screening**

* Where possible, STI screening and prevention should become routine and integrated into all health visits.16
* Chlamydia antibody test to detect the antibodies to *Chlamydia Trachoma* should not be done as its clinical utility is limited.
* This should align with the STI Guidelines.

# Genetic screening

This should be done in accordance to the genetic screening guidelines. Routine testing is not recommended. The treating physicians should discuss and offer genetic counselling and appropriate testing.

Men with non-obstructive azoospermia and severe oligozoospermia are at increased risk of having genetic and chromosomal abnormalities.17 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. Recommended algorithm for genetic testing through the stages of reproduction is shown in Figure 4 below.

**Testing through the stages of conception**

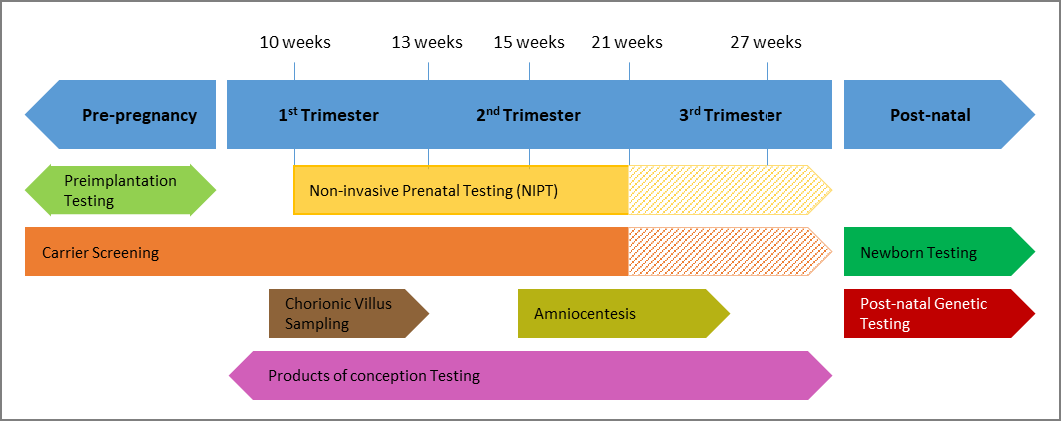
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Figure 4. Recommended genetic testing

**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.
* Genetic screening should be offered where appropriate.

# Counselling and psychological support

## 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 is often very lengthy and cumbersome. They also have to manage the psychological stress of the knowledge that they may not achieve a 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.18

**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.

# Causes of infertility and treatment options

The causes of infertility and their percentage contribution are depicted in Figure 5 below.

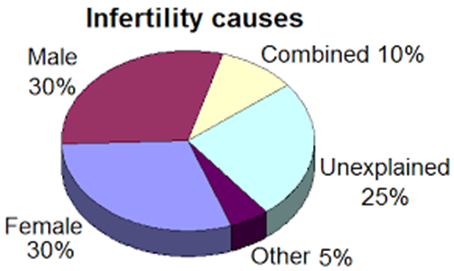


Figure 5. Causes of infertility

## Female factor infertility

**Introduction**

One third of all causes of infertility are solely due to female factors.14 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

**Causes of female factor infertility**

Figure 6. 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.19, 20 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.

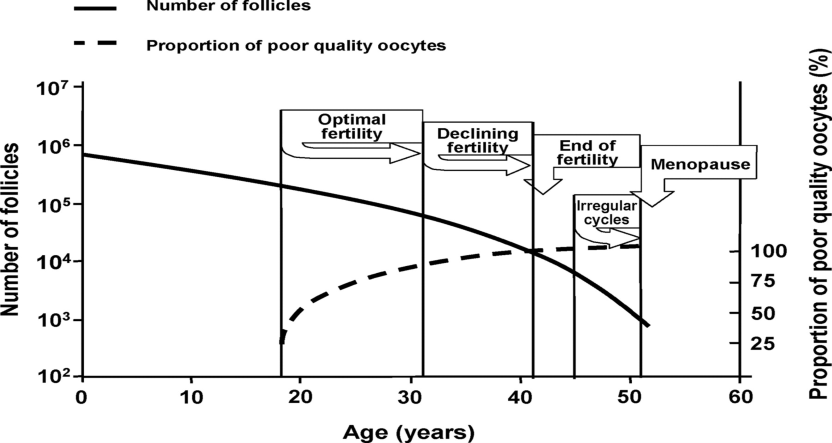


Figure 7. Schematic representation of the number of primordial follicles present in the ovaries and the chromosomal quality of oocytes in relation to female age and corresponding reproductive events21

Patients, whose ovarian reserve is reduced or very 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.22 When a woman does not ovulate spontaneously, she will not conceive without medical intervention. The WHO classifies anovulatory disorders into 3 groups: 22, 23
* Group 1: Hypothalamic amenorrhea
  + Hypothalamo-hypophyseal dysfunction
  + Anorexia Nervosa
  + Excessive exercise (top athletes)
* Group 2: Hypothalamic – pituitary – ovarian dysfunction
  + Polycystic ovary syndrome (PCOS)
* Group 3: Ovarian dysfunction
  + Turner’s syndrome
  + Primary ovarian insufficiency

Table 6. WHO classification of ovulatory disorder and management

|  |  |  |
| --- | --- | --- |
| WHO group 1  (Hypothalamic Amenorrhoea) | WHO group 2  (PCOS) | WHO group 3  (Ovarian dysfunction) |
| 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 5 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 |  |

* **Tubal factor infertility:** Blocked fallopian tubes are the most common cause of female factor infertility in South Africa.24-27 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. The tubal reconstruction surgery should be done by an experienced gynaecological surgeon. 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 8. 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, 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. Submucosal fibroids are mostly symptomatic. They cause heavy menstrual bleeding, prolonged menstrual bleeding, implantation failure, and recurrent pregnancy losses. The Submucosal fibroids, ESGE type 0, 1 and 2, should be removed preferably through hysteroscopy or laparoscopy according to the type.28, 29 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 a number of patients who conceived after UAE. However, serious morbidity has been reported.There is a high risk of morbidly adherent placenta, miscarriage, low birth weight, intra-uterine growth restriction, preterm delivery, and post-partum haemorrhage post UAE.30 Its effect on future pregnancy and fertility remains unclear. All patients who conceived after UAE require close monitoring.

**Uterine anomalies**

* **Septate Uterus:** The septate uterus causes most reproductive failures. Women with a septate uterus should be referred to a reproductive medicine specialist for evaluation and assessment if hysteroscopic removal will improve fertility outcomes.
* **Uterus Didelphus and unicornuate uterus:** Patients with a double uterus (Didelphus) and those with a unicornuate 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 postpartum 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 our setting, Genital tuberculosis (TB) as a cause of severe Asherman’s syndrome should be investigated.

**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 7. 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
* Persons seeking fertility treatment should be advised that lifestyle modification may improve fertility
* 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

## Male factor infertility

Twenty percent of cases of infertility are solely due to male factor infertility, and male factory infertility contributes in 30%-40% of couples overall.15 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 be in parallel to the evaluation of the female. No invasive procedures like laparoscopy or myomectomy should 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, including 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.

**Semen Analysis**

Semen analysis is the most important test for the evaluation of male subfertility. A standardized instruction for semen collection is 2-3 days abstinence.31 Semen can be collected by means of 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.14

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

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 6 weeks. Should the reproductive history be abnormal or the semen parameters remain abnormal evaluation by an urologist or reproductive medicine specialist is indicated.

**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.32
* 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 is not recommended because there is no evidence of efficacy. Corticosteroids should not be used for antisperm antibodies.
* Men with hypogonadotropic hypogonadism should be treated with gonadotrophins.
* The use of anti-oxidant therapy may be beneficial in idiopathic semen abnormalities.

**Surgical management**

* Surgery for varicoceles may be considered when there is a clinically palpable varicocele, with abnormal semen parameters and no female factor for infertility is present.33 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 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**

* Intra uterine insemination (IUI) should be considered in cases of mild male factor. Maximum of 3-6 cycles should be done.
* Intra cytoplasmic 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 9. 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

## Unexplained infertility

This 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 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 fertilization (IVF). IUI still remains a reasonable treatment option for unexplained infertility in the South African setting because of the lower costs and safety. It is a 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 patient’s wishes should also be taken into consideration.

## 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.34 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 the 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 risks to ensure optimal maternal and perinatal outcomes. When couples are seroconcordant, the aim is to assist with conception without risk of superinfection and aim for unaffected offspring. When the couple is serodifferent (serodiscordant), the priority is to prevent infection of the uninfected partner while assisting with safe conception and to also aim 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 revolutionized 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 considerations that the treating healthcare provider must take into account 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 discussed 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 infected from having children.35

**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 increase 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.36
  + HIV-infected women may also have reduced ovarian reserve.37
  + Reduced coital frequency because of increased morbidity and poor nutrition may result in low spermatogenesis in men. There may be progressive damage to sperm morphology, motility, quality, and function as the disease progresses.38
  + Antiretroviral treatment may cause alteration in sperm characteristics such as a decrease in ejaculate volume and percentage of motile spermatozoa.39
  + 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.40

**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.41

* Undetectable viral load is defined as an HIV-1 RNA viral load less than 200 copies/mL.41
* Sperm washing is also an option for safe reproduction for serodifferent couples where the male is HIV-positive.42
* The HIV virus is present in semen as free virus in the seminal plasma and as 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.43
* 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 full range of investigations.
* Antiretroviral (ARV) therapy must be optimised before attempting to conceive.
* Consistent use of ARVs with good adherence to treatment.
* Viral load must be undetectable< 200 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 HIV-positive client is virally suppressed.
* All clients should be screened for active STI and both partners treated, should there be evidence of STI in either or both partners.
* Offer assisted reproductive technology when indicated.
* These guidelines are aligned with recommendations of the Southern African HIV Clinicians Society (SAHIVCS) guidelines and PMTCT guidelines.

Table 8. Reproductive options for people living with HIV

|  |  |  |
| --- | --- | --- |
| HIV+ woman HIV- man | HIV+ man HIV- woman | Both HIV+ |
| * Antiretroviral therapy * Undetectable viral load <200 copies/ml   Fertility not an issue   * Condomless sex * Timed condomless sex\*/Self-insemination * Consider PrEP if female not on ARVs > 6 months and viral load >200 copies/ml   Infertility an issue   * IUI/IVF/ICSI * Adoption | * Antiretroviral therapy * Undetectable viral load <200 copies/ml   Fertility not an issue   * Condomless sex * Timed condomless sex\* * Consider PrEP if female not on ARVs > 6 months and viral load >200 copies/ml   Infertility an issue   * Sperm washing * IUI/IVF/ICSI * Donor sperm * Adoption | * Antiretroviral therapy * Undetectable viral load <200 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 is recommended if:   * Positive partner(s) not confirmed VL <200 copies/ml * Viral load monitoring available * Client preference * No STI’s | | |

## Fertility treatment for single person or LGBTQI



Single persons and the LBGTI 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 10 depicts the standard of care to be provided.

Figure 10. Standard of care for single and LGBTQI persons

## Fertility treatment for the medically complicated persons

Persons with medical conditions 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. Important issues to be taken into consideration are; the effects of pregnancy on the disease, the effects of the disease on the pregnancy, effects of the disease on reproduction, and the effects of ART on the disease. All persons with medical diseases should be optimised before initiating ART. 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

**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

# 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 is: all treatments or procedures that include the *in vitro* handling of both human oocytes and sperm, or embryos, for the purpose of establishing a pregnancy. This includes, but is not limited to, *in vitro* fertilization and embryo transfer, gamete intrafallopian transfer, zygote intrafallopian transfer, tubal embryo transfer, gamete and embryo cryopreservation, 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.1 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 the purposes of surgical removal for ART. Indications and complications of the different ART procedures are shown in Table 9 below.

Table 9. ART procedures

|  |  |  |
| --- | --- | --- |
|  | Indications | Complications |
| IUI | * Unexplained Infertility * Mild male factor * Mild endometriosis * Cervical factors * Sexual disorders * Same sex/single persons | * Multiple pregnancy * OHSS |
| IVF | * Tubal factor * Failed IUI * Severe endometriosis * Diminished ovarian reserve | * OHSS * Multiple pregnancy * Side effects of drugs * Egg retrieval procedure complications * Ectopic pregnancy * Obstetric complications * Cost of treatment |
| ICSI | * Male factor * Fertilisation failure with IVF * Fertilisation with epidydimal/testicular sample * Fertilisation with cryopreserved sperm * Fertilisation with immotile sperm | * OHSS * Multiple pregnancy * 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 |

# Reproductive laboratory

## Introduction

Laboratory procedures form a fundamental part of assisted reproduction technologies (ART) and selection thereof is crucial. To determine the appropriate procedure to follow, both the male and female components involved should be evaluated. 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.44-47

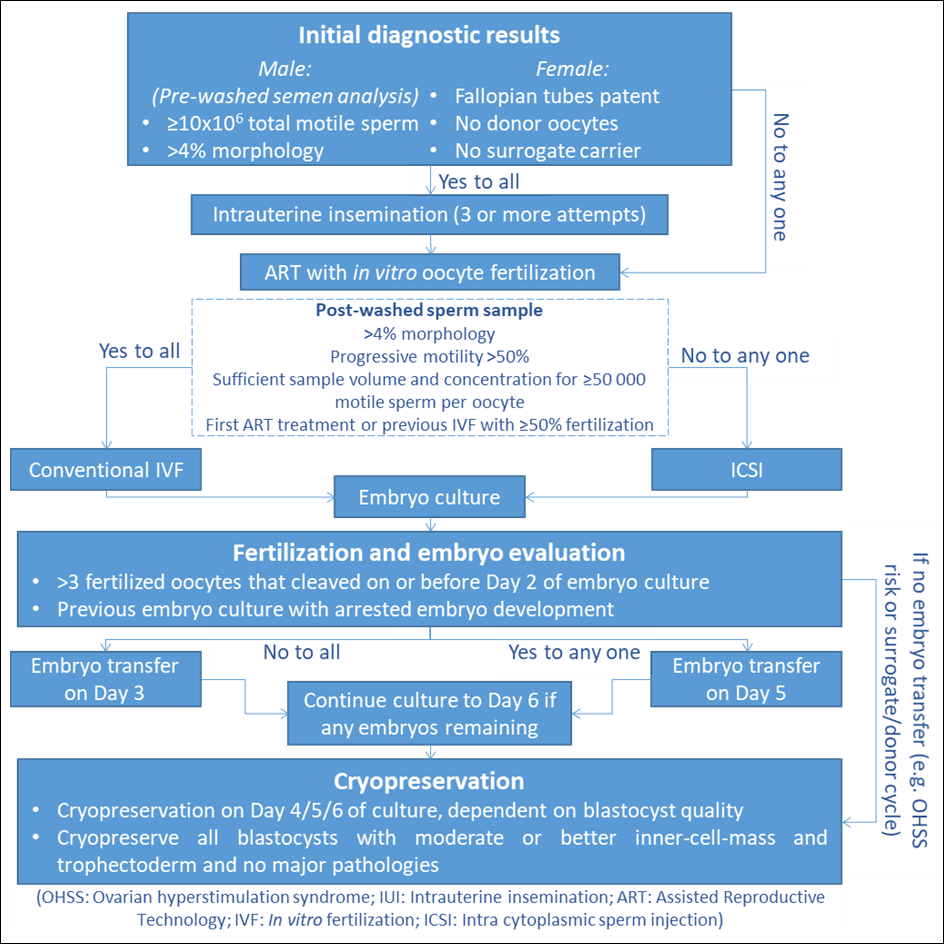


Figure 11. Algorithm for selection of laboratory procedures14, 45, 46, 48-52

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* fertilization (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 their 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

## 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 10. To safeguard this embryo-safe environment, access control to the laboratory must be maintained.

Table 10. Minimum staff and equipment requirements for ART procedures

|  |  |  |  |
| --- | --- | --- | --- |
| Level of care | Procedures | Staff | Equipment |
| Diagnostic | Semen analysis | * Andrologist/Embryologist   **OR** | * Class II Biological Safety Cabinet * Appropriate sized pipettes * Appropriate counting chamber * Upright microscope with:   + Phase contrast   + 20x, 40x, 100x magnification |
| * Trained laboratory technologist  (in conjunction with a calibrated Computer Assisted Sperm Analyzer (CASA) and satellite laboratory to confirm results if no Andrologist/Embryologist is available) | * Same as above, with camera and CASA system attached to microscope * Internet access to send CASA video |
| Therapeutic - Basic | Semen processing (IUI) | * Andrologist/Embryologist | * 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). Sufficient number of trained embryologists must be available for appropriate laboratory operations (Table 10).53-55

**Environment**

The location, structure, building materials, fittings and furniture inside the laboratory should be chosen to limit volatile organic contaminants.56 A “burning-in” period of six weeks is advised for any new equipment prior to the initiation of ART cycles.56

*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 55, 57-59 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.53, 58 Yearly particulate testing and microbial culture can be done to monitor and validate air quality.60

*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.44

*Sterility*

Scheduled cleaning and sterilization of laboratory, workstations, and all equipment must be performed before any procedure.57, 59, 61

**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).53, 54

## 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 semen14 and as described in “Section 9: Male factor infertility”, can be used to determine mild or severe mild factor infertility.14, 52, 53

Mild male infertility can be defined when either one or more of the following falls 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.45-47

**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 needs to be removed prior to ART treatment.62 This could include males with blood borne viruses (BBV) (e.g. HIV, Hepatitis C etc.) with detectable blood or seminal viral loads.62, 63 For the ART treatment of patients with BBV, separate facilities or batching of positive patients is advised.62, 63

Certain males presenting with azoospermia may benefit from surgical retrieval of spermatozoa. Sperm aspiration from the epididymis or testis can be performed or if needed a biopsy of testicular tissue containing seminiferous tubules are removed.14, 52-54 The retrieved tissue and fluids are microscopically evaluated and used for ART on the same day or after cryopreservation.

## 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.52-54 Common identifiers used in conjunction are 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.52, 53 The selection of insemination procedure (IVF vs. ICSI) should be according to sperm profile, as well as patient history and previous ART cycles.52, 53 Standard operational procedural details on performing an ICSI and IVF insemination are described in detail by Freour (2017) and Montag (2017).

**Embryo culture**

Embryo culture conditions are influenced by 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 fertilization (2PN2PB: the presence of two pronuclei and two polar bodies), as well as embryonic development provides information to be used for embryo selection at time of embryo transfer or cryopreservation. Figure 12 illustrates the sequence of embryo evaluations.49, 53

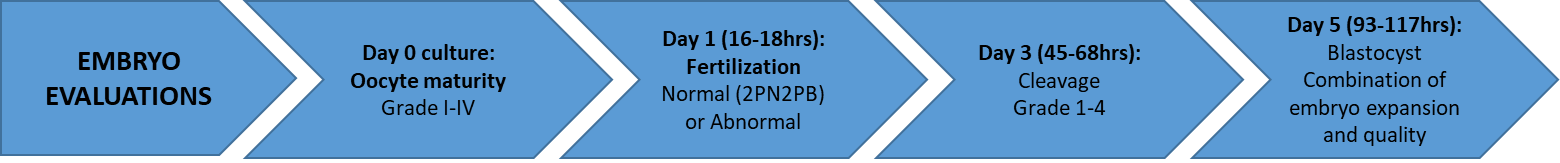


Figure 12. Overview of embryo evaluation timepoints

**Embryo Transfer**

Each patient is unique and factors influencing the day of embryo transfer (ET) and 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 less oocytes fertilized normally or embryo culture cannot be supported for five days, ET can be performed on Day 3 of culture.49-51, 53

Embryo evaluation and recommendation of number for transfer should be discussed with the patients. No more than 2 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.50, 52, 53

**Cryopreservation**

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

* Strict criteria for selection of cryopreservable material is essential49, 52, 53
* 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 warming47
* Closed cryopreservation systems and separate storage dewars are advised to be used when working with gametes or embryos from BBV positive patients62, 63
* 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

## 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.53, 54, 64 The use of flow diagrams for ease of understanding procedural steps is recommended. This system forms the backbone of an ART Unit, to ensure:

* Adherence and consequently consistency through the standardization of a procedure
* Optimizing procedural and environmental conditions
* Safety measures (e.g. double witnessing of samples; difficult ART procedure)
* How to deal with emergencies (e.g. equipment malfunctioning/human errors/power failures etc.)

These policies and laboratory guidelines 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
* This forms part of internationally accepted good laboratory practices and is essential to operate and provide the best clinical outcome for patients.53, 54, 64

**Key Areas:**

* Male and female diagnostic workup to identify 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

# Service delivery platform

Table 11. 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 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 * 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 * Appropriate 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 12. 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 | * Clinician with appropriate qualifications * Registered nurses * Facility for tubal testing e.g. HSG * NHLS laboratory services * Psychologist * Administrative staff | * Procedure room * Ultrasound with 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 Analyzer (CASA) * Satellite laboratory to confirm results (if no Andrologist / Embryologist is available) | * Class II Biological Safety Cabinet * Appropriate sized pipettes * Appropriate counting chamber * Upright microscope with: * Phase contrast * 20x, 40x, 100x magnification * CASA system attached to microscope * Internet access to send CASA video Centrifuge with swing-out rotors | * Semen Analysis * Diagnostic * Semen processing for IUI |

# Addendum

Table 13. Example of equipment used in ART laboratory, with proposed maintenance and quality control schedule



Table 14. Sperm preparation technique

|  |  |  |
| --- | --- | --- |
| **Simple washing** | Removal of sperm from the seminal plasma through several washes and centrifugation cycles. Using 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 liquefied 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.65 | **Illustration 7** |
| **Density gradient** | Discontinuous density gradient centrifugation offers the best selection of consistent 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.65 | **Illustration 6** |
| **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. | **Illustration 4** |
| **Semen decontamination** | Semen from males who tested positive for the hepatitis C (HCV) or human immunodeficiency virus can be decontaminated using density gradient centrifugation with a tube insert for the harvesting of a purified sperm pellet.48 An aliquot can be frozen for therapeutic ART procedures and a portion submitted for virological evaluation (e.g. HIV-1 DNA & RNA).66 | **two syringe....png** |

Table 15. Summary of critical elements during embryo culture

|  |  |  |
| --- | --- | --- |
| Culture media type  (Hardarson *et al.,* 2015) | 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 developmental stage * Media change necessary on day three of culture |
| Oil overlay  (Sifer *et al.,* 2009) | With | * Temperature and osmolarity buffer * Physical barrier * Takes longer for media to gas, but also to out-gas |
| Without | * Media in direct contact with ambient air * Incubation system must be humidified |
| Embryo evaluation  (Basille *et al*., 2015; Meseguer *et al*., 2012; Zhang *et al*., 2010;) | Time-lapse | * Timing of evaluations are flexible * Embryos remain in culture during evaluation * More information available per embryo * Better visualisation of morphokinetic events * Automated algorithms to assist in embryo selection * Expensive |
| Static | * Single time-point evaluation of embryos - morphokinetic events can easily be missed * Embryo has to be removed from incubation during evaluation * Less expensive |
| Duration of culture  (Chang *et al.,* 2009; Kovacic et al., 2010) | Three days | * Incubation with carbon dioxide in air * Cleavage stage transfer & cryopreservation |
| Five days | * Tri-gas incubation * Blastocyst stage transfer & cryopreservation |

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