Semen Analysis and Sperm Preparation
Semen Analysis

- SA are mainly carried out to determine whether:
  - A man has a reproductive problem that is causing infertility.
  - A vasectomy or vasectomy reversal has been successful.
  - SA is the singular test for fertility in male that can provide information on:
    - sperm production.
    - patency of the male ducts.
    - the function of the accessory glands.
    - and ejaculative function.

Since semen samples may vary from day to day, 2 or 3 samples may be evaluated within a 3-6 month period for accurate testing.

A semen analysis to test the effectiveness of a vasectomy is usually done 6 weeks after the vasectomy.
Semen Analysis

- Semen analysis is a relatively inexpensive test to perform in Hong Kong. But because it is inexpensive and simple, many doctors and patients believe it is an easy test to perform.

- In reality, it is a test that requires skill and expertise. It is easy to perform the test badly as it is subjective.

- Besides containing sperms, normal semen also contains water, simple sugars such as fructose for nourishment of sperms and alkaline substances that buffer sperms against acidic environment in the vagina. Semen also contains prostaglandin which can cause contractions in the uterine muscles, vitamin C, zinc, cholesterol and a few additional compounds which may also be tested.
Semen can be divided into 4 fractions:

1. Pre-ejaculatory – secretion from urethral and bulbourethral gland (no sperms)
2. Dilute fluid from prostate gland (no sperms)
3. Major portion of sperms from vas deferens and distal epididymis (volume 5%)
4. Seminal vesicle secretion (few sperms)

- *Fraction 1, 2, 3 made up of the first portion of the ejaculate (30% of total volume). Therefore important to ensure the first portion is collected.*
- *IMPORTANT – Mix semen well before analysis*
Ways to Collect Semen

- Masturbation, directing the semen into a clean sample cup. Do not use a lubricant which can kill sperms.
- Coitus interruptus - withdrawing the penis from the partner just before ejaculating follow by ejaculating into a clean sample cup.
- Coitus - by using a condom. A special (silicon) condom that does not contain any substance that kills sperm (spermicide). After ejaculation, carefully remove the condom from the penis. Tie a knot in the open end of the condom and place it in a container that can be sealed in case the condom leaks or breaks. Ordinary condoms should not be used since they usually contain spermicides.
- Assisted ejaculation – electro-ejaculation used in paralegics.
Semen Collection

Good and reliable SA results starts from semen collection, preferably by masturbation

- Abstinence days 2-6
- Pass urine
- Wash hands with soap, dry
- Collect the entire sample into the wide mouth sterile container, 70% of sperms is in the first part of the ejaculate
- Keep the sample at body temperature, no sunlight
- Deliver the sample within one hour of ejaculation
WHO 2010

- Fifth edition (30 years from 1st edition, 1980)
- Reference range is derived from 4500 SA characterizing semen quality of fertile men whose partner had time to pregnancy of 12 months or less
- Raw data on from recent fathers in 14 countries on four continents. Chinese data on 429 SA and 4 Singaporean SA was included. No Indians and other Asian nationalities.
- The 5th percentile is given as the lower reference range
- No high value reference range is given as it is not considered detrimental to fertility


The WHO 2010 criteria is a guideline to improve semen analysis and sperm preparation, not obligatory
## WHO 2010

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lower Reference Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume (ml)</td>
<td>1.5</td>
</tr>
<tr>
<td>Sperm concentration (10^6/ml)</td>
<td>15</td>
</tr>
<tr>
<td>Total sperm number (10^6/ejaculate)</td>
<td>39</td>
</tr>
<tr>
<td>Progressive motility (PR, %)</td>
<td>32</td>
</tr>
<tr>
<td>Total motility (PR +NP, %)</td>
<td>40</td>
</tr>
<tr>
<td>Vitality (live sperms, %)</td>
<td>58</td>
</tr>
<tr>
<td>Sperm morphology (NF, %)</td>
<td>4</td>
</tr>
<tr>
<td>pH*</td>
<td>&gt;/=7.2</td>
</tr>
<tr>
<td>Leucocyte* (10^6/ml)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>MAR/Immunobead test* (%)</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

*Parameters agreed on consensus
Terminologies of SA

- Oligospermia – sperm concentration < 15 million/ml
- Asthenozoospermia – < 40% grade (PR+NP) or < 32 PR%
- Teratozoospermia – < 4% spermatozoa
- OAT = Oligo-astheno-teratozoospermia
- Azoospermia – no spermatozoa in semen
- Polyzoospermia – ++ high sperm concentration, > 200M/ml
- Hypospermia – semen volume < 1.5 ml
- Hyperspermia – semen volume > 6.0 ml
- Aspermia – no semen volume
- Pyospermia – leukocytes present in semen, > 1M/ml
- Hematospermia – red blood cell present in semen
- Necrozoospermia – “dead” sperm
## Macroscopic Examination

<table>
<thead>
<tr>
<th>WHO criteria 2010</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Normal: Whitish to grey opalescent</td>
</tr>
<tr>
<td>Yellow (urine, jaundice); Pink/Reddish/Brown (RBCs)</td>
<td></td>
</tr>
<tr>
<td>Liquefaction</td>
<td>Normal: 15–30 minutes after collection</td>
</tr>
<tr>
<td></td>
<td>Lumpy &gt;60 min – sperms may be trapped in unliquefied jelly; maybe sign of prostatic infection, lack of prostatic protease</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Normal Smooth and watery</td>
</tr>
<tr>
<td></td>
<td>Abnormal –, thick with long threads (21G needle). High viscosity impede sperm movements</td>
</tr>
</tbody>
</table>

MIX SEMEN THOROUGHLY BEFORE ANALYSIS – USE A PIPETTE
**Macroscopic Examination**

<table>
<thead>
<tr>
<th>Volume</th>
<th>Normal:</th>
<th>1.5 ml per ejaculation</th>
</tr>
</thead>
</table>

*Low volume (<1ml) reflect a problem with the seminal vesicles and prostate – a block, retrograde ejaculation, infection or lack of androgen.*

*Low semen volume cannot neutralize vaginal acidity*

*High semen volume dilute sperms/ active infection*

<table>
<thead>
<tr>
<th>pH</th>
<th>Normal:</th>
<th>=/&gt;7.2 (alkaline)</th>
</tr>
</thead>
</table>

*Acidic pH (<7.0) in a low volume & density sample indicates – congenital bilateral absence of vas deferens (in which seminal vesicles are also poorly developed) and ejaculatory duct obstruction. pH increases with time as natural buffering capacity of semen decreases – therefore high ph is not clinically useful*
Sperm Motility Assessment

- A very important parameter
- Glass slide and coverslip 22 x 22mm
- Add 10ul semen
- Area = 484mm², depth 20.7um
- Wait 1-2 minutes before reading, use 40x magnification
- Count 200 sperms in total from several fields
- Count only complete sperms, not pinheads

- PR=Progressive motility (forward movement, large circles)
- NP=Non progressive (on the spot movement, twitching)
- IM=Immotile (no movement)
Sperm Density Assessment

- Add 10 ul semen to 190 ul water = 20x dilution.
- Mix well
- Add 10 ul of mixture to the chamber
- Wait 2-3 min to settle
- Sum of 5 squares (central grid) = sperm density x 10^6/ml
- Count only whole sperms, not pinheads
- Read at x20 objective
- Use the “L” rule

Neubauer haemocytometer chamber is the WHO 2010 recommended standard
Sperm Density Calculation

- With chamber depth of 100um, each large grid holds 100nl.
- The central grid contains 5 x 5 squares = 25 squares = 4nl per square
- Count = Number of sperms / volume x dilution factor

Example: \( N \text{ (per 5 squares)} \times 20 \text{ (DF)} = C \times 10^6 \text{ sperms per ml} \)
\[
\frac{(1+19)}{20 \text{ nl}}
\]

Example: \( N \text{ (per 5 squares)} \times 5 \text{ (DF)} = C \times 10^6 \text{ sperms per ml} \)
\[
\frac{(1+4)}{20 \text{ nl}} \times \frac{1}{4}
\]

*1 ml = 1x10^6 nanolitre
(At 20x objective~16nl, at 40x objective ~4nl)
Makler Chamber

- A 10 ul of semen
- Allow 1 minute to settle and spread evenly.
- Use x20 objective.
- A row of 10 squares = sperm density x 10^6/ml.
- Read motility and sperm density at the same time.
- Usually 3 to 5 randomly selected rows are read.
- If < 5 sperms per row of 10 squares is seen, then the entire grid is counted.

A chamber specially designed for semen analysis
Azoospermia

- In cases of very low sperm count – perform sperm count using a lower dilution factor example 1+4
- In cases of azoospermia, concentrate entire semen volume into a small pellet, spread the pellet onto a glass slide and observe again.
- Use the “SS” pattern
- IMPORTANT – Repeat SA for confirmation
Vitality Assessment

1. Eosin-nigrosin (dead sperm stain pink/red)
2. Eosin (1%) (dead sperm stain pink/red)
3. Trypan (0.4%) blue (dead sperm stain blue)
4. Hypo-osmotic swelling test (HOS) (live sperm shows tail curling)

Test 1, 2 and 3 for diagnostic uses.

- Usually 1:1 ratio of semen to dye mixture, mix well and smear onto a slide. Read immediately at x40 objective, count 200 sperms

Test 4 is use to choose live (immotile) sperm for ICSI

- Dead sperms will not react in HOS while live sperm will take up fluids causing their tails to curl within 5 min and stabilize at 30 min. Therefore viable sperms may be selected for ICSI [Lin et al, 1998; Cayan et al, 2001].
Vitality Assessment

Dead sperms are stained pink/red

Live sperms show curling tails
Sperms must be stained for an accurate assessment
Strict criteria /Kruger /Tygerberg
The sperm head is oval, smooth-symmetrical outline, a length of 3-5 um and a width of 2-3um.
The head should have a well defined acrosome area of 40-70% and vacuoles (=/<2) that occupies ~ 20% head area.
The mid-piece must be straight and slender, 0.5 um in width and 7-8um long, straightly aligned to the head.
The tail must be straight and 45-50 um long.
To be classified as normal, the sperm must be normal in all portions (head, mid-piece, tail).
At least 400 sperms must be scored on randomly chosen fields.

Normal Forms (%) = normal sperms / the total number of sperms evaluated x 100.
### Hematoxylin-Eosin Staining

- **Hematoxylin-Eosin**
  - Fix slide in EtOH/MeOH 95% for 20 min
  - Wash in running tap water for 5 min
  - Dry on absorbent paper
  - Hematoxylin (Sigma, HHS-128) for 20 min
  - Wash in running tap water for 5 min
  - Acid alcohol (99 ml 70% EtOH + 1 ml H$_2$SO$_4$) Dip (2)
  - Eosin (Sigma, HT1102128) for 5 min
  - EtOH 70% for 2 min
  - EtOH 90% for 2 min

- **Fairly good differentiation**
  - The acrosomal area and cytoplasmic fragments is stained pink and the post-acrosomal area is stained dark purple.
  - Abnormally stained sperms (nuclear/chromatin material) may be differentiated.
  - Takes longer and need experience to produce good staining
Diff-Quik Staining

- **Diff-Quik Staining (Baxter, Australia)**
- Simple, quick staining method.
- Moderate differentiation of structures --the acrosome is stained red and the post acrosomal area dark red. Very thin smears are necessary as the background is influenced by the presence of protein in the seminal fluid and tends to be dark.
- The slides are fixed with fixative (Cytospray, Kinetik, Australia).
- Diff Quik 1 for 5-6 seconds.
- Clean water x 2. Removed excess moisture.
- Diff Quik 2 for 10 seconds
- 2-3 dips in clean water.
- It is left to dry by standing the slide on one end on absorbent paper towel.
- Sperms stained by Diff-Quik are larger by comparison to H&E staining as the cells did not undergo dehydration by alcohol.
Abnormal Sperms

PMN

Globozoospermic

Senescence
Abstinence > 20 days

Strict Normal Morphology Type A

Acrosome-defective with 'normal' appearance

Multi-Head
Abnormal Sperms

1. Triple head sperm
2. Acrosome reacted sperm
3. Sperm with no acrosome
4. Sperm with a tapering head and swollen mid-piece
Morphology Assessment

- Assessment is subjective due to the wide variation in sperm sizes and shapes.
- % NF correlated well with the fertilization rate in-vitro and pregnancy rate [Kruger et al, 1996; Morgenthaler et al, 1995]
- Sperms with defective heads are more likely to be immotile than sperms without defects, and defective motile sperms tend to show sluggish motility as compared to normal sperms [Aitken et al, 1995].
Round Cells Assessment

- Round cell concentration can be assessed from slides. If 400 sperms are counted and the number of round cells are N and sperm concentration is S. Then the formula for concentration (C) of round cells (x10^6 per ml) :-
  \[ C = \frac{N}{400} \times S \]

- Only Neutrophils are stained by peroxidase, other WBCs such as lymphocytes and macrophages, epithelial cells as well as spermatogenic cells remain unstained.

- The presence of neutrophils (multi-lobated nuclei) denotes infection/inflammatory reaction

Mixed Anti-globulin Reaction Test

- Place 10 ul of semen, SpermMar latex particles and antiserum on a glass slide.
- Mix well semen and latex particles (5 seconds), followed by the antiserum (5 seconds).
- Place a coverslip on top of the mixture and incubate the slide at RT for 2-3 minutes in a damp chamber (e.g. a Petri dish with a moist cotton or filter paper)
- Count at least 200 sperms.
- Assume clinical significance (infertility due to immune reaction) if >50% of sperms have beads attached.
Computer Assisted SA (CASA)

- **Advantages**
  - More objective and reproducible measurement
  - Superior documentation of laboratory values
  - Superior in measurement of sperm motility

- **Disadvantages**
  - Not reliable if sperm density is \(<2\times10^6/\text{ml} \text{ or } >50\times10^6/\text{ml}\), lots of debris/immotile sperm. Require dilution if \(>40 \times 10^6/\text{ml}\) in isotonic buffer to avoid sperm collision
  - Need to record 200 sperms for accurate distribution of velocity.
  - Parameters not standardized between laboratories – difficult to interpret results
  - No improvement on the manual method in distinguishing fertilizing capacity of semen
Computer Assisted SA (CASA)

- Uses video and computer software technology to capture the types and speed of sperm motility.
- Automatic image digitization and processing
- Additional parameters can be measured such as curvilinear velocity (VCL), straightline velocity (VSL), linearity and flagellar beat frequency and amplitude of lateral head (ALH)
  - VCL = mean distance between 1st sperm-2nd sperm position/time
  - VSL = distance between 1st-last sperm position/time
  - VAP = average path velocity
  - ALH = mean deviation from average path
  - LIN = linearity (VSL/VCL)
  - STR = straightness (VSL/VAP)
CASA and SQA-V

- Twelve fresh semen samples from fertile volunteers and 73 from infertility patients were compared for sperm concentration and motility, sperm motility index (SMI), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), linearity (LIN = VSL/VCL) and straightness (STR = VSL/VAP).

- Significant differences were observed in the above parameters between the fertile and infertile groups. An obvious consistency was noted between the results from SQA-V and those from CASA in sperm concentration (r = 0.58, P < 0.01), motile sperm concentration (r = 0.75, P < 0.01) and average sperm velocity (r = 0.59, P < 0.01). Significant correlations were found between the SMI from SQA-V and STR, LIN, BCF, VCL, VSL and VAP from CASA (P < 0.05).

- There is a consistency between the results from SQA-V and those from CASA. Both the devices can detect the seminal differences between different cohorts of patients.

Zhong Nan Ke Xue 2008 by Ge YF et al
This study was conducted in Calcutta, India, - a selected group of subjects, designated as normozoospermic in routine analysis, but under risk factor exposure, were selected for a repeat computer aided semen analysis (CASA) and were compared with a control group. The parameters considered among CASA results were: curvilinear velocity (VCL), straight-line velocity, average path velocity (VAP), straightness index (STR), lateral head displacement (ALH) and beat cross frequency. The results depict a significant decline in the mean values of VCL (P = 0.029) and STR (P = 0.007) in the tobacco-exposed group when compared with the unexposed group. On the other hand, there was a significant decline in the mean values of VCL (P = 0.014) and ALH (P = 0.040) in the heavy metal-exposed group when compared with the unexposed group. The other parameters did not show significant change in either group. Semen samples that had been designated normozoospermic in conventional analysis were seen to be influenced by risk factors at the level of sperm motion kinetics.

Andrologia 2010 by Mukhopadhyay D et al
Retrograde Ejaculation

- Retrograde ejaculation occurs whereby the semen is ejaculated into the bladder. The acidity of the urine will kill sperms quickly. Alkalination of the urine is very important in order to recover live motile sperms.
- The patient is instructed to alkalinize his urine by intake of sodium bicarbonate, 3g (two table spoons) dissolved in a glass of water in the evening before bed. In the morning, the patient must empty his bladder completely and drink another glass of sodium bicarbonate before coming directly to the laboratory.
- Ask the patient to empty his bladder before semen collection. Provide two containers for collection, a small one for semen and a larger one for urine. Instruct the patient to collect the semen by masturbation and to urinate immediately after masturbation.
- The urine is divided into tubes and centrifuged for 10 min at 1500 rpm.
- The supernatant is removed leaving behind the pellet. The contents of all tubes are pooled.
- Analyzed as SA
- Add 2-3 ml of culture medium before proceeding with a gradient column.
Transient Poor SA

Poor SA can result from factors such as:-

- Incorrect semen collection technique – spillage, dirty container, long delay in delivering sample
- Poor technical expertise
- History of recent illness – flu or high fever may depress sperm counts
- Long period of abstinence – increased abnormal sperm morphology and decrease motility
- Short abstinence period – lower semen volume and sperm count

As it take 10 weeks (64-70 days) for a new batch of sperm to be generated by the testes, it is best to repeat SA after a period
Factors affecting SA results

- Medicines, such as cimetidine (Tagamet), male and female hormones (testosterone, estrogen), sulfasalazine, nitrofurantoin, and some chemotherapy medicines.
- Caffeine, alcohol, cocaine, marijuana, and smoking tobacco.
- Herbal medicines, such as St. John's wort and high doses of echinacea.
- Temperature - sperm motility value will be inaccurately low if the semen sample gets cold.
- Exposure to radiation, some chemicals (such as certain pesticides or spermicides), and prolonged heat exposure.
- An incomplete semen sample -- more common if a sample is collected by methods other than masturbation.
- Not ejaculating for several days -- affect the semen volume
Conditions with Abnormal SA

- Certain conditions may be linked with a low or absent sperm count. These conditions include:
  - Orchitis
  - Varicocele
  - Klinefelter syndrome
  - Radiation treatment to the testicles
  - Diseases that can cause shrinking (atrophy) of the testicles (such as mumps).
  - Long term illness such as diabetes which may cause retrograde ejaculation
  - Hormonal imbalance (testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), or prolactin
  - Azoospermia - A small sample (biopsy) of the testicles may be needed for further evaluation.
Improving SA

- Quality Assurance Program
  - Standard Operating Procedures
  - Laboratory Manual
  - Documentation
  - Sample ID and Tracking

- External QC
  - Comparison of tests with an external source

- Internal QC
  - Minimized variation by training
  - Purchased QC samples with known values
  - Video recordings for motility
Sperm preparation

- The semen is a mixture of motile and dead spermatozoa with cells, cellular debris and sometimes micro-organisms present.
- A variety of methods have been developed to separate the motile sperms from the ejaculate. The most common methods are washing and centrifugation which has been shown to cause some damage to the sperm.
- Simple sperm wash
- Swim up
- Gradient
- All preparations should done in a laminar flow for sterility [Dale & Elder, 1997].
- The clean sperm suspension is used for IUI, IVF and ICSI and certain special sperm tests.
Simple wash

- This method is used if the semen sample is very poor. It mainly removes seminal plasma from the sperms.
- One volume of semen is placed in a 15 ml test tube and diluted with 2 volume of culture medium. The tube is gently inverted twice to mix the components.
- The tube is then centrifuged at 250-300g for 5-7 min.
- The supernatant is removed and the pellet is re-suspended in 2 ml of culture medium.
- The centrifugation is repeated at 250-300g for 5-7 min and the supernatant removed. About 0.4 ml of media is added to the final sperm pellet for re-suspension.
- The sample is suited for intra-cervical insemination
Swim up method

- This technique relies on the ability of the sperms to swim. This method is suitable for semen with high to moderate motility.
- Semen is diluted with 1:2 ratio of culture medium and centrifuged at 250-300g for 5-7 min.
- The supernatant is removed leaving the pellet.
- Pipette 0.8-1 ml of media into a new test tube. Carefully layer the semen pellet beneath the media.
- Stand at 37° for 45-60 min. Placement of tube at 45° angle creates a larger surface area for sperms to swim-up.
- Carefully take up the top 0.5-0.6 ml without disturbing the lower layer and transfer into a new test tube.
- To concentrate the sperms, pooled several tubes and centrifuged at 250-300g for 5-7 min. The supernatant is removed and the resultant pellet re-suspended in 0.4 ml of media.
Gradient systems

- Gradient systems use solutions with a higher density than semen to separate the debris, cells, micro-organisms and non-motile sperms from the motile ones.
- Among the dense solutions used are colloidal silica (Percoll, Puresperm), poly-sucrose (Ficoll, Ixaprep) and other dense solutions (Optiprep, Nycodenz).
- All could be obtained commercially
- Centrifugal force is applied to enable the motile sperms to swim from a less dense seminal fluid into a denser solution.
- Cellular debris and non-motile microorganisms will be trapped at the interphase between the two solutions
- Select more normal sperms than swim up method.
- Recovery may be poor in viscous semen and severe teratozoospermia (small heads but good swimmers)
If a large volume of semen is placed on top of the column, seminal fluid will mix with the gradients due to its weight.

A prior concentration step to reduce seminal volume can be carried out (centrifugation at 350g for 5-7 min) if the initial volume was high. This will reduce the number of gradient column necessary for semen processing.

However, centrifugation should be kept to a minimum as it has been shown to induce sub-lethal damage in sperms.

Density gradients must be prepared just before usage, gradients will mixed after standing for an hour or so.

The volume of semen being processed should not exceed the volume of the gradient in order to get a good recovery.
Two Gradient Column

- Usually comprise 2ml of each solution i.e. 40% and 80%
- Gently pipette 80% solution followed by 40% solution in a 15 ml test tube. Two visible layers should be seen.
- Layer 2-4 ml of semen on top of the column.
- Centrifuge for 10 min at 600g to assist the sperms in reaching the bottom layer.
- All supernatant is removed leaving the sperm pellet.
- The pellet is transferred to a new tube and resuspended in 3.0 ml of media.
- The tube is centrifuged again for 5-7 min at 250g in order to remove the silica particles found in gradient solutions.
- Remove all supernatant leaving the pellet and resuspend it with 0.4 ml of media.
- This method can be used to harvest motile sperms from poor quality samples. Usually a two gradient column is adequate for the processing of most semen samples.
Mini gradient procedure

- A three gradient column is used to remove the cryopreservatives from thawed semen samples
- 1 ml each of 40%, 70% and 90% is used.
- Overlay 2-3 ml of semen onto each gradient column
- Centrifuge the tubes at 600g for 10 minutes to assist the sperms in reaching bottom.
- All supernatant is removed leaving the sperm pellet. The contents of several tubes may be pooled.
- The pellet is transferred to a new tube and resuspended in 3.0 ml of media. The tube is centrifuged again for 5-7 min at 250g in order to remove the silica particles
- Remove all supernatant leaving the pellet and resuspend it with 0.4 ml of media.

FOR IVF/ICSI, a swim up is performed after gradient processing

<table>
<thead>
<tr>
<th>Density (%)</th>
<th>Stock solution (ml)</th>
<th>Media (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>70</td>
<td>3.5</td>
<td>1.5</td>
</tr>
<tr>
<td>90</td>
<td>4.5</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>5.0</td>
</tr>
</tbody>
</table>
WHO 2010 laboratory manual for the Examination and processing of human semen

The End
Thank You