- What is semen?
- Semen is the fluid formed at ejaculation.
- Made of secretions of all the accessory glands of the male genital tract and testicular sperm component
- Semen quality is maintained by all the accessory glands



### Formation

- Semen is formed by the four fractions of the testes
- Epididymis, seinal vesicles, prostate gland and bulbourethral glands
- Each fraction differs and mixing during ejaculation is essential for normal semen

#### SUMMARY 10-1 Semen Production

#### Structure

Function

Seminiferous tubules of testes Epididymis

Ductus deferens Seminal vesicles

Prostate gland

Bulbourethral glands

### Spermatogenesis

Sperm maturation

Propel sperm to ejaculatory ducts Provide nutrients for sperm and fluid Provide enzymes and proteins for coagulation and liquefaction

Add alkaline mucus to neutralize prostatic acid and vaginal acidity

| Table 10–1     | Semen Composition |
|----------------|-------------------|
| Spermatozoa    | 5%                |
| Seminal fluid  | 60% to 70%        |
| Prostate fluid | 20% to 30%        |
| Bulbourethral  | glands 5%         |

- Purpose of the test
- Semen analysis mainly measures the amount of semen a man produces and determines the number and quality of sperm
- One of the routine tests done to determine male infertiliy
- To determine the effectiveness of vasoctomy
- To assess suitability of semen for artificial insemination
- Forensic and medico legal cases requires semen analysis in sex crimes

- Sample collection
- Samples are collected after a period of abstinence of 2 days
  - If not false results may appear
- Complete sample collection after ejaculation is essential
  - If not false results may appear
- Sample should be collected in to pre warmed sterile, non-toxic, wide mouth container
- Should be tested within 1-2 hours
- Many methods are in practice

#### Seminal fluid analysis

#### Following parameters are routinely checked

Volume, Viscocity, pH, Sperm count, Motility and morphology

#### Volume

Normal volume is 2-5 ml. A low volume is generally a result of problem with any of the four glands secretions and is associated with infertiligy

#### **Color and viscocity**

Normal semen has a gray-white /yellow color and has musty odor. Viscosity refers to the consistency of the fluid and is direct relation with liquefection.

Normal semen should be drawn in to pipet and forms discrete droplets that are not clumped.

Viscosity can be reported as low (watery) to high (gel like)

### рΗ

Normal pH range is 7.2-8.0 (increases with time) Abnormal pH indicates abnormal secretion of any of the Four glands



#### **Microsocpic examination**

### **Concentration (count)**

This is the measurement of how many million sperms are present in 1 ml of the sample

Various techniques are in practice

Average sperm concentration is more than 60 million / ml (60-150 mil/ml) Counts lessthan 20 million are considered sub-fertile

#### Azoospermia

Total absence of sperms in the sample



Oligozoospermia Reduced number of sperms (<20 mil/ml) of 5-6 sperms / HPF Severe Oligospermia 1-2 sperms / HPF

#### Polyzoospermia

Increased number of spermatozoa i. e in excess of 350 mil/ml



#### **Microsocpic examination**

#### Motility – or Mobility

Describes the percentage of sperm which are movint. Generally 50% of the sperm should be moving For normal fertilization sperm not only moving but must be capable to move in forward progression (progressive activity)

| Rapid progressive         | Slow or sluggish           | Immobility         |
|---------------------------|----------------------------|--------------------|
| The sperm are moving      | Straight line mobility but | No movement at all |
| swiftly across field in a | slow                       |                    |
| straight line             | Non-progressive            |                    |
|                           | Sperm not moving in        |                    |
|                           | straight line (twitching   |                    |
|                           | or shaking)                |                    |

Live and dead sperms can be differentiated by eosin stain (dead - stained)

| Table 10–4                 | Alternativ<br>Criteria <sup>1</sup> | e Sperm Motility Grading                       |
|----------------------------|-------------------------------------|--|
| Progressive<br>motility (I | PM)                                 | Sperm moving linearly or in a large circle     |
| Nonprogress<br>motility (1 | sive<br>NP)                         | Sperm moving with an absence<br>of progression |
| Immotility (               | IM)                                 | No movement                                    |



#### **Microsocpic examination**

#### Other cells in semen

Leukocytes normally 1-4 / HPF

High number (leukocytospermia) indicates infection

Epithelial cells normally 1-2 /HPF

Spermatocytes (immature germ cells) 1-2 / HPF

Erythrocytes 1-2 / HPF. Increased number indicates trauma or infection

Bacteria or protozoan such as trichomonas vaginalis are rare but presence indicates infection

#### **Agglutination or clumping**

Presence of agglutination indicates immunological infertility

Presence of anti-sperm antibody



#### **Microsocpic examination**

#### Morphology – describes the shape of the sperm

At least 30% of the sperm should be normal for fertility Must meet specific sets of criteria to be classified as normal

Normal spermatozoa should have oval shaped head (4-5.5 µm X 2.5-3 µm)

The middle piece should be cylindrical (45-50  $\mu$ m long and 0.5  $\mu$ m wide) The tail should also be cylindrical (45-50  $\mu$ m long and 0.5  $\mu$ m wide)

#### Head shape/size

Large, small, tapering, pinhead form, amorphours, vacuolated, multiple heads

#### Neck & middle piece

Irregular, bent middle piece, thin middle piece (no mitochondria),

#### **Tail defects**

Short, multiple, hairpin, broken, irregular width, coiled tails **Cytoplasmic droplets** 

#### **Microsocpic examination**

Morphology – describes the shape of the sperm



#### **Biochemical examination**

#### Fructose

Main energy source for sperm is fructose

If spermatozoa are separated by centrifugation and not provided with fructose cells will die

Tested spectrophotometrically

Other chemicals like zinc, citric acid and acid phosphatase enzyme can also be tested

#### Seminal Fructose Screening Test<sup>6</sup>

- Prepare reagent (50 mg resorcinol in 33 mL concentrated HCl diluted to 100 mL with water).
- 2. Mix 1 mL of semen with 9 mL of reagent.
- 3. Boil.

#### Table 10–6 Reference Semen Chemical Values1 4. Observe for orange-red color.

| Neutral $\alpha$ -glucosidase | ≥20 mU/ejaculate     |
|-------------------------------|----------------------|
| Zinc                          | ≥2.4 µmol/ejaculate  |
| Citric acid                   | ≥52 µmol/ejaculate   |
| Acid phosphatase              | ≥200 Units/ejaculate |

### Next class....

• 2<sup>nd</sup> mid exam

The sample brought from home.

- The semen analysis appear that all parameter with in normal range, it was Normozoospermia.
- Low semen volume is characteristic of obstruction of the ejaculatory duct or congenital bilateral absence of the vas deferens (CBAVD), a condition in which the seminal vesicles are also poorly developed.
- Low semen volume can also be the result of collection problems (loss of a fraction of the ejaculate), partial retrograde ejaculation or androgen deficiency.
- High semen volume may reflect active exudation in cases of active inflammation of the accessory organs.
- The pH is less than 7.0 in a semen sample with low volume and low sperm numbers; there may be ejaculatory duct obstruction (Acidic ejaculate (lower pH value) may indicate one or both of the seminal vesicles are blocked.) or congenital bilateral absence of the vas deferens, a condition in which seminal vesicles are also poorly developed.
- The PH exceeds 8.0 it was indicated of infection or should be suspected with decreased secretion of acidic products by prostate. High pH values may be providing little clinically useful information.
- The High viscosity can interfere with determination of sperm motility. The viscosity present and may be related to prostate dysfunction (chronic inflammation) and also may indicated infection in prostate or epdidymes or Antisperm Ab.
- The semen analysis appears that the sperm counts fewer than 15,000,000/ ml, it was Oligozoospermia..
- The semen analysis appear that the sperm motility fewer than 40 % spermatozoa with forward progression (Stage PR and NP), it was Asthenozoospermia. In Asthenozoosperia there were frequent causes like abnormal spermatogenesis', epididymal sperm maturation problem, transport abnormalities, varicocele. These conditions should all be looked for if sperm motility is repeatedly low..
- The semen analysis appear that the sperm counts fewer than 39,000,000/ Ejaculate and the sperm motility fewer than 40 % spermatozoa with forward progression (Stage PR and NP), it was Oligoasthenozoospermia. In Asthenozoosperia there were frequent causes like abnormal spermatogensis, epididymal sperm maturation problem, transport abnormalities, varicocele. These conditions should all be looked for if sperm motility is repeatedly low.
- The semen analysis appear that no spermatozoa in the ejaculate, it was Azoospermia.
- In azoospermia, the presence of spermatogenesis cells indicates a testicular malfunction

- The presence of leucocytes in the absence of any spermatogenesis cells suggests that the azoospemia might be due to an obstruction problem.
- There is no spermatozoa are observed in either wet preparation, but after centrifuged the sample to determine the spermatozoa in a larger sample it was present sperms, it was indicates cryptozoospermia.
- The semen analyses appear that the leukocytes (germ fighter cells) present in semen, it was Pyospermia.
- Excessive numbers of leukocytes in the ejaculate (leukocytospermia or pyospermia) may be associated with infection and poor sperm quality.
- The viscosity present and may be related to prostate dysfunction (chronic inflammation) and also may indicated infection in prostate or epdidymes or Antisperm Ab.
- Agglutination of sperm present in examination in percentage more than 10%, so the Sperm culture must be performed in order to exclude infection with e.g. *E.coli*. After exclude infection is suggestive of the presence of anti-sperm antibodies; so further testing is required.
- Sperm morphology: gives information for the function of the reproductive tract and is a predictor of man fertility potential.