# *Semen analysis*

Semen (or seminal fluid) is a fluid that is emitted from the male genital tract and contains sperms that are capable of fertilizing female ova. Structures involved in production of semen are (Box 15.1):

- Testes: Male gametes or spermatozoa (sperms) are produced by testes; constitute 2-5% of semen volume.
- Epididymis: After emerging from the testes, sperms are stored in the epididymis where they mature; potassium, sodium, and glycerylphosphorylcholine (an energy source for sperms) are secreted by epididymis.
- Vas deferens: Sperms travel through the vas deferens to the ampulla which is another storage area. Ampulla secretes ergothioneine (a yellowish fluid that reduces chemicals) and fructose (source of nutrition for sperms).
- Seminal vesicles: During ejaculation, nutritive and lubricating fluids secreted by seminal vesicles and prostate are added. Fluid secreted by seminal vesicles consists of fructose (energy source for sperms), amino acids, citric acid, phosphorous, potassium, and prostaglandins. Seminal vesicles contribute 50% to semen volume.
- Prostate: Prostatic secretions comprise about 40% of semen volume and consist of citric acid, acid phosphatase, calcium, sodium, zinc, potassium, proteolytic enzymes, and fibrolysin.
- Bulbourethral glands of Cowper secrete mucus.

## EXAMINATION OF SEMINAL FLUID

The tests that can be done on seminal fluid are shown in Box 15.2. Tests commonly done in infertility are shown in Box 15.3.

The usual analysis consists of measurement of semen volume, sperm count, sperm motility, and sperm morphology.

Terminology in semen analysis is shown in Box 15.4.

**Table 15.1:** Normal values of semen analysis (World Health Organization, 1999)

| *Test* | *Result* |
|---|---|
| 1. Volume | ≥2 ml |
| 2. pH | 7.2 to 8.0 |
| 3. Sperm concentration | ≥20 million/ml |
| 4. Total sperm count per ejaculate | ≥40 million |
| 5. Morphology | ≥30% sperms with normal morphology |
| 6. Vitality | ≥75% live |
| 7. White blood cells | ≤1 million/ml |
| 8. Motility within 1 hour of ejaculation | |
| • Class A | ≥25% rapidly progressive |
| • Class A and B | ≥50% progressive |
| 9. Mixed antiglobuiln reaction (MAR) test | <50% motile sperms with adherent particles |
| 10. Immunobead test | <50% motile sperms with adherent particles |

**Table 15.2:** Biochemical variables of semen analysis (World HelathOrganization,1992)

| | |
|---|---|
| 1. Total fructose (seminal vesicle marker) | ≥13 μmol/ejaculate |
| 2. Total zinc (Prostate marker) | ≥2.4 μmol/ejaculate |
| 3. Total acid phosphatase (Prostate marker) | ≥200 U/ejaculate |
| 4. Total citric acid (Prostate marker) | ≥52 μmol/ejaculate |
| 5. α-glucosidase (Epididymis marker) | ≥20 mU/ejaculate |
| 6. Carnitine (Epididymis marker) | 0.8-2.9 μmol/ejaculate |

**Box 15.2:** Tests done on seminal fluid

- **Physical examination:** Time to liquefaction, viscosity, volume, pH, color
- **Microscopic examination:** Sperm count, vitality, motility, morphology, and proportion of white cells
- **Immunologic analysis:** Antisperm antibodies (SpermMAR test, Immunobead test)
- **Bacteriologic analysis:** Detection of infection
- **Biochemical analysis:** Fructose, zinc, acid phosphatase, carnitine.
- **Sperm function tests:** Postcoital test, cervical mucus penetration test, Hamster egg penetration assay, hypo-osmotic swelling of flagella, and computer-assisted semen analysis

**Box 15.3:** Semen analysis for initial investigation of infertility

- Volume
- pH
- Microscopic examination for (i) percentage of motile spermatozoa, (ii) sperm count, and (iii) sperm morphology

**Box 15.4:** Terminology in semen analysis

- Normozoospermia: All semen parameters normal
- Oligozoospermia: Sperm concentration <20 million/ml (mild to moderate: 5-20 million/ml; severe: <5 million/ml)
- Azoospermia: Absence of sperms in seminal fluid
- Aspermia: Absence of ejaculate
- Asthenozoospermia: Reduced sperm motility; <50% of sperms showing class (a) and class (b) type of motility OR <25% sperms showing class (a) type of motility.
- Teratozoospermia: Spermatozoa with reduced proportion of normal morphology (or increased proportion of abnormal forms)
- Leukocytospermia: >1 million white blood cells/ml of semen
- Oligoasthenoteratozoospermia: All sperm variables are abnormal
- Necrozoospermia: All sperms are non-motile or non-viable

## Physical Examination

Examination is carried out after liquefaction of semen that occurs usually within 20-30 minutes of ejaculation.

1. Visual appearance: Normal semen is viscous and opaque gray-white in appearance. After prolonged abstinence, it appears slightly yellow.
2. Viscosity: Immediately following ejaculation, normal semen is thick and viscous. It becomes liquefied within 30 minutes by the action of proteolytic enzymes secreted by prostate. If liquefaction does not occur within 60 minutes, it is abnormal. The viscosity of the sample is assessed by filling a pipette with semen and allowing it to flow back into the container. Normal semen will fall drop by drop. If droplets form 'threads' more than 2 cm long, then viscosity is increased. Increased semen viscosity affects sperm motility and leads to poor invasion of cervical mucus; it results from infection of seminal vesicles or prostate.
3. Volume: Volume of ejaculated semen sample should normally be > 2 ml. It is measured after the sample has liquefied. Volume < 2.0 ml is abnormal, and is associated with low sperm count.
4. pH: A drop of liquefied semen is spread on pH paper (of pH range 6.4-8.0) and pH is recorded after 30 seconds. Normal pH is 7.2 to 8.0 after 1 hour of ejaculation. The portion of semen contributed by seminal vesicles is basic, while portion from prostate is acidic. Low pH (< 7.0) with absence of sperms (azoospermia) suggests obstruction of ejaculatory ducts or absence of vas deferens. **Low pH is usually associated with low semen volume (as most of the volume is supplied by seminal vesicles).**

## Microscopic Examination

The most important test in semen analysis for infertility is microscopic examination of the semen.

### *Sperm Motility*

The first laboratory assessment of sperm function in a wet preparation is sperm motility (ability of the sperms to move). Sperm motility is essential for penetration of cervical mucus, traveling through the fallopian tube, and penetrating the ovum. Only those sperms having rapidly progressive motility are capable of penetrating ovum and fertilizing it.

*Principle*: All motile and non-motile sperms are counted in randomly chosen fields in a wet preparation under 40× objective. Result is expressed as a percentage of motile spermatozoa observed.

*Method*: A drop of semen is placed on a glass slide, covered with a coverslip that is then ringed with petroleum jelly to prevent dehydration, and examined under 40× objective. **At least 200 spermatozoa are counted in several different microscopic fields.** Result is expressed as a percentage of (a) rapidly progressive spermatozoa (moving fast forward in a straight line), (b) slowly progressive spermatozoa (slow linear or non-linear, i.e. crooked or curved movement), (c) non-progressive spermatozoa (movement of tails, but with no forward progress), and (d) immotile spermatozoa (no movement at all) (WHO critera). Sperms of grades (c) and (d) are considered to be poorly motile (asthenospermia). Normally, ≥25% of sperms show rapid progressive motility, or ≥50% of sperms show rapid progressive and slow progressive motility.

If the proportion of motile spermatozoa is < 50%, then proportion of viable sperms should be determined by examining an eosin preparation.

### *Sperm Viability or Vitality*

*Principle*: A cell with intact cell membrane (a vital or viable cell) will not take up the **eosin Y** and will not be stained, while a non-viable or dead cell will have damaged cell membrane, will take up the dye, and will be stained pink-red (Fig. 15.1). Another stain (e.g. nigrosin) may be used to stain the background material. The test is performed if motility is abnormal.

### *Method*

1. Mix one drop of semen with 1 drop of eosin-nigrosin solution and incubate for 30 seconds.
2. A smear is made from a drop placed on a glass slide.
3. The smear is air-dried and examined under oil- immersion objective. White sperms are classified as live or viable, and red sperms are classified as dead or non-viable. At least 200 spermatozoa are examined.
4. The result is expressed as a proportion of viable sperms against non-viable as an integer percentage. Seventy-five percent or more of sperms are normally live or viable.

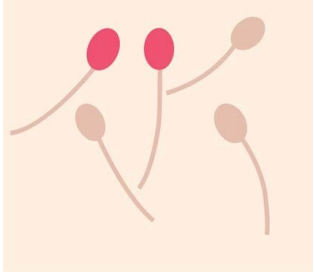


**Fig. 15.1: Eosin-nigrosin stain. Dead sperms are stained pink-red, while live sperms are stained white**

***Sperm Count***

*Principle:* The sperm count is done after liquefaction in a counting chamber following dilution and the total number of spermatozoa is reported in millions/ml ($10^6$/ ml).

***Method***

Semen is diluted 1:20 with sodium bicarbonate- formalin diluting fluid (Take 1 ml liquefied semen in a graduated tube and fill with diluting fluid to 20 ml mark. Mix well). A coverslip is placed over the improved Neubauer counting chamber and the counting chamber is filled with the well-mixed diluted semen sample using Pasteur pipette. The chamber is then placed in a humid box for 10-15 minutes for spermatozoa to settle.

Sperm count per ml is calculated as follows:

$$\text{Sperm count} = \frac{\text{Sperms counted} \times \text{correction factor for dilution}}{\text{Number of squares counted} \times \text{Volume of 1 square}} \times 1000$$

$$= \frac{\text{Sperms counted} \times 20}{4 \times 0.1} \quad 1000$$

$$= \text{Sperms counted} \times 50,000$$

Normal sperm count is ≥ 20 million/ml (i.e. ≥ $20 \times 10^6$/ml). Sperm count < 20 million/ml may be associated with infertility in males.

***Sperm Morphology***

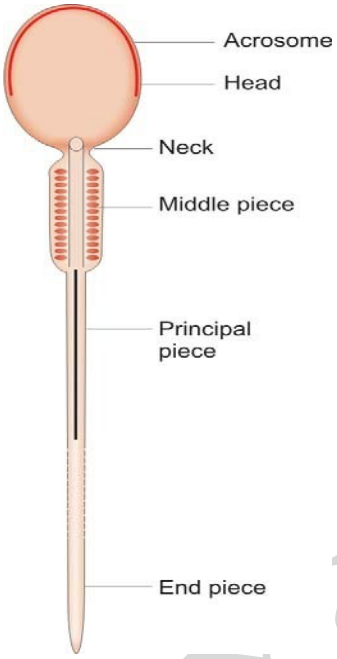
A smear is prepared by spreading a drop of seminal fluid on a glass slide, stained, and percentages of normal and abnormal forms of spermatozoa are counted. The staining techniques used are **Papanicolaou, eosin- nigrosin, hematoxylin-eosin, and Rose Bengal-toluidine blue stain**. At least 200 spermatozoa should be counted under oil immersion. Percentages of normal and abnormal spermatozoa should be recorded.

Normal morphology: A spermatozoon consists of three main components: head, neck, and tail. Tail is further subdivided into midpiece, main (principle) piece, and end piece (Fig. 15.2 and Box 15.5).

Head is pear-shaped. Most of the head is occupied by the nucleus which has condensed chromatin and few areas of dispersed chromatin (called nuclear vacuoles). The anterior 2/3rds of the nucleus is surrounded by acrosomal cap. **Acrosomal cap is a flattened membrane- bound vesicle containing glycoproteins and enzymes**. These enzymes are required for separation of cells of corona radiata and dissolution of zona pellucida of ovum during fertilization.

Neck is a very short segment that connects the head and the tail. Centriole in the neck gives rise to axoneme of the flagellum. Axoneme consists of 20 microtubules (arranged as a central pair surrounded by 9 peripheral doublets) and is surrounded by condensed fibrous rings.

Middle piece is the first part of the tail and consists of central axoneme surrounded by coarse longitudinal fibers. These are surrounded by elongated mitochondria that provide energy for movement of tail.
Principle or main piece constitutes most of the tail and is composed of axoneme that is surrounded by 9 coarse fibers. This central core is surrounded by many circularly arranged fibrous ribs.
Endpiece is the short tapering part composed of only axoneme.
Normally, ≧ 30% of spermatozoa should show normal morphology (WHO, 1999). The defects in morphology that are associated with infertility in males include defective mid-piece (causes reduced motility), an incomplete or absent acrosome (causes inability to penetrate the ovum), and giant head (defective DNA condensation).




**Box 15.5:** Normal sperm morphology

- Total length of sperm: About 60 μ
- Head:
  - Length: 3-5 μ
  - Width: 2-3 μ
  - Thickness: 1.5 μ
- Neck: Length: 0.3 μ
- Middle piece:
  - Length: 3-5 μ
  - Width: 1.0 μ
- Principal piece:
  - Length: 40-50 μ
  - Width: 0.5 μ
- End piece: 4-6 μ

**Fig. 15.2: Morphology of spermatozoa**

***Abnormal morphology*** **(Fig. 15.3): WHO morphological classification of human spermatozoa (1999) is given below:**

1. Normal sperm
2. Defects in head:
   - Large heads
   - Small heads
   - Tapered heads
   - Pyriform heads
   - Round heads
   - Amorphous heads
   - Vacuolated heads (> 20% of the head area occu- pied by vacuoles)
   - Small acrosomes (occupying < 40% of head area)
   - Double heads
3. Defects in neck:

- Bent neck and tail forming an angle >90° to the long axis of head

4. Defects in middle piece:
   - Asymmetric insertion of midpiece into head
   - Thick or irregular midpiece
   - Abnormally thin midpiece
5. Defects in tail:
   - Bent tails
   - Short tails
   - Coiled tails
   - Irregular tails
   - Multiple tails
   - Tails with irregular width
6. Pin heads: Not to be counted
7. Cytoplasmic droplets
   - > 1/3rd the size of the sperm head
8. Precursor cells: Considered abnormal

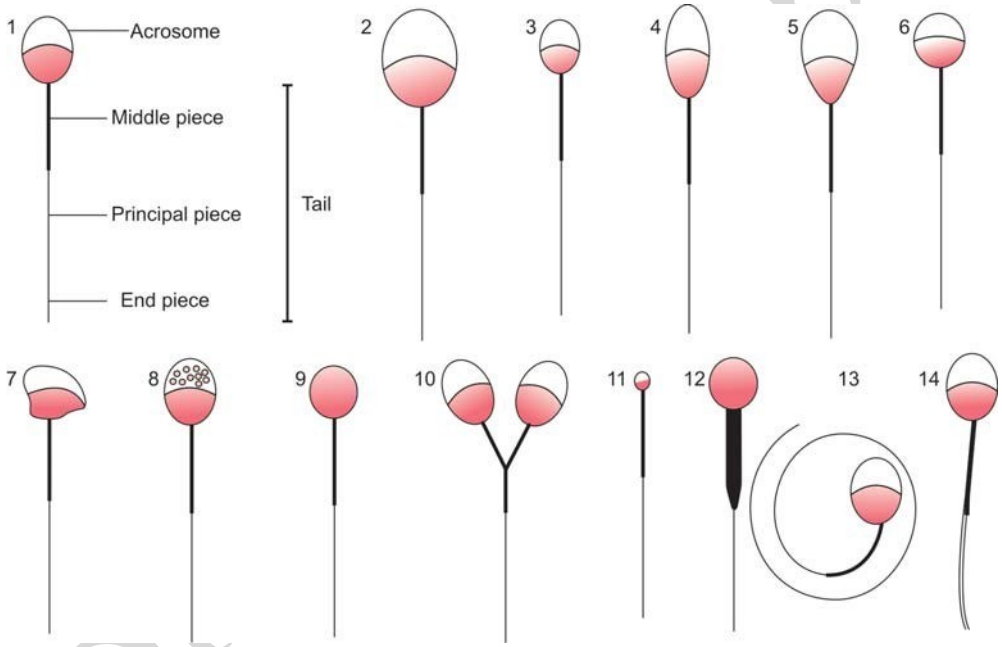



**Fig. 15.3:** Abnormal morphological sperm forms: (1) Normal sperm, (2) Large head, (3) Small head, (4) Tapered head, (5) Pyriform head, (6) Round head, (7) Amorphous head, (8) Vacuoles in head, (9) Round head without acrosome, (10) Double head, (11) Pin head, (12) Round head without acrosome and thick midpiece, (13) Coiled tail, and (14) Double tail

***Round Cells***

Round cells on microscopic examination may be white blood cells or immature sperm cells. Special stain (peroxidase or Papanicolaou) is required to differentiate between them. White blood cells >1 million/ml indicate presence of infection.

Presence of large number of immature sperm cells indicates spermatogenesis dysfunction at the testicular level.

**Immunologic Analysis**

*Antisperm Antibodies*

The role of antisperm antibodies in causation of male infertility is controversial. The immunological tests done on seminal fluid include mixed antiglobulin reaction (MAR test) and immunobead test.

The antibodies against sperms immobilize or kill them, thus preventing their passage through the cervix to the ovum. The antibodies can be tested in the serum, seminal fluid, or cervical mucus. If the antibodies are present bound to the head of the sperm, they will prevent the penetration of the egg by the sperm. If antibodies are bound to the tail of the sperm, they will retard motility.

- **SpermMARTM *test***: This test can detect IgG and IgA antibodies against sperm surface in semen sample. In direct SpermMARTM IgG test, a drop each of semen (fresh and unwashed), IgG-coated latex particles, and anti-human immunoglobulin are mixed together on a glass slide. At least 200 motile spermatozoa are examined. If the spermatozoa have antibodies on their surface, antihuman immunoglobulin will bind IgG-coated latex particles to IgG on the surface of the spermatozoa; **this will cause attachment of latex particles to spermatozoa, and motile, swimming sperms with attached particles will be seen.** If the spermatozoa do not have antibodies on their surface, they will be seen swimming **without attached particles**; the latex particles will show clumping due to binding of their IgG to antihuman immuno-globulin.
- ***In direct SpermMARTM IgA test***, a drop each of fresh unwashed semen and of IgA-coated latex particles, are mixed on a glass slide. The latex particles will bind to spermatozoa if spermatozoa are coated with IgA antibodies. In indirect SpermMARTM tests, fluid without spermatozoa (e.g. serum) is tested for the presence of antisperm antibodies. First, antibodies are bound to donor spermatozoa which are then mixed with the fluid to be analyzed. These antibodies are then detected as described above for direct tests.

Atleast 200 motile spermatozoa should be counted. If >50% of spermatozoa show attached latex particles, immunological problem is likely.

**Biochemical Analysis of Semen**

Biochemical markers (Table 15.2) can be measured in semen to test the secretions of accessory structures. These include fructose (seminal vesicles), zinc, citric acid or acid phosphatase (prostate), and □-glucosidase or carnitine (epididymis).

***Test for Fructose***

**Resorcinol method** is used for detection of fructose. In this test, 5 ml of resorcinol reagent (50 mg resorcinol dissolved in 33 ml concentrated hydrochloric acid; dilute up to 100 ml with distilled water) is added to 0.5 ml of seminal fluid. The mixture is heated and brought to boil. If fructose is present, a red-colored precipitate is formed within 30 seconds.

Absence of fructose indicates obstruction proximal to seminal vesicles (obstructed or absent vas deferens) or a lack of seminal vesicles. In a case of **azoospermia**, if fructose is absent, it is due to the obstruction of ejaculatory ducts or absence of vas deferens, and if present, azoospermia is due to failure of testes to produce sperm.

## MALE INFERTILITY

The male reproductive system consists of testes (paired organs located in the scrotal sac that produce spermatozoa and secrete testosterone), a paired system of ducts comprising of epididymis, vasa deferentia, and ejaculatory ducts (collect, store, and conduct spermatozoa), paired seminal vesicles and a single prostate gland (produce nutritive and lubricating seminal fluid), bulbourethral glands of Cowper (secrete lubricating mucus), and penis (organ of copulation).

The hypothalamus secretes gonadotropin releasing hormone (GnRH) that regulates the secretion of the two gonadotropins from the anterior pituitary: luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Fig. 14.1). **Luteinizing hormone** primarily stimulates the production and secretion of testosterone from Leydig cells located in the interstitial tissue of the testes. Testosterone stimulates spermatogenesis, and plays a role in the development of secondary sexual characters. Testosterone needs to be converted to an important steroidal metabolite, dihydrotestosterone within cells to perform most of its androgenic functions. Testosterone inhibits LH secretion by negative feedback. **Follicle stimulating hormone** acts on Sertoli cells of seminiferous tubules to regulate the normal maturation of the sperms. Sertoli cells produce inhibin that controls FSH secretion by negative feedback.

During sexual intercourse, semen is deposited into the vagina. Liquefaction of semen occurs within 20-30 minutes due to proteolytic enzymes of prostatic fluid. For fertilization to occur *in vivo*, the sperm must undergo capacitation and acrosome reaction. **Capacitation refers to physiologic changes in sperms that occur during their passage through the cervix of the female genital tract**. With capacitation, the sperm acquires (i) ability to undergo acrosome reaction, (ii) ability to bind to zona pellucida, and (iii) hypermotility. Sperm then travels through the cervix and uterus up to the fallopian tube. Binding of sperm to **zona pellucida induces acrosomal reaction** (**breakdown of outer plasma membrane by enzymes of acrosome and its fusion with outer acrosomal membrane, i.e. loss of acrosome**). This is necessary for fusion of **sperm and oocyte membranes**. Acrosomal reaction and binding of sperm and ovum surface proteins is followed by penetration of zona pellucida of ovum by the sperm. Following penetration by sperm, hardening of zona pellucida occurs that inhibits penetration by additional sperms. A sperm penetrates and fertilizes the egg in the ampullary portion of the fallopian tube (Fig. 14.2).

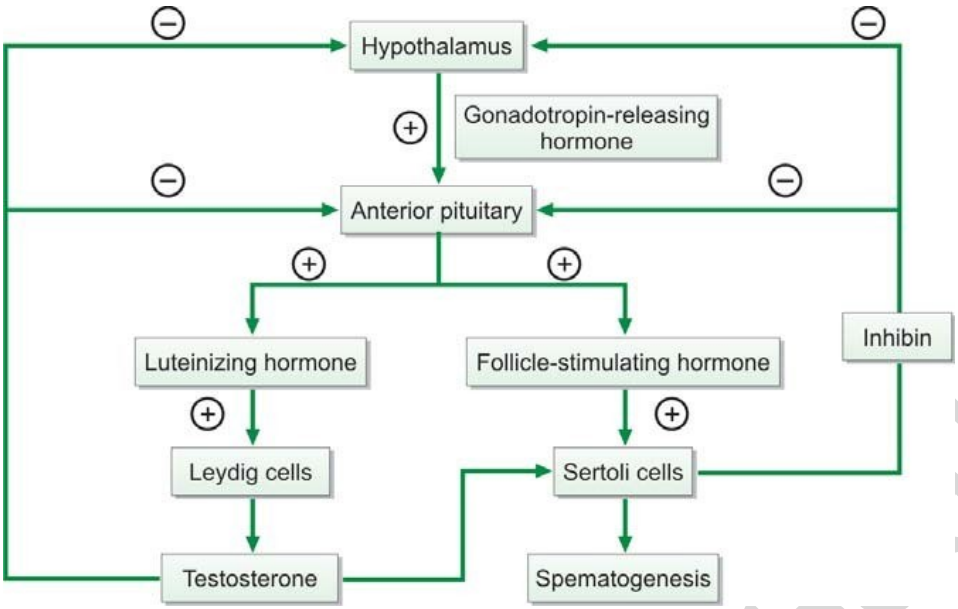



Fig. 14.1: Hypothalamus-pituitary-testis axis. + indicates stimulation; – indicates negative feedback

**Table 14.1:** Causes of male infertility

1. Idiopathic
2. Hypothalamic-pituitary dysfunction (hypogonadotropic hypogonadism)
3. Testicular dysfunction:
   - Radiation, cytotoxic drugs, antihypertensives, antidepressants
   - General factors like stress, emotional factors, drugs like marijuana, anabolic steroids, and cocaine, alcoholism, heavy smoking, undernutrition
   - Mumps orchitis after puberty
   - Varicocele (dilatation of pampiniform plexus of scrotal veins)
   - Undescended testes (cryptorchidism)
   - Endocrine disorders like diabetes mellitus, thyroid dysfunction
   - Genetic disorders: Klinefelter's syndrome, microdeletions in Y chromosome, autosomal Robertsonian translocation, immotile cilia syndrome (Kartagener's syndrome), cystic fibrosis, androgen receptor gene defect
4. Dysfunction of passages and accessory sex glands:
   - Infections of epididymis: tuberculosis, gonorrhea, *Chlamydia*
   - Congenital bilateral absence of vasa deferentia (cystic fibrosis), vasectomy
   - Prostatitis
5. Dysfunction of sexual act:
   - Impotence, erectile dysfunction
   - Defects in ejaculation: retrograde (semen is pumped backwards in to the bladder), premature, or absent
   - Hypospadias

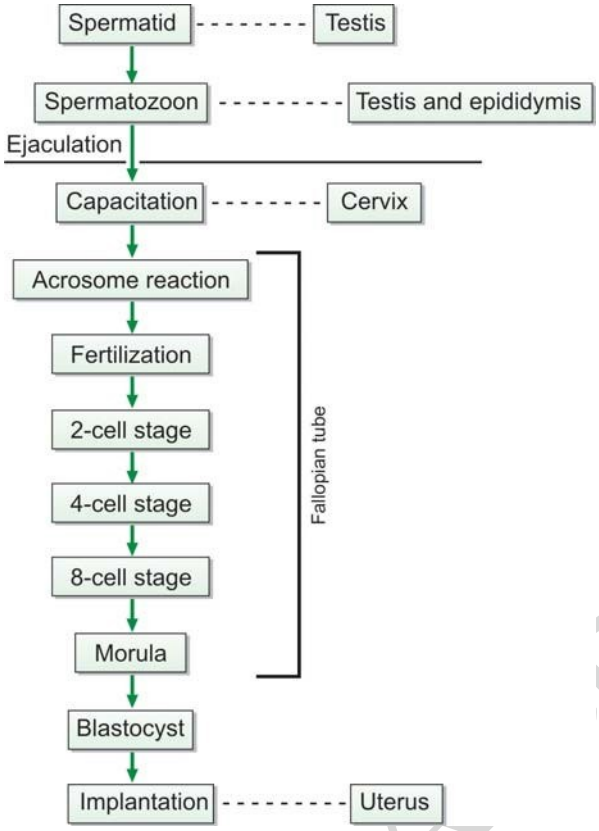



**Fig. 14.2: Steps before and after fertilization of ovum**

### *Pregnancy Tests*

Pregnancy tests detect human chorionic gonadotropin (hCG) in serum or urine. Although pregnancy is the most common reason for ordering the test for hCG, measure- ment of hCG is also indicated in other conditions as shown in Box 13.1.

Human chorionic gonadotropin is a glycoprotein hormone produced by placenta that circulates in maternal blood and excreted intact by the kidneys. It consists of two polypeptide subunits: α (92 amino acids) and β (145 amino acids) which are non-covalently bound to each other. Structurally, hCG is closely related to three other glycoprotein hormones, namely, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid-stimulating hormone (TSH). The α subunits of hCG, LH, FSH, and TSH are similar, while β subunits differ and confer specific biologic and immunologic properties. Immunological tests use antibodies directed against β-subunit of hCG to avoid cross-reactivity against LH, FSH, and TSH.

**Syncytiotrophoblastic cells** of conceptus and later of placenta synthesize hCG. Human chorionic gonadotropin supports the corpus luteum of ovary during early pregnancy. Progesterone, produced by **corpus luteum, prevents ovulation and thus maintains pregnancy**. After 7-10 weeks of gestation, sufficient amounts of progesterone are synthesized by placenta, and hCG is no longer needed and its level declines.

**Box 13.1:** Indications for measurement of β human chorionic gonadotropin

- Early diagnosis of pregnancy
- Diagnosis and management of gestational trophoblastic disease
- As a part of maternal triple test screen
- Follow-up of malignant tumors that produce β human chorionic gonadotropin.

## CLINICAL APPLICATIONS OF TESTS FOR HUMAN CHORIONIC GONADOTROPIN

1. Early diagnosis of pregnancy: Qualitative serum hCG test becomes positive 3 weeks after last menstrual period (LMP), while urine hCG test becomes positive 5 weeks after LMP.
2. Exclusion of pregnancy before prescribing certain medications (like oral contraceptives, steroids, some antibiotics), and before ordering radiological studies, radiotherapy, or chemotherapy. This is necessary to prevent any teratogenic effect on the fetus.
3. Early diagnosis of ectopic pregnancy: Transvaginal ultrasonography (USG) and quantitative estimation of hCG are helpful in early diagnosis of ectopic pregnancy (before rupture).
4. Evaluation of threatened abortion: Serial quantitative estimation of hCG is helpful in following the course of threatened abortion.
5. Diagnosis and follow-up of gestational trophoblastic disease (GTD).
6. Maternal triple test screen: This consists of measurement of **hCG, α-fetoprotein, and unconjugated estriol in maternal serum at 14-19 weeks** of gestation. The maternal triple screen identifies pregnant women with

increased risk of Down syndrome and major congenital anomalies like neural tube defects.

7. Follow-up of ovarian or testicular germ cell tumors, which produce hCG.

**Normal Pregnancy**

In women with normal menstrual cycle, conception (fertilization of ovum to form a zygote) occurs on day 14 in the fallopian tube. Zygote travels down the fallopian tube into the uterus. Division of zygote produces a **morula**. At 50-60-cell stage, morula develops a primitive yolk sac and is then called as a **blastocyst**. About 5 days after fertilization, implantation of blastocyst occurs in the uterine wall. Trophoblastic cells (on the outer surface of the blastocyst) penetrate the endometrium and develop into chorionic villi. There are two main forms of trophoblasts—syncytiotrophoblast and cytotrophoblast. Placental development occurs from chorionic villi. After formation of placenta, the conceptus is called as an embryo. When embryo develops most major organs, it is called as fetus (after 10 weeks of gestation).

Human chorionic gonadotropin is synthesized by syncytiotrophoblasts (of placenta) and detectable amounts (~5 mIU/ml) appear in maternal serum about 8 days after conception (3 weeks after LMP). In the first trimester (first 12 weeks, calculated from day 1 of LMP) of pregnancy, hCG levels rapidly rise with a doubling time of about 2 days. Highest or peak level is reached at 8-10 weeks (about 100,000 mIU/ml). This is followed by a gradual fall, and from 15-16 weeks onwards, a steady level of 10,000-20,000 mIU/ml is maintained for the rest of the pregnancy (Fig. 13.1). After delivery, hCG becomes non-detectable by about 2 weeks.

Box 13.2 shows minimum time required for the earliest diagnosis of pregnancy by hCG test and ultrasonography (USG).

*Two types of pregnancy tests are available*:

- **Qualitative tests:** These are positive/negative result types that are done on urine sample.
- **Quantitative tests:** These give numerical result and are done on serum or urine. They are also used for evaluation of ectopic pregnancy, failing pregnancy, and for follow-up of gestational trophoblastic disease.

**Box 13.2:** Diagnosis of early pregnancy

- Positive serum hCG test: 8 days after conception or 3 weeks after last menstrual period (LMP)
- Positive urine hCG test: 21 days after conception or 5 weeks after LMP
- Ultrasonography for visualization of gestational sac:
  - Transvaginal: 21 days after conception or 5 weeks after LMP
  - Transabdominal: 28 days after conception or 6 weeks after LMP

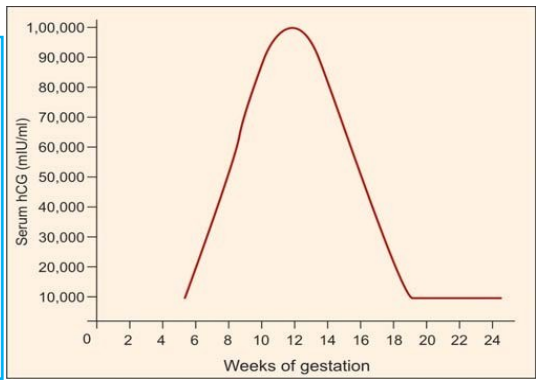



Fig. 13.1: Level of human chorionic gonadotropin during pregnancy

**Immunological Assays**

These are rapid and sensitive tests for detection and quantitation of hCG. Variable results are obtained by different immunological tests with the same serum sample; this is due to differences in specificity of different immunoassays to complete hCG, β-subunit, and β-core fragment. A number of immunological tests are commercially available based on different principles like agglutination inhibition assay, enzyme immunoassay including enzyme linked immunosorbent assay or ELISA, radioimmunoassay (RIA), and immunoradiometric assay.

A commonly used qualitative urine test is agglutination inhibition assay. Early morning urine specimen is preferred because it contains the highest concentration of hCG. Causes of false-positive test include red cells, leukocytes, bacteria, some drugs, proteins, and excess luteinizing hormone (menopause, midcycle LH surge) in urine.

- Some patients have anti-mouse antibodies (that are used in the test), while others have hCG-like material in circulation, producing false-positive test.
- Anti-mouse antibodies also interfere with other antibody-based tests and are known as 'heterophil' antibodies.
- Fetal death, abortion, dilute urine, and low sensitivity of a particular test are causes of false-negative test.
- Renal failure leads to accumulation of interfering substances causing incorrect results.

In latex particle agglutination inhibition test (Fig. 13.2), anti-hCG antibodies are incubated with patient's urine. This is followed by addition of hCG- coated latex particles. If hCG is present in urine, anti- hCG serum is neutralized, and no agglutination of latex particles occurs (positive test). If there is no hCG in urine, there is agglutination of latex particles (negative test). This is commonly used as a slide test and requires only a few minutes.

Sensitivity of agglutination inhibition test is >200 units/liter of hCG.

Radioimmunoassay, enzyme immunoassay, and radioimmunometric assay are more sensitive and reliable than agglutination inhibition assay.

Quantitative tests are employed for detection of very early pregnancy, estimation of gestational age, diagnosis of ectopic pregnancy, evaluation of threatened abortion, and management of GTD.

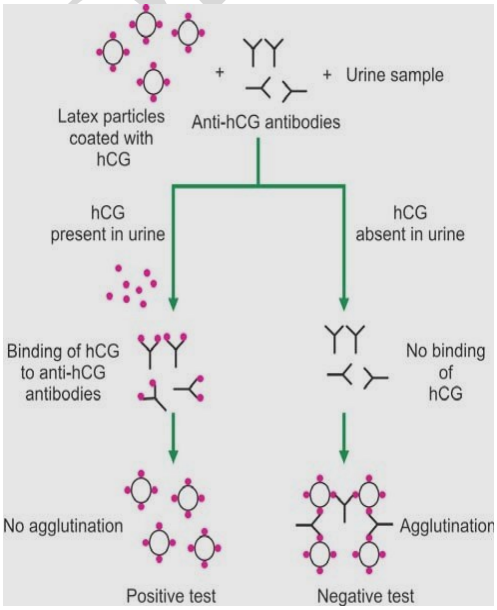



Fig. 13.2: Principle of agglutination inhibition test for diagnosis of pregnancy