



TRAINING ON

INFERTILITY AND ASSISTED REPRODUCTION

AT KAIRUKI GREEN IVF

BUNJU 'A' MIANZINI

DAR ES SALAAM, TANZANIA

KHGIVF

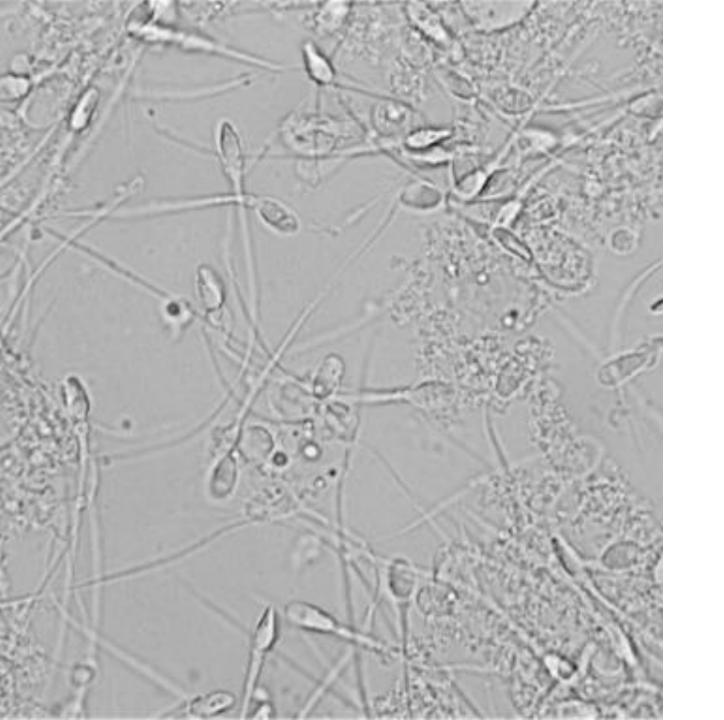
16 & 17th SEPTEMBER

Basic semen analysis and interpretation

George Tryphone:

(BSc. MLT, MSc. Biotechnology & Human assisted reproduction

in Progress)



OBJECTIVES:

- Evaluation of the infertility
 Man
- Interpretation of Semen
 report and additional tests
- Microbiological Culture
- Karyotype and Sperm
 FISH
- Special Protocol

The definition of a **fertile/infertile** male presents an extreme complexity, since the situation can be variable in short periods of time, and even with different partners.

The only commonly accepted tool for studying the fertile potential of men is semen analysis according to the criteria of the World Health Organization (WHO, 2010), based on concentration, mobility and morphology. (2021 new revision)



Introduction

Evaluation of the classic parameters for the correct diagnosis of fertility. WHO

normality criteria (WHO, 1999/2010)



World Health Organization

1.Collection of the sample and transport to the laboratory

2. Macroscopic examination

3. Microscopic examination

4. Normality criteria and nomenclature

Collection of sample by masturbation and transport to the laboratory

Sexual abstinence of 3-7 days.

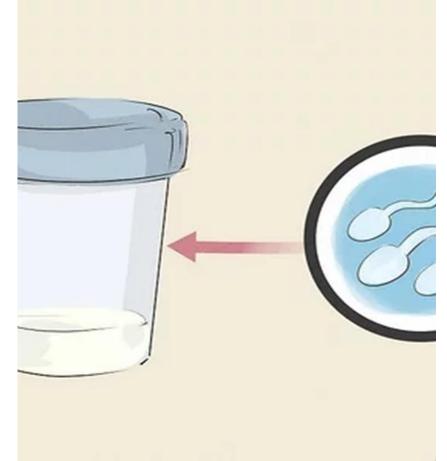
Sterile and non-toxic plastic container. Never use

normal condoms.

Clear labelling. Special cases:

- Collection in split.
- collected in urine.

Keep T near 37ºC until the laboratory



Macroscopic Examination

- Liquefaction
- Viscosity Appearance Odor
- Volume
- pH



Microscopic Examination

- 1. Concentration
- 2. Motility
- 3. Agglutination
- 4. Morphology
- 5. Vitality
- 6. Other semen cells
- 7. Antisperm antibodies



Microscopic Examination. Concentration: (20x

Makler's chamber:

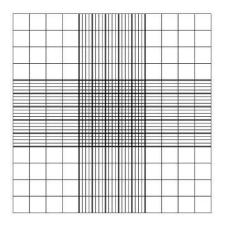
 Indicated for discharges, such as fresh semen under Phase contrast

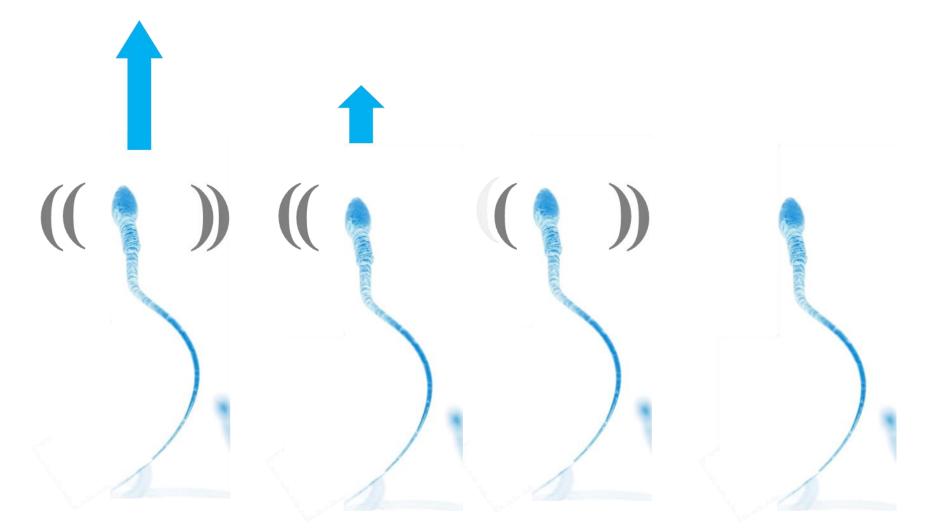
Improved Neubauer Chamber:

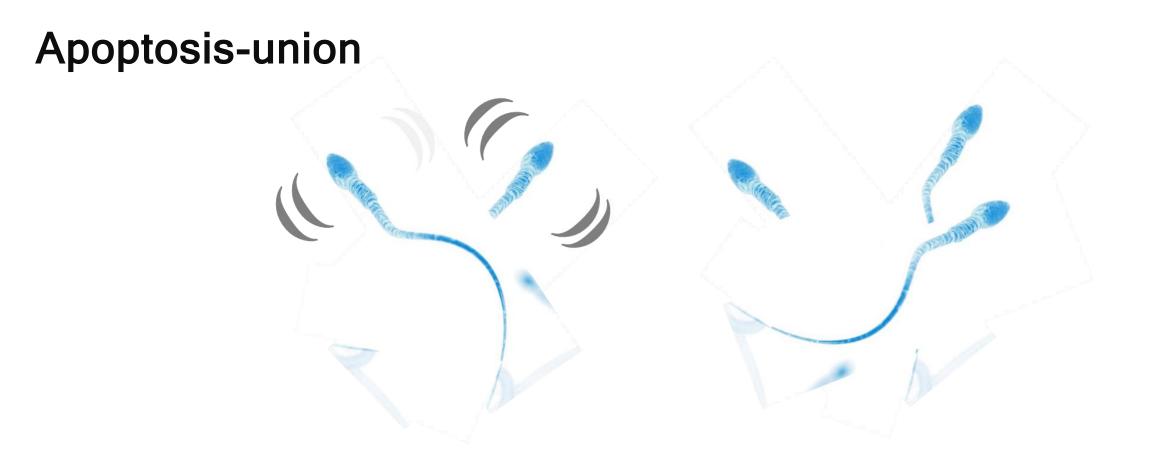
 Indicated to adjust concentrations in insemination drops where minor number are seen











Microscopic Examination. Concentration: (20x

Dyes:

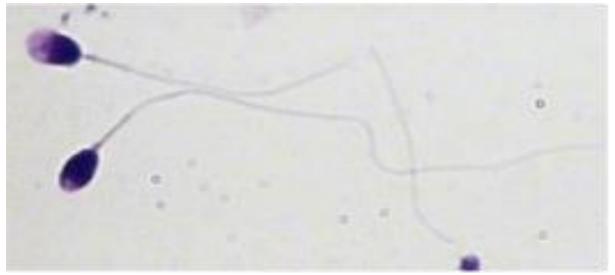
Diff Quick Quick panoptic Papanicolau Shorr

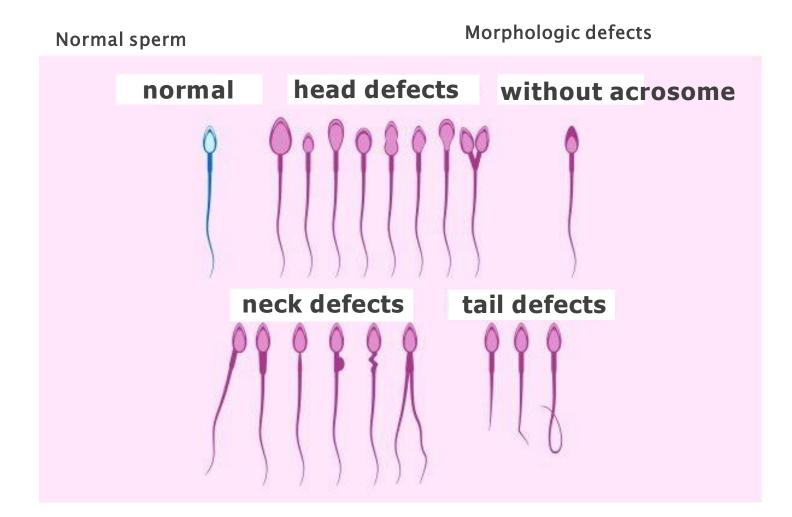
Normal Characteristics:

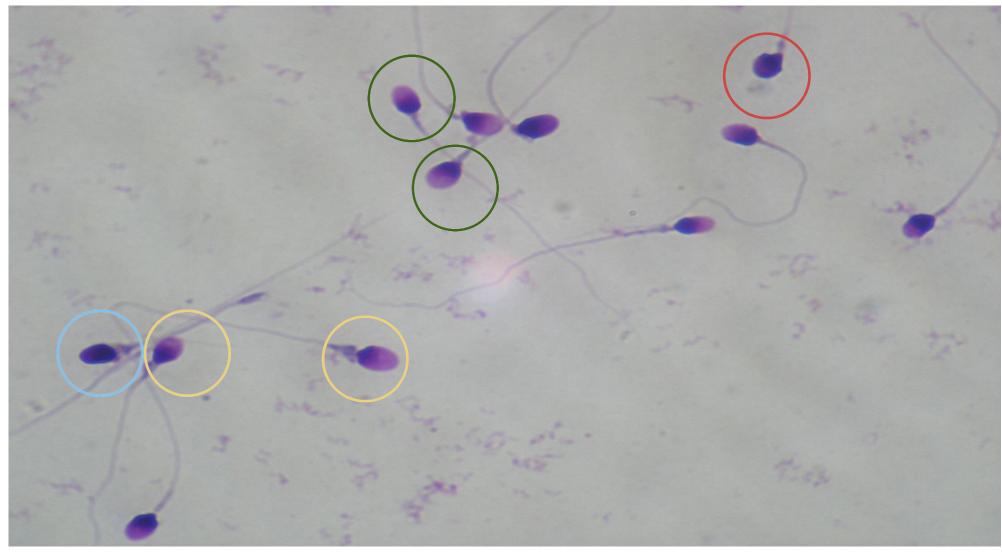
Oval head Acrosome 40-70% Without >50% vacuoles

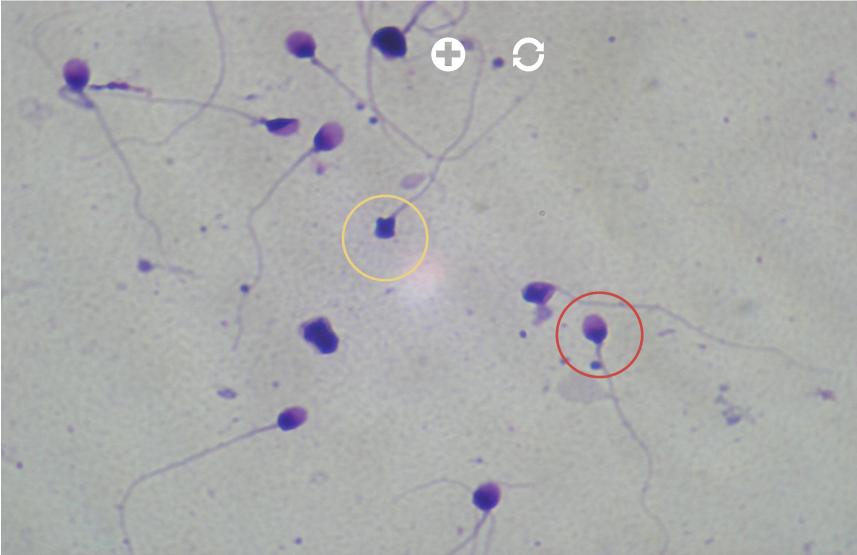
Axial midpiece without breakage or thickening

Extended tail, approx. 7-8 times the head









Microscopic Examination. Concentration: (20x

Microscopic Examination. Morphology: (100x)

Results:

Normal

Abnormal

- Head defects
- Mid-piece defects
- Tail defects

Sum of defects Abormal

Teratospermia Index =

Evaluation of the infertile man: Spermiogram Microscopic Examination. Concentration: (20x

Microscopic Examination. Vitality: (20x)

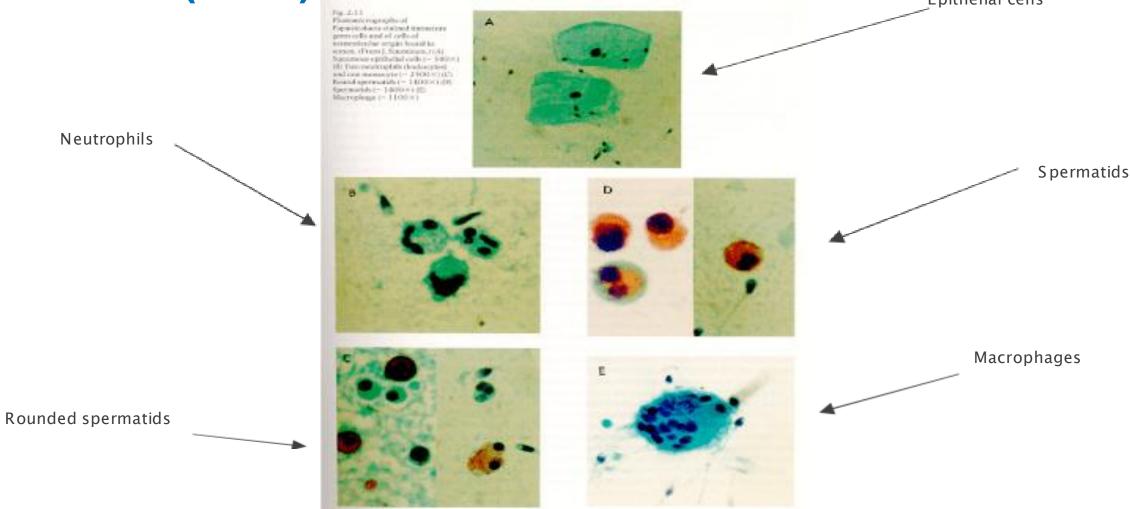
Methods:

A.Vital stain (eosin, trypan blue)

B.Hypoosmotic Test

A. Live Dead B. Live Dead

Evaluation of the infertile man: Spermiogram Microscopic Examination. Other cells present in semen.(100x)



Normality criteria and nomenclature (1999)

| Volume: | 2 ml |
|--------------------|-----------------------------------|
| Concentration: | > 20 mill/ml |
| Motility: | > 50% forms A+B or 25% A |
| Morphology: | >14% (Kruger's strict criterion) |
| Normozoospermia: | normal values |
| Oligozoospermia: | <20mill/ml concentration |
| Asthenozoospermia: | <50% grade (PR+NP) or < 25 PR% |
| Teratozoospermia: | <14% morphology |
| Cryptozoospermia: | less than 100000 sp/sample |
| Leucospermia: | more than 1 mill/ml |
| Necrospermia: | all spermatozoa dead |
| Azoospermia: | no spermatozoa in the ejaculate |

Normality criteria and nomenclature (2010)

| Volume: | 1.5 ml |
|--------------------|----------------------------------|
| Concentration: | > 15 mill/ml or 39 mill total |
| Motility: | > 32% forms A+B or 25% A |
| Morphology: | >4% (Kruger's strict criterion) |
| Normozoospermia: | normal values |
| Oligozoospermia: | <15 mill/ml or 39 mill total |
| Asthenozoospermia: | <40% grade (PR+NP) or < 32 PR% |
| Teratozoospermia: | morphology |
| Cryptozoospermia: | less than 100,000 sp/sample |
| Leucospermia: | more than 1 mill/ml |
| Necrospermia: | >58% vitality or >70 % D |
| Azoospermia: | no spermatozoa in the ejaculate |

Normality criteria and nomenclature (2021)

| Volume: | 1.4 ml | Progressive Motility | >30% of all sperm |
|--------------------|----------------------------------|----------------------|-------------------|
| Concentration/ml: | > 16 mill/ml or 39 mil/ejaculate | | |
| Total Motility: | > 42% forms A+B or 27% A | | |
| Morphology: | >4% (Kruger's strict criterion) | | |
| Normozoospermia: | normal values | | |
| Oligozoospermia: | <16mill/ml concentration | | |
| Asthenozoospermia: | <42% grade (PR+NP) or < 30 PR% | | |
| Teratozoospermia: | <4% nol morphology | | |
| Cryptozoospermia: | less than 100,000 sp/sample | | |
| Leucospermia: | more than 1 mill/ml | | |
| Necrospermia: | >54% (vitality) | | |
| Azoospermia: | no spermatozoa in the ejaculate | | |

Summary of normality criteria and nomenclature

| Semen parameter | WHO 1980 | WHO 1987 | WHO 1992 | WHO 1999 | WHO 20101 | WHO 2021 |
|---|----------|----------|------------------|------------------|-----------|----------|
| Volume (mL) | ND | ≥2 | ≥2 | ≥2 | 1.5 | 1.4 |
| Sperm concentration (x10 ⁴ /mL) | 20-200 | ≥20 | ≥20 | ≥20 | 15 | 16 |
| Total sperm number (x10 ⁶) | ND | ≥40 | ≥40 | ≥40 | 39 | 39 |
| Total motility (%) | ≥60 | ≥50 | ≥50 | ≥50 | 40 | 42 |
| Progressive motility (%) ² | ≥23 | ≥25 | ≥25 (grade a) | ≥25 (grade a) | 32 (a+b) | 30 |
| Vitality (%) | ND | ≥50 | ≥75 | ≥75 | 58 | 54 |
| Normal morphology (%) | 80,5 | ≥50 | ≥30 | (14) | 4 | 4 |

Severe concentration alterations: severe oligozoospermia (<3 million/ml)

Additional diagnostic tests:

Hormone levels such:

• FSH, LH, Testosterone, Prolactin, and

TSH etc.

Karyotype

- FISH studies on sperm
- Cystic brosis gene mutation studies Microdeletion

studies

Very severe alterations in concentration (cryptozoospermia and azoospermia):

Diagnostic tests:

Semen pH analysis, fructose test, Inhibin B

Hormone levels

Karyotype

FISH studies of sperm

Cystic brosis gene mutation studies

Microdeletion studies

Testicular biopsy

Therapeutic action:

Freeze semen in which motile spermatozoa are

found

Testicular biopsy/ICSI

Epididymis aspiration/ICSI

Severe motility alterations (asthenozoospermia):

Diagnostic tests

Perform microbiological culture

Rule out ciliary immotility syndrome (Kartagener)

Rule out the presence of urine in the sample (pH)

Vitality test (total immotility)

Therapeutic actions

Hypoosmotic test and ICSI

Pentoxifylline and ICSI

There are different occasions in which tests are required to complement it and provide more information about the etiology of the disease, as well as the necessary treatments, and this occurs mainly when the quality of the semen is extremely low.

In these cases, physical examination is necessary in case of finding very pathological spermiogram results.

