

# Chapter 6

# Reproductive System

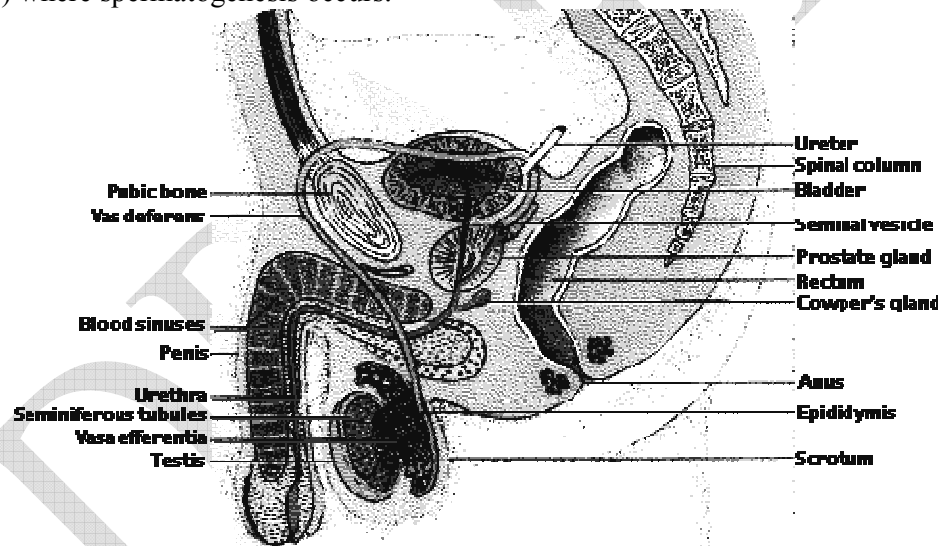
## 8.1. The Problems to be Solved

Sexual reproduction is the formation of a new individual following the union of two **gametes**. In humans and the majority of other eukaryotes — plants as well as animals — the two gametes differ in structure ("anisogamy") and are contributed by different parents. Gametes need motility to be able to meet and unite and food to nourish the developing embryo. In animals (and some plants), these two rather contrasting needs are met by anisogametes: sperm that are motile (small) and eggs that contain food.

## 8.2. Sex Organs of the Human Male

The reproductive system of the male has two major functions: the production of sperm and delivery of these to the reproductive tract of the female.

Sperm production — spermatogenesis — takes place in the **testes**. Each testis is packed with **seminiferous tubules** (laid end to end, they would extend more than 20 meters) where spermatogenesis occurs.



*Fig. 8.1. The Male Reproductive Organs*

### 8.2.1. Spermatogenesis

The walls of the seminiferous tubules consist of **diploid** spermatogonia, **stem cells** that are the precursors of sperm.

#### (a) Spermatogonia

Spermatogonia divide by **mitosis** to produce more spermatogonia or differentiate into **spermatocytes**. **Meiosis** of each spermatocyte produces 4 haploid **spermatids**. This process takes over three weeks to complete. Then the spermatids differentiate into sperm, losing most of their cytoplasm in the process. For simplicity, the figure shows the behaviour of just a single pair of homologous chromosomes with a single crossover. With 22 pairs of **autosomes** and an average of two crossovers between each pair, the variety of gene combinations in sperm is very great.

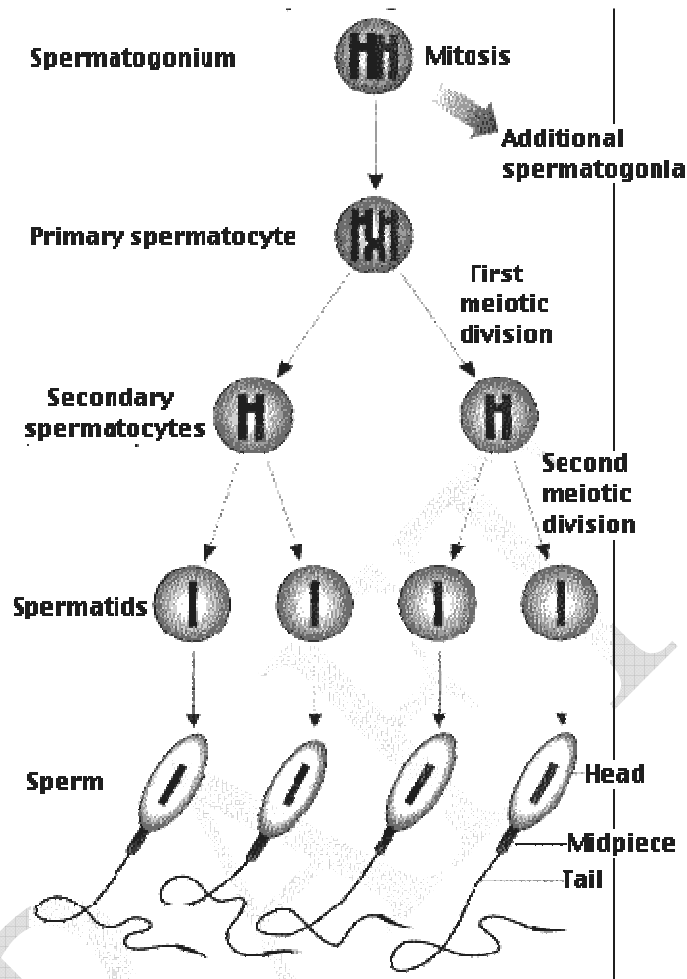


Fig.8.2. Spermatogenesis

### (b) Sperm

Sperm cells are little more than flagellated nuclei. Each consists of a head, which has an **acrosome** at its tip and contains a haploid set of chromosomes in a compact, inactive, state, a **midpiece** containing **mitochondria** and a single **centriole** and a **tail**. Mitochondria supply the **ATP** to power the whiplike motion of the tail. An adult male manufactures over 100 million sperm cells each day. These gradually move into the **epididymis** and the first portion of the **vas deferens**, where they undergo further maturation and are stored. In addition to making sperm, the testis is an **endocrine gland**. Its principal hormone, **testosterone**, is responsible for the development of the secondary sex characteristics of men such as the beard, deep voice, and masculine body shape. **Testosterone is also essential for making sperm**. Testosterone is made in the **interstitial cells** that lie between the seminiferous tubules.

### (c) LH

Interstitial cells are, in turn, the targets for a hormone often called interstitial cell stimulating hormone (**ICSH**). It is a product of the anterior lobe of the **pituitary gland**. However, ICSH is identical to the **luteinizing hormone (LH)** found in females, and I prefer to call it LH.

## (d) FSH

Follicle-stimulating hormone (also named for its role in females) acts directly on spermatogonia to stimulate sperm production (aided by the LH needed for testosterone synthesis).

## 8.3. Sex Organs of the Human Female

The responsibility of the female mammal for successful reproduction is considerably greater than that of the male.

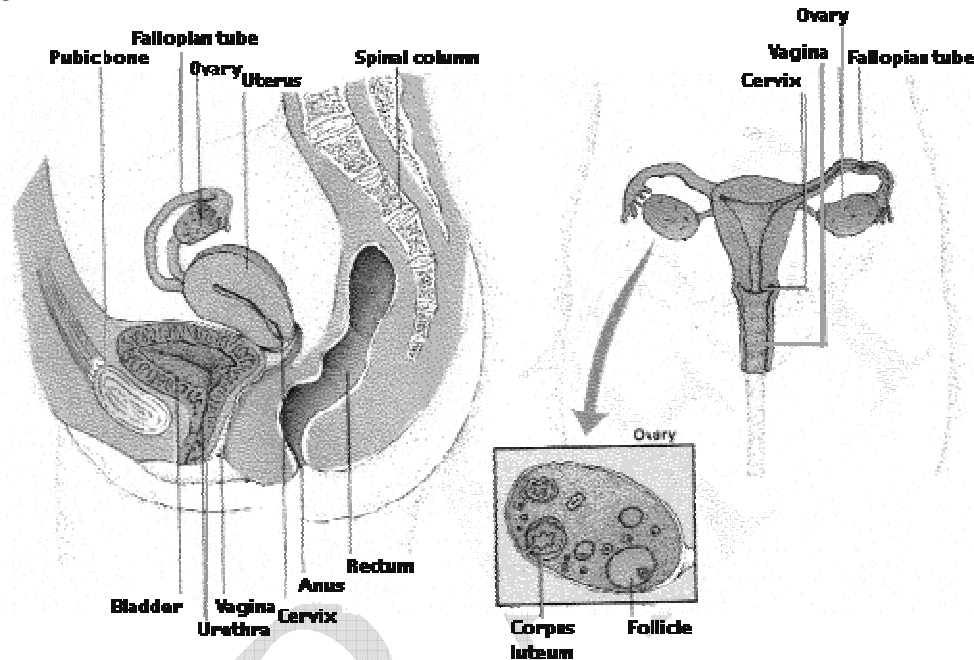


Fig.8.3. Female Sex Organs

She must manufacture eggs and be equipped to receive sperm from the male. She must provide an environment conducive to fertilization and **implantation** and nourish the developing baby not only before birth but after.

### 8.3.1. Oogenesis

Egg formation takes place in the **ovaries**. In contrast to males, the initial steps in egg production occur prior to birth. Diploid stem cells called **oogonia** divide by mitosis to produce more oogonia and **primary oocytes**. By the time the foetus is 20 weeks old, the process reaches its peak and all the oocytes that she will ever possess (~4 million of them) have been formed. By the time she is born, 1–2 million of these remain. Each has begun the first steps of the first meiotic division (**meiosis I**) and then stopped. No further development occurs until years later when the girl becomes sexually mature. Then the primary oocytes recommence their development, usually one at a time and once a month. The primary oocyte grows much larger and completes the **meiosis I**, forming a large **secondary oocyte** and a small **polar body** that receives little more than one set of chromosomes. Which chromosomes end up in the egg and which in the polar body is entirely a matter of chance. In humans (and most vertebrates), the first polar body does not go on to meiosis II, but the secondary oocyte does proceed as far as **metaphase of meiosis II** and then stops.

Only if **fertilization** occurs will meiosis II ever be completed. Entry of the sperm restarts the cell cycle breaking down **MPF** (M-phase promoting factor) and turning on the **anaphase promoting complex** (APC). Completion of meiosis II converts the

secondary oocyte into a fertilized egg or zygote (and also a second polar body). As in the diagram for spermatogenesis, the behaviour of the chromosomes is greatly simplified.

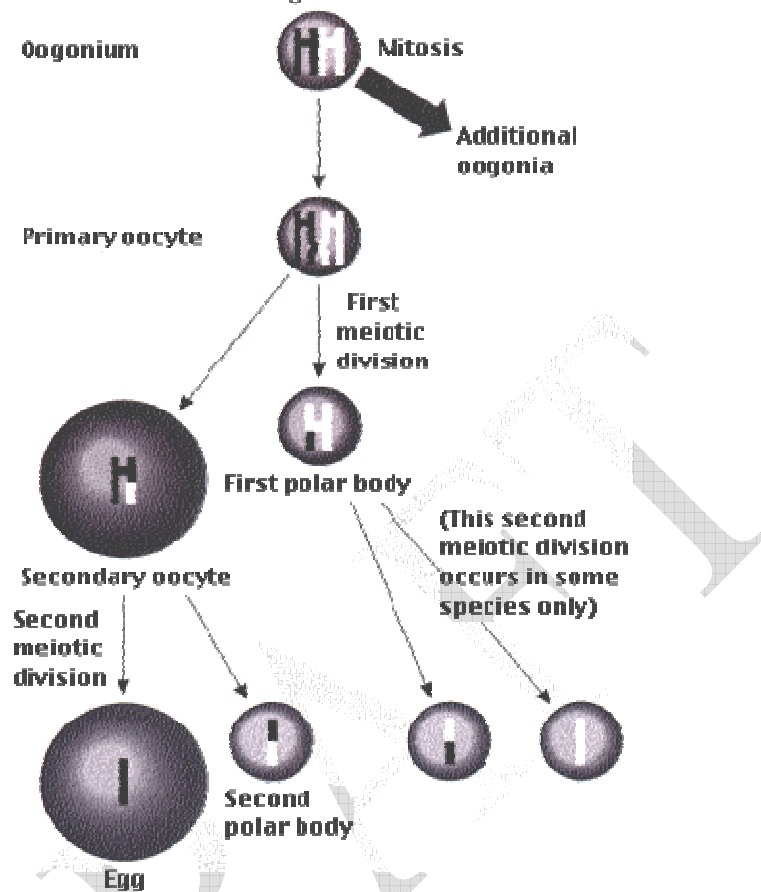


Fig.8.4. Oogenesis

These events take place within a **follicle**, a fluid-filled envelope of cells surrounding the developing egg. The ripening follicle also serves as an **endocrine gland**. Its cells make a mixture of **steroid** hormones collectively known as **estrogen**. Estrogen is responsible for the development of the secondary sexual characteristics of a mature woman, e.g., a broadening of the pelvis, development of the breasts, growth of hair around the genitals and in the armpits and development of **adipose tissue** leading to the more rounded body contours of adult women. Estrogen continues to be secreted throughout the reproductive years of women. During this period, it plays an essential role in the monthly **menstrual cycle**.

### 8.3.2. Ovulation

Ovulation occurs about two weeks after the onset of menstruation. In response to a sudden surge of LH, the follicle ruptures and discharges a secondary oocyte. This is swept into the open end of the **fallopian tube** and begins to move slowly down it. Several sexually-transmitted diseases (STDs), especially **gonorrhoea** and infections by **chlamydia** can cause scarring and blocking of the tubes and are a major cause of infertility. In tubal ligation, the fallopian tubes are surgically cut and their ends tied to prevent pregnancy.

### 8.3.3. Copulation and Fertilization

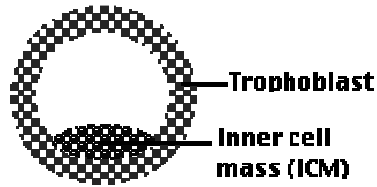
For fertilization to occur, sperm must be deposited in the vagina within a few days before or a day or two after ovulation. Sperm transfer is accomplished by copulation. Sexual excitation dilates the arterioles supplying blood to the penis. Blood accumulates in three cylindrical spongy sinuses that run lengthwise through the penis. The resulting pressure causes the penis to enlarge and erect and thus able to penetrate the vagina. Movement of the penis back and forth within the vagina causes sexual tension to increase to the point of **ejaculation**. Contraction of the walls of each **vas deferens** propels the sperm along. Fluid is added to the sperm by the **seminal vesicles**, Cowper's glands, and the **prostate gland**. These fluids provide a source of energy (fructose) and perhaps in other ways provide an optimum chemical environment for the sperm. The mixture of sperm and accessory fluids is called **semen**. It passes through the **urethra** and is expelled into the vagina. Physiological changes occur in the female as well as the male in response to sexual excitement, although these are not as readily apparent. In contrast to the male, however, such responses are not a prerequisite for copulation and fertilization to occur. Once deposited within the vagina, the sperm proceed on their journey into and through the uterus and on up into the fallopian tubes. It is here that fertilization may occur if an "egg" is present (strictly speaking, it is still a secondary oocyte until after completion of meiosis II).

Although sperm can swim several millimetres each second, their trip to and through the fallopian tubes may be assisted by muscular contraction of the walls of the uterus and the tubes. There is also evidence that they respond to a chemical attractant produced by the egg or the tissues surrounding it. In any case, sperm may reach the egg within 15 minutes of ejaculation. The trip is also fraught with heavy mortality. An average human ejaculate contains over one hundred million sperm, but only a few dozen complete the journey. And of these, only one will succeed in fertilizing the egg. Fertilization begins with the binding of a sperm head to the outer coating of the egg (called the **zona pellucida**). **Exocytosis** of the **acrosome** at the tip of the sperm head releases enzymes that digest a path through the zona and enable the sperm head to bind to the plasma membrane of the egg. Fusion of their respective membranes allows the entire contents of the sperm to be drawn into the cytosol of the egg. (Even though the sperm's mitochondria enter the egg, they are almost always destroyed and do not contribute their genes to the embryo. So human **mitochondrial DNA** is almost always inherited from mothers only.) Within moments, enzymes released from the egg cytosol act on the zona making it impermeable to the other sperm that arrive. Soon the nucleus of the successful sperm enlarges into the **male pronucleus**. At the same time, the egg (secondary oocyte) completes meiosis II forming a **second polar body** and the **female pronucleus**. The male and female pronuclei move toward each other. Their nuclear envelopes disintegrate. A spindle is formed (following replication of the sperm's centriole), and a full diploid set of chromosomes assembles on it. The fertilized egg or **zygote** is now ready for its first mitosis. In sea urchins, at least, the block to additional sperm entry and the fusion of the pronuclei are triggered by nitric oxide generated in the egg by the sperm acrosome.

### 8.4. Pregnancy

Development begins while the fertilized egg is still within the **fallopian tube**. Repeated mitotic divisions produces a solid ball of cells called a **morula**. Further mitosis and some migration of cells converts this into a hollow ball of cells called the **blastocyst**. Approximately one week after fertilization, the blastocyst embeds itself in

the thickened wall of the uterus, a process called **implantation**, and pregnancy is established.



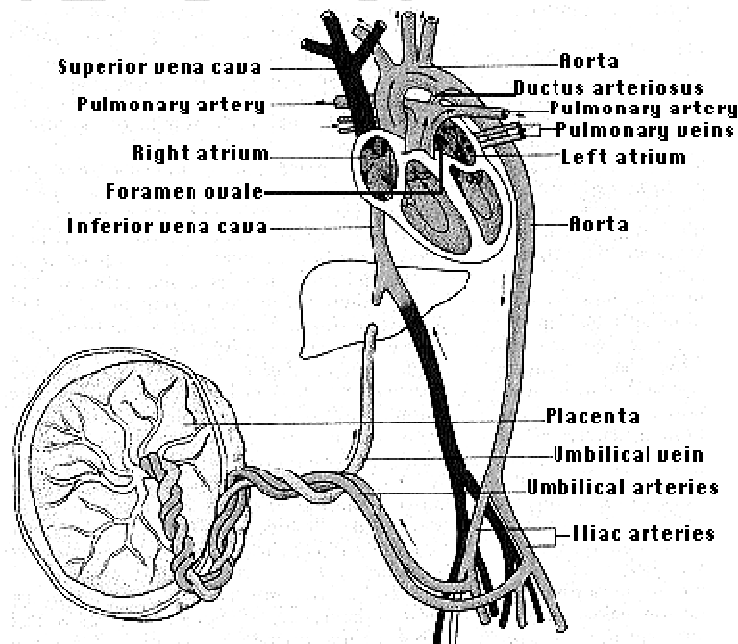
*Fig.8.5. Blastocyst*

The blastocyst produces two major divisions of cells:

1. Three or four blastocyst cells develop into the **inner cell mass**, which will, in about 2 months, become the foetus and, ultimately, the baby.
2. The remaining 100 or so cells form the **trophoblast**, which will produce the **four extraembryonic membranes**. These will play vital roles during development but will be discarded at the time of birth.

The extraembryonic membranes form the **amnion, placenta and umbilical cord**. The placenta grows tightly fused to the wall of the uterus. Its blood vessels, supplied by the foetal heart, are literally bathed in the mother's blood. Although there is normally no mixing of the two blood supplies, the placenta does facilitate the transfer of a variety of materials between the foetus and the mother: receiving food, oxygen and discharging carbon dioxide, urea and other wastes. It also receives **antibodies** (chiefly of the **IgG** class). These remain for weeks after birth, protecting the baby from the diseases to which the mother is immune. But the placenta is not simply a transfer device. Using raw materials from the mother's blood, it synthesizes large quantities of proteins and also some hormones.

The metabolic activity of the placenta is almost as great as that of the foetus itself. The **umbilical cord** connects the foetus to the placenta. It receives deoxygenated blood from the iliac arteries of the foetus and returns oxygenated blood to the liver and on to the inferior vena cava.



*Fig.8.6. Umbilical Circulation*

Because its lungs are not functioning, circulation in the foetus differs dramatically from that of the baby after birth. While within the uterus, blood pumped by the right

ventricle bypasses the lungs by flowing through the **foramen ovale** and the **ductus arteriosus**. Although the blood in the placenta is in close contact with the mother's blood in the uterus, intermingling of their blood does not normally occur. However, some of the blood cells of the foetus usually do get into the mother's circulation — where they have been known to survive for decades. This raises the possibility of doing **prenatal diagnosis** of genetic disorders by sampling the mother's blood rather than having to rely on the more invasive procedures of **amniocentesis** and **chorionic villus sampling (CVS)**.

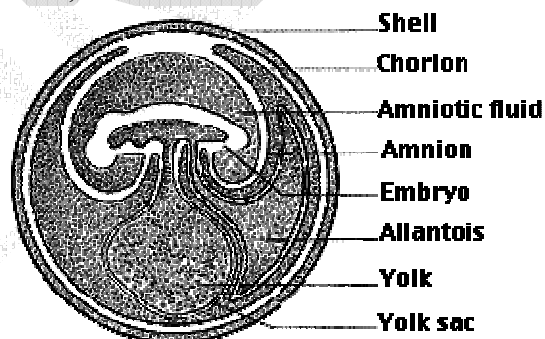
Far rarer is the leakage of mother's blood cells into the foetus. However, it does occur. A few pregnant women with leukaemia or lymphoma have transferred the malignancy to their foetus. Some babies have also acquired melanoma from the transplacental passage of these highly-malignant cells from their mother.

During the first 2 months of pregnancy, the basic structure of the baby is being formed. This involves cell division, cell migration, and the differentiation of cells into the many types found in the baby. During this period, the developing baby — called an **embryo** — is very sensitive to anything that interferes with the steps involved. Virus infection of the mother, e.g., by rubella ("German measles") virus or exposure to certain chemicals may cause malformations in the developing embryo. Such agents are called **teratogens** ("monster-forming"). The tranquilizer, **thalidomide**, taken by many pregnant European women between 1954 and 1962, turned out to be a potent teratogen and was responsible for the birth of several thousand deformed babies.

After about two months, all the systems of the baby have been formed, at least in a rudimentary way. From then on, development of the **foetus**, as it is now called, is primarily a matter of growth and minor structural modifications. The foetus is less susceptible to teratogens than is the embryo. Pregnancy involves a complex interplay of **hormones**.

## 8.5. Extraembryonic Membranes

The embryos of reptiles, birds, and mammals produce 4 extraembryonic membranes, the amnion, yolk sac, chorion, and allantois



*Fig.8.7. Extraembryonic Membranes*

In birds and most reptiles, the embryo with its extraembryonic membranes develops within a shelled egg.

- The **amnion** protects the embryo in a sac filled with **amniotic fluid**.
- The **yolk sac** contains yolk — the sole source of food until hatching. Yolk is a mixture of proteins and **lipoproteins**.
- The **chorion** lines the inner surface of the shell (which is permeable to gases) and participates in the exchange of O<sub>2</sub> and CO<sub>2</sub> between the embryo and the outside air.

- The **allantois** stores metabolic wastes (chiefly **uric acid**) of the embryo and, as it grows larger, also participates in gas exchange.

With these four membranes, the developing embryo is able to carry on essential metabolism while sealed within the egg. Surrounded by amniotic fluid, the embryo is kept as moist as a fish embryo in a pond.

Although (most) mammals do not make a shelled egg, they do also enclose their embryo in an amnion. For this reason, the reptiles, birds, and mammals are collectively referred to as the **amniota**.

Mammals fall into three groups that differ in the way they use the amniotic egg.

- **Monotremes**

These primitive mammals produce a shelled egg like their reptilian ancestors. Only three species exist today: two species of spiny anteater (echidna) and the duckbill platypus.

- **Marsupials** Marsupials do not produce a shelled egg. The egg, which is poorly supplied with yolk, is retained for a time within the reproductive tract of the mother. The embryo penetrates the wall of the **uterus**. The yolk sac provides a rudimentary connection to the mother's blood supply from which it receives food, oxygen, and other essentials. However, this interface between the tissues of the uterus and the extraembryonic membranes never becomes elaborately developed, and the young are born in a very immature state. Despite their tiny size, they are able to crawl into a pouch on the mother's abdomen, attach themselves to nipples, and drink milk from her mammary glands. Marsupials are still abundant in Australia, but only the opossum is found in North America.

- **Placental**

**mammals**

In placental mammals, the extraembryonic membranes form a **placenta** and **umbilical cord**, which connect the embryo to the mother's uterus in a more elaborate and efficient way. The blood supply of the developing foetus is continuous with that of the placenta. The placenta extracts food and oxygen from the uterus. Carbon dioxide and other wastes (e.g., urea) are transferred to the mother for disposal by her excretory organs. Humans are placental mammals.

## 8.6. The Placenta is an Allograft

One of the greatest unsolved mysteries in immunology is how the placenta survives for 9 months without being rejected by the mother's immune system. Every cell of the placenta carries the father's genome (a haploid set of his chromosomes); including one of his #6 chromosomes where the genes for the major histocompatibility antigens are located. One partial exception: none of the genes on the father's X chromosome are expressed. While X-chromosome inactivation is random in the cells of the foetus, it is NOT random in the cells of the trophoblast. In every cell of the trophoblast — and its descendants — it is the paternal X chromosome that is inactivated. But this does not solve our problem because the genes for all the major histocompatibility antigens are located on chromosome 6, which is not inactivated. Thus the placenta is immunologically as foreign to the mother as a kidney transplant would be. Yet it thrives. Despite a half-century of research, the mechanism for this immunologically privileged status remains uncertain. But one thing is clear:

The mother is **not** intrinsically tolerant of the father's antigens. Some evidence:

1. She will promptly reject a skin transplant from the father.
2. She develops antibodies against his histocompatibility antigens expressed by the foetus.

3. The placenta does not express class II histocompatibility antigens. Nor does it express the strongly-immunogenic **class I histocompatibility antigens** (HLA-A, HLA-B). It does express **HLA-C**, but this is only weakly immunogenic.
4. The cells of the placenta secrete **progesterone**, which is immunosuppressive.

## 8.7. Assisted Reproductive Technology ("ART")

**Louise Brown** recently celebrated her 25th birthday in 2004. She was the first of what today number around one million "test tube babies"; that is, she developed from an egg that was fertilized outside her mother's body — the process called *in vitro* fertilization (IVF).

### 8.7.1. *In Vitro* Fertilization (IVF)

IVF involves

1. Harvesting mature eggs from the mother. This is not an easy process. The mother must undergo hormonal treatments to produce multiple eggs, which then must be removed (under anaesthesia) from her ovaries.
2. Then there is harvesting sperm from the father. Harvesting is usually no problem, but often the sperm are defective in their ability to fertilize (so setting the stage for ICSI);
3. Mixing sperm and eggs in a culture vessel ("*in vitro*");
4. culturing the fertilized eggs for several days until they have developed to at least the 8-cell stage;
5. Placing two (usually) of these into the mother's uterus (which her hormone treatments have prepared for **implantation**);
6. Keeping one's fingers crossed — only about one-third of the attempts result in a successful pregnancy)

### 8.7.2. Intracytoplasmic Sperm Injection (ICSI)

Successful IVF assumes the availability of healthy sperm. But many cases of infertility arise from defects in the father's sperm. Often these can be overcome by directly injecting a single sperm into the egg.

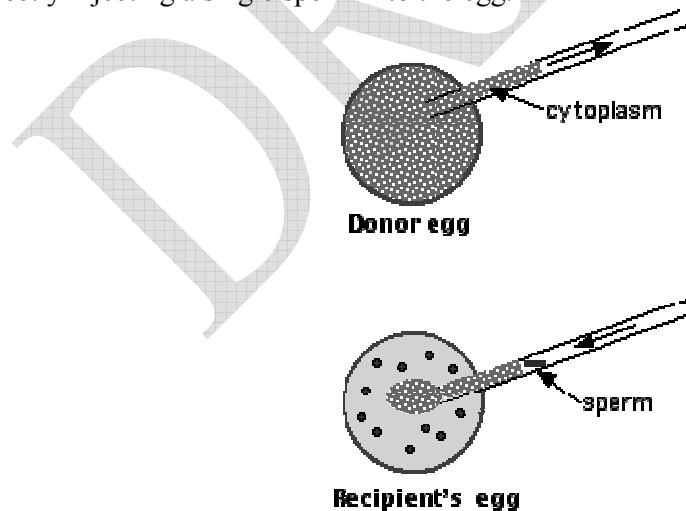


Fig.8.8. Assisted Reproductive Technology

In the U.S. today, some 40% of ART procedures employ ICSI.

### 8.7.3. Ooplasmic Transfer

Infertility in some cases may stem from defects in the cytoplasm of the mother's egg. To circumvent these, cytoplasm can be removed from the egg of a young, healthy woman ("Donor egg") and injected — along with a single sperm — into the

prospective mother's egg. Several dozen children have been born by this method, but it is not yet approved for general use in the U.S. One reason for concern is that ooplasmic transfer results in an egg carrying both the mother's mitochondria and mitochondria from the donor. This condition — called **heteroplasmy** — creates a child having two different **mitochondrial DNA genomes** in all of its cells. In normal fertilization, all the mitochondria in the father's sperm are destroyed in the egg, and perhaps this is important. Although a few healthy children have been born following ooplasmic transfer, the jury is still out on its safety.

#### **8.7.4. The Upside of ART**

1. It has allowed hundreds of thousands of previously-infertile couples to have children.
2. It permits screening (on one cell removed from the 8-celled morula) for the presence of genetic disorders — thus avoiding starting a pregnancy if a disorder is found.
3. One can use frozen sperm allowing fatherhood for a man who is no longer able to provide fresh sperm.
4. Because a number of morulas are created, the extras can be frozen, stored, and used later
  - a. if the initial attempt fails (the prospective mother must still receive hormones to prepare her uterus for implantation and the success rate is lower with thawed morulas).
  - b. Where regulations permit, the extras can be used as a source of embryonic stem (ES) cells.

#### **8.7.5. The Downside of ART**

1. Although improving, the success rate is still sufficiently low (~30%) that the process often has to be repeated.
2. Because several morulas are usually transferred, multiple births are common (about 40%), and as is the case with most multiple births, the babies weigh less. To reduce the number of twins, triplets, etc., more ART centres are turning to "**single-embryo transfer**" (SET). Some ART centres find that they can increase the success rate — and thus rely more on SET — by culturing the morulas for 5–6 days, instead of the usual 2–3 days, before transferring them (by now they have become **blastocysts**) to the mother.
3. The risk of birth defects is about doubled (from ~4% in "normal" pregnancies to ~8% in ART pregnancies).
4. ART procedures in experimental animals often result in a failure of correct **gene imprinting**. Whether this will pose a problem for humans remains to be seen.

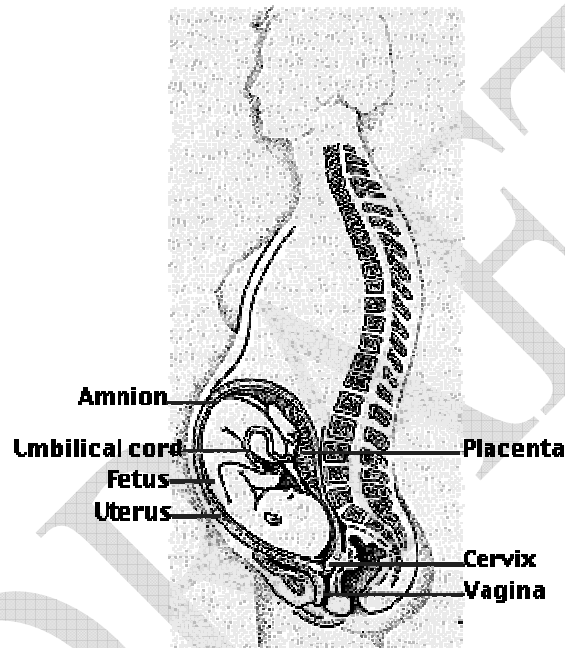
### **8.8. Birth and Lactation**

Exactly what brings about the onset of labour is still not completely understood. Probably a variety of integrated hormonal controls are at work. The first result of labour is the opening of the cervix. With continued powerful contractions, the amnion ruptures and the amniotic fluid (the "waters") flows out through the vagina. The baby follows, and its umbilical cord can be cut. The infant's lungs expand, and it begins breathing. This requires a major switchover in the circulatory system. Blood flow through the **umbilical cord, ductus arteriosus, and foramen ovale** ceases, and the **adult pattern** of blood flow through the heart, aorta, and pulmonary arteries begins. In some infants, the switchover is incomplete, and blood flow through the pulmonary arteries is inadequate. Failure to synthesize enough **nitric oxide** (NO) is one cause.

Shortly after the baby, the placenta and the remains of the umbilical cord (the "afterbirth") are expelled.

At the time of birth, and for a few days after, the mother's breasts contain a fluid called **colostrum**. It is rich in calories and protein, including antibodies that provide passive immunity for the newborn infant. Three or four days after delivery, the breasts begin to secrete milk.

1. Its synthesis is stimulated by the pituitary hormone **prolactin (PRL)**.
2. Its release is stimulated by a rise in the level of **Oxytocin** when the baby begins nursing.
3. Milk contains an **inhibitory peptide**. If the breasts are not fully emptied, the peptide accumulates and inhibits milk production. This **autocrine** action thus matches supply with demand.



*Fig.8.9. Woman at Term*

## **8.9. Screening for Genetic Disease**

Many tests are now available to detect genetic diseases. Some examples include sickle cell disease, cystic fibrosis and phenylketonuria (PKU). Most of these tests can not only be performed on cells removed from adults but also on cells removed from the foetus and even from a pre-implantation embryo.

### **8.9.1. Amniocentesis**

During its development, the foetus sheds cells into the **amniotic fluid**. After 14–22 weeks of pregnancy, a small volume of this fluid can be removed (using a needle inserted through the abdominal wall). Separating the cells and culturing them enables the clinician to look for chromosome abnormalities (e.g., the three number 21 chromosomes of **Down syndrome**); certain enzymatic defects (e.g., an inability to metabolize **galactose**, hence milk); and the sex of the foetus. Over 100 genetic abnormalities can be diagnosed by amniocentesis and the pregnancy deliberately ended if the parents wish it.

### **8.9.2. Chorionic Villus Sampling (CVS)**

This is an alternate method of prenatal diagnosis. A small amount of placental tissue is sucked out by a tube inserted through the abdominal wall or through the vagina (the latter avoiding the need for an incision). For some tests the foetal cells can be examined immediately without the need to culture them. Another advantage of CVS is that it can be performed earlier in pregnancy (after only 10–12 weeks) than amniocentesis. If an abortion is to be performed, it is a simpler process early in pregnancy.

### **8.9.3. Noninvasive Prenatal Diagnosis**

Although the blood in the placenta is in close contact with the mother's blood in the uterus, intermingling of their blood does not normally occur. However, some of the blood cells of the foetus do manage to get into the mother's circulation where they may represent 1 in a million of her white blood cells (so only some 2–6 cells per ml of blood). Fragments of foetal DNA (~ 300 bp long) also are found in the mother's plasma as early as 5 weeks after implantation. This raises the possibility of using genetic tests (e.g., **PCR**) to identify mutations or chromosomal abnormalities in the foetus. However, only genes contributed by the father (e.g., *SRY*) can be detected because there is as yet no way to separate the mother's DNA from the foetal DNA. And the tests are not yet as sensitive as amniocentesis and CVS.

### **8.9.4. Preimplantation Genetic Diagnosis (PGD)**

One of the remarkable facts about mammalian development is that all the cells in the early (e.g., 8-cell) embryo are not needed to produce a healthy foetus (which is why a single fertilized egg can on occasions produce identical twins, triplets, etc.).

So couples using in vitro fertilization (IVF) also can take advantage of genetic screening. While the embryo is in culture, a cell or two can safely be removed and tested for its genotype. For example: The sex of the embryo can be determined with a probe for Y-specific DNA. This permits prospective mothers carrying a severe **X-linked trait** like **haemophilia A** to choose a female rather than a male embryo for attempted implantation. Fluorescent probes specific for the DNA of particular chromosomes can detect (by FISH) if there is an abnormal number (**aneuploidy**) such as the three #21 chromosomes of **Down syndrome**. In fact the entire **karyotype** of the embryo can be determined. Random fragments of DNA prepared by the **polymerase chain reaction** (PCR) of all the DNA of a cell from the **embryo** can be given a fluorescent label and applied to the metaphase **chromosomes** of a standard reference cell that has a normal karyotype along with DNA fragments from the reference cell labelled with a different colour.

### **8.9.5. Genetic Diagnosis Before in vitro Fertilization (IVF)**

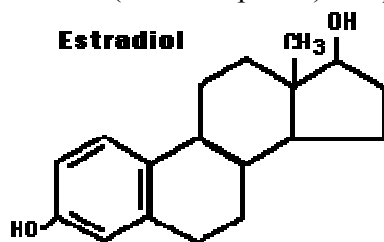
Thanks to the **polymerase chain reaction** (PCR), the genotype of an egg can be determined **before** it is fertilized. As **meiosis I** is completed, one set of chromosomes is extruded into the **first polar body**. If the mother is **heterozygous** for a trait, the DNA of the polar body can be amplified by PCR and tested for **both** alleles. If the test is **positive** for the **mutant** allele **AND negative** for the **healthy** allele, no **crossover** has occurred. Both copies of the mutant allele have been sequestered in the polar body so the egg may safely be fertilized. For simplicity, the figure shows only the pair of homologues carrying the locus of concern. So it is not sufficient to test only for the presence of the mutant gene in the first polar body. You must also demonstrate that the healthy gene is absent. For if crossing over had occurred, the first polar body would contain one mutant and one healthy allele.

# Chapter 9

## Hormones of Reproduction

### 9.1. In Females

The **ovaries** of sexually-mature females secrete a mixture of **estrogens** of which **17 $\beta$ -estradiol** is the most abundant (and most potent) and **progesterone**.

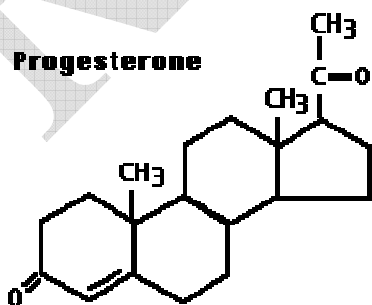


#### 9.1.1. Estrogens

Estrogens are **steroids**. They are primarily responsible for the conversion of girls into sexually-mature women in development of breasts, further development of the uterus and vagina, broadening of the pelvis, growth of pubic and axillary hair and increase in adipose (fat) tissue. They participate in the monthly preparation of the body for a possible pregnancy and in pregnancy if it occurs. Estrogens also have non-reproductive effects. They antagonize the effects of the **parathyroid hormone**, minimizing the loss of calcium from bones and thus helping to keep bones strong. They promote blood clotting.

#### 9.1.2. Progesterone

Progesterone is also a steroid. It has many effects in the body, some having nothing to do with sex and reproduction. Progesterone plays a major role in the **menstrual cycle** and **pregnancy**.



It is secreted by the **corpus luteum** and by the **placenta** and is responsible for preparing the body for pregnancy and, if pregnancy occurs, maintaining it until birth.

#### (a) Corpus Luteum

Progesterone secretion by the corpus luteum occurs after **ovulation** and continues the preparation of the **endometrium** for a possible pregnancy. It inhibits the contraction of the uterus and also inhibits the development of a new follicle. If pregnancy does **not** occur, secretion wanes toward the end of the menstrual cycle, and menstruation begins.

## **(b) Placenta**

If pregnancy does occur, the placenta begins to secrete progesterone which supplements that of the corpus luteum. In fact, by the fifth month of pregnancy, the placenta secretes sufficient progesterone by itself that the corpus luteum is no longer essential to maintain pregnancy.

## **(c) Method of Action**

Progesterone, like all steroids, is a small **hydrophobic** molecule. Thus it diffuses freely through the plasma membrane of all cells. However, in **target cells**, like those of the endometrium, it becomes tightly bound to a cytoplasmic protein the **progesterone receptor**. The complex of receptor and its hormone moves into the nucleus, where it binds to a **progesterone response element**. The progesterone response element is a specific sequence of DNA in the **promoters** of certain genes that is needed to turn those genes on (or off). Thus, the complex of progesterone with its receptor forms a **transcription factor**.

Some target cells also have other progesterone receptors that are exposed at the surface of the cell embedded in the plasma membrane. These are **G-protein-coupled receptors**, and binding of the hormone to them produces more rapid effects than those of the nuclear receptors.

## **(d) Progestins**

Progestins are synthetic modifications of the progesterone molecule. Several different ones are prescribed for birth control pills (and other forms of contraceptives); for **hormone replacement therapy (HRT)** to reduce the unpleasant symptoms of the menopause; to treat young women who cease to menstruate normally; and to prevent premature birth. Some examples: norgestrel (Trade name = "Orvette"); used as an oral contraceptive levonorgestrel used in the "Norplant" system: an implantable contraceptive; the ingredient in "Plan B", an oral contraceptive taken after unprotected intercourse. released from Mirena®, an **intrauterine device (IUD)**. norethindrone (Trade name = "Aygestin"; used in HRT (hormone replacement therapy)

## **(e) RU-486**

RU486 (also known as **mifepristone**) is a synthetic steroid related to progesterone. Unlike the progestins discussed above, that mimic the action of progesterone, RU-486 **blocks** the action of progesterone. (Synthetic molecules that mimic the action of a natural molecule are called **agonists**; those that oppose it are **antagonists**.) **RU-486 is a progesterone antagonist**. It binds to the progesterone receptor, and in so doing prevents progesterone itself from occupying its receptor. Thus the gene transcription normally turned on by progesterone is blocked, and the proteins necessary to begin and maintain pregnancy are not synthesized. If RU-486 is taken shortly after intercourse, it prevents pregnancy. If taken early in pregnancy, it causes the embryo to be aborted. This result has caused RU-486 to be widely used in Europe to terminate early pregnancy. It has not found widespread acceptance in the U.S.

## **9.1.3. How estrogens and progesterone achieve their effects**

Steroids like estrogens and progesterone are small, **hydrophobic** molecules that are transported in the blood bound to a **serum globulin**. In "target" cells, i.e., cells that change their gene expression in response to the hormone, they bind to receptor proteins located in the cytoplasm and/or nucleus. The hormone-receptor complex enters the nucleus (if it formed in the cytoplasm) and binds to specific sequences of DNA, called the estrogen (or progesterone) **response elements**. Response elements are located in the **promoters** of genes. The hormone-receptor complex acts as a **transcription factor** (often recruiting other transcription factors to help) which turns

on (sometimes off) transcription of those genes. Gene expression in the cell produces the response. Some "target" cells also have other types of estrogen and progesterone receptors that are embedded in a membrane (endoplasmic reticulum and plasma membrane respectively). These are **G-protein-coupled receptors**, and binding of the hormone to them produces more rapid effects than those of the nuclear receptors.

### 9.1.4. Regulation of Estrogen and Progesterone

The synthesis and secretion of **estrogens** is stimulated by **follicle-stimulating hormone (FSH)**, which is, in turn, controlled by the hypothalamic **gonadotropin releasing hormone (GnRH)**.

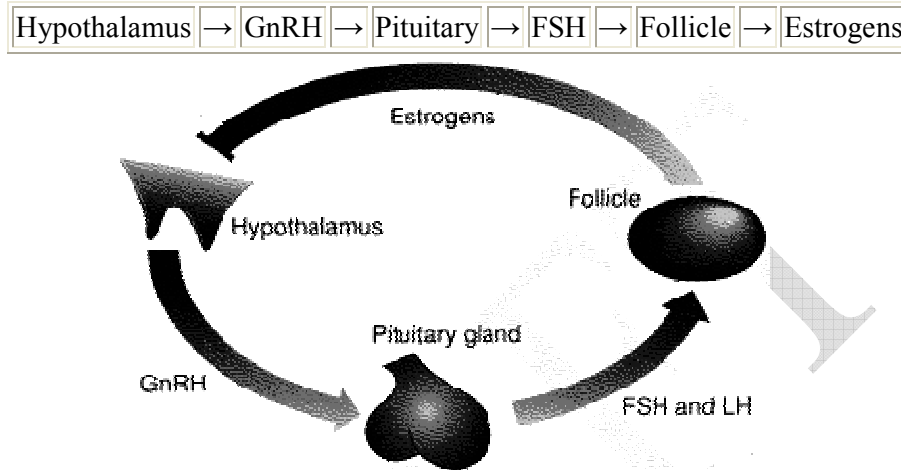
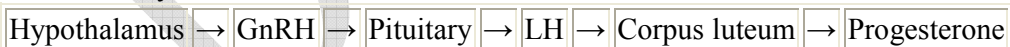


Fig.9.1. Regulation of Estrogen and Progesterone

High levels of estrogens **suppress** the release of GnRH (bar) providing a negative-feedback control of hormone levels. It works like this: Secretion of GnRH depends on certain neurons in the hypothalamus which express a gene (*KiSS-1*) encoding a protein of 145 amino acids. From this are cut several short peptides collectively called **kisspeptin**. These are secreted and bind to **G-protein-coupled receptors** on the surface of the GnRH neurons stimulating them to release GnRH. However, high levels of estrogen (or progesterone or testosterone) inhibit the secretion of kisspeptin and suppress further production of those hormones.

**Progesterone** production is stimulated by **luteinizing hormone (LH)**, which is also stimulated by GnRH.



Elevated levels of progesterone control themselves by the same negative feedback loop used by estrogen (and **testosterone**).

### 9.1.5. The Menstrual Cycle

About every 28 days, some blood and other products of the disintegration of the inner lining of the uterus (the **endometrium**) are discharged from the uterus, a process called **menstruation**. During this time a new **follicle** begins to develop in one of the ovaries. After menstruation ceases, the follicle continues to develop, secreting an increasing amount of **estrogen** as it does so. The rising level of estrogen causes the endometrium to become thicker and more richly supplied with blood vessels and glands. A rising level of **LH** causes the developing egg within the follicle to complete the first meiotic division (**meiosis I**), forming a **secondary oocyte**. After about two weeks, there is a sudden surge in the production of LH. This surge in LH triggers **ovulation**: the release of the secondary oocyte into the **fallopian tube**.

Under the continued influence of LH, the now-empty follicle develops into a **corpus luteum** (hence the name luteinizing hormone for LH). Stimulated by LH, the corpus luteum secretes **progesterone** which continues the preparation of the endometrium for a possible pregnancy, inhibits the contraction of the uterus and inhibits the development of a new follicle. If fertilization does **not** occur (which is usually the case), the rising level of progesterone inhibits the release of GnRH which, in turn, inhibits further production of progesterone.

As the progesterone level drops, the corpus luteum begins to degenerate; the endometrium begins to break down, its cells committing programmed cell death (**apoptosis**); the inhibition of uterine contraction is lifted, and the bleeding and cramps of menstruation begin.

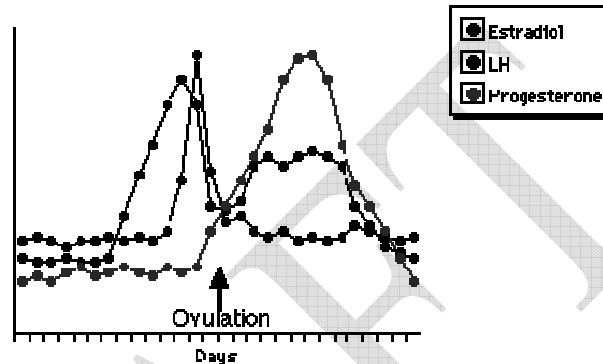


Fig.9.2. The Menstrual Cycle

### 9.1.6. Pregnancy

Fertilization of the egg takes place within the **fallopian tube**. As the fertilized egg passes down the tube, it undergoes its first **mitotic divisions**. By the end of the week, the developing embryo has become a hollow ball of cells called a **blastocyst**. At this time, the blastocyst reaches the uterus and embeds itself in the endometrium, a process called **implantation**. With implantation, pregnancy is established.

The blastocyst has two parts: the **inner cell mass**, which will become the baby, and the **trophoblast**, which will develop into the **extraembryonic membranes**, the **amnion placenta**, and **umbilical cord** and begin to secrete **human chorionic gonadotropin (HCG)**.

**HCG** is a **glycoprotein**. It is a dimer of the same alpha subunit (of 89 amino acids) used by TSH, FSH, and LH) and a unique beta subunit (of 148 amino acids). HCG behaves much like FSH and LH with one crucial exception: it is **NOT** inhibited by a rising level of progesterone. Thus HCG prevents the deterioration of the corpus luteum at the end of the fourth week and enables pregnancy to continue beyond the end of the normal menstrual cycle. Because only the implanted trophoblast makes HCG, its early appearance in the urine of pregnant women provides the basis for the most widely used test for pregnancy (which can provide a positive signal even before menstruation would have otherwise begun). As pregnancy continues, the placenta becomes a major source of **progesterone**, and its presence is essential to maintain pregnancy. Mothers at risk of giving birth too soon can be given a synthetic **progestin** to help them retain the foetus until it is full-term.

### 9.1.7. Birth

Toward the end of pregnancy, Secretion of **estrogen** by the placenta **rises**. This rise is triggered by the foetus itself: The placenta releases **CRH** which stimulates the pituitary of the foetus to secrete **ACTH**, which acts on the adrenal glands of the foetus

causing them to release the estrogen precursor **dehydroepiandrosterone sulfate (DHEA-S)**. This is converted into estrogen by the placenta.

The rising level of estrogen causes the **smooth muscle cells** of the uterus to synthesize **connexins** and form **gap junctions**. Gap junctions connect the cells electrically so that they contract together as labour begins. express receptors for **oxytocin**. **Oxytocin** is secreted by the posterior lobe of the pituitary as well as by the uterus. A number of **prostaglandins** also appear in the mother's blood as well as in the amniotic fluid. Both oxytocin and prostaglandins cause the uterus to contract and labour begins. Three or four days after the baby is born, the breasts begin to secrete milk. Milk synthesis is stimulated by the pituitary hormone **prolactin (PRL)**, and its release from the breast is stimulated by **oxytocin**. Milk contains an **inhibitory peptide**. If the breasts are not fully emptied, the peptide accumulates and inhibits milk production. This **autocrine** action thus matches supply with demand.

### **9.1.8. Other Hormones**

#### **(a) Relaxin**

As the time of birth approaches in some animals (e.g., pigs, rats) , this polypeptide has been found to: relax the pubic ligaments soften and enlarge the opening to the cervix. Relaxin is found in pregnant humans but at higher levels early in pregnancy than close to the time of birth. Relaxin promotes **angiogenesis**, and in humans it probably plays a more important role in the development of the interface between the uterus and the placenta that it does in the birth process.

#### **(b) Activins, Inhibins, Follistatin**

These proteins are synthesized within the follicle. Activins and inhibins bind to follistatin. Activins increase the action of FSH; inhibins, as their name suggests, inhibit it. How important they are in humans remains to be seen. However the important role that **activin** and follistatin play in the embryonic development of vertebrates justifies mentioning them here.

### **9.1.9. Oral Contraceptives: The "Pill"**

The feedback inhibition of GnRH secretion by estrogens and progesterone provides the basis for the most widely-used form of contraception. Dozens of different formulations of **synthetic estrogens** or **progestins** (progesterone relatives) — or both — are available. Their **inhibition of GnRH** prevents the mid-cycle surge of LH and ovulation. Hence there is no egg to be fertilized. Usually the preparation is taken for about three weeks and then stopped long enough for normal menstruation to occur.

The main side-effects of the pill stem from an increased tendency for blood clots to form (estrogen enhances clotting of the blood).

#### **9.1.10. RU-486**

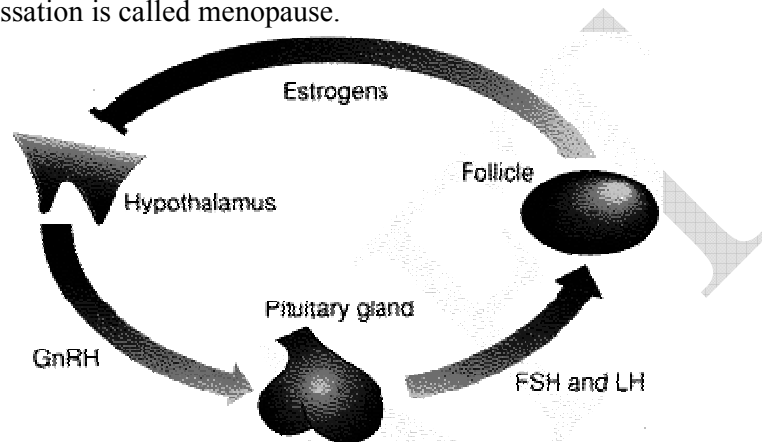
RU-486 (also known as mifepristone) is a synthetic steroid related to **progesterone**. Unlike the synthetic progestins used in oral contraceptives that mimic the actions of progesterone, RU-486 is a progesterone **antagonist**; that is, it blocks the action of progesterone. It does this by binding more tightly to the **progesterone receptor** than progesterone itself but without the normal biological effects: The RU-486/receptor complex is not active as a transcription factor. Thus genes that are turned on by progesterone are turned off by RU-486. The proteins needed to establish and maintain pregnancy are no longer synthesized. The endometrium breaks down. The embryo detaches from it and can no longer make chorionic gonadotropin (**HCG**). Consequently the corpus luteum ceases its production of progesterone. The inhibition

on uterine contraction is lifted. Soon the embryo and the breakdown products of the endometrium are expelled.

These properties of RU-486 have caused it to be used to **induce abortion** of an unwanted foetus. In practice, the physician assists the process by giving a synthetic **prostaglandin** (e.g., **misoprostol** [Cytotec®]) 36–48 hours after giving the dose of RU-486. Use of RU-486 is generally limited to the first seven weeks of pregnancy. RU-486 has been used for many years in some countries.

### 9.1.11. Menopause

The menstrual cycle continues for many years. But eventually, usually between 42 and 52 years of age, the follicles become less responsive to FSH and LH. They begin to secrete less estrogen. Ovulation and menstruation become irregular and finally cease. This cessation is called menopause.



*Fig.9.3. Hormonal Control of Menopause*

With levels of estrogen now running one-tenth or less of what they had been, the **hypothalamus** is released from their inhibitory influence (bar). As a result it now stimulates the **pituitary** to increased activity. The concentrations of FSH and LH in the blood rise to ten or more times their former values. These elevated levels may cause a variety of unpleasant physical and emotional symptoms.

### 9.1.12. Hormone Replacement Therapy (HRT)

Many menopausal women elect to take a combination of estrogen and progesterone after they cease to make their own. The benefits are: reduction in the unpleasant symptoms of the menopause a reduction in the loss of calcium from bones and thus a reduction in **osteoporosis** and the fractures that accompany it. It was also believed that HRT reduced the risk of cardiovascular disease. However, a recent study of 16,000 menopausal women was stopped 3 years early when it was found that, in fact, HRT increased (albeit only slightly) not decreased the incidence of cardiovascular disease. Perhaps synthetic **selective estrogen response modulators** or SERMs (raloxifene is an example) will provide the protective effects without the harmful ones. Stay tuned.

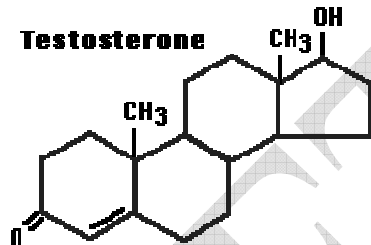
### 9.1.13. Environmental Estrogens

Some substances that find their way into the environment, such as **DDE**, a breakdown product of the once widely-used insecticide DDT, **DDT** itself (still used in some countries (e.g., Mexico), and **PCBs**, chemicals once used in a wide variety of industrial applications can bind to the estrogen (and **androgen**) receptors and mimic (weakly) the effects of the hormone. This has created anxiety that they may be responsible for harmful effects such as cancer and low sperm counts.

However, there is as yet little evidence to support these worries. No epidemiological relationship has been found between the incidence of breast cancer and the levels of these compounds in the body. As for laboratory studies that found a synergistic effect of two of these substances on receptor binding (findings that created the great alarm), these have not been replicated in other laboratories, and the authors of the original report have since withdrawn it as invalid.

## 9.2. Males

The principal **androgen** (male sex hormone) is **testosterone**. This steroid is manufactured by the interstitial (Leydig) cells of the **testes**. Secretion of testosterone increases sharply at puberty and is responsible for the development of the so-called secondary sexual characteristics (e.g., beard) of men.



Testosterone is also essential for the production of sperm. Production of testosterone is controlled by the release of **luteinizing hormone (LH)** from the anterior lobe of the pituitary gland, which is in turn controlled by the release of GnRH from the **hypothalamus**. LH is also called interstitial cell stimulating hormone (**ICSH**).

Hypothalamus → GnRH → Pituitary → LH → Testes → Testosterone

The level of testosterone is under negative-feedback control: a rising level of testosterone suppresses the release of GnRH from the hypothalamus. This is exactly parallel to the control of estrogen secretion in females.

### 9.2.1. Males Need Estrogen, Too!

In 1994, a man was described who was **homozygous** for a mutation in the gene encoding the **estrogen receptor**. A single **nonsense mutation** had converted a **codon** (CGA) for arginine early in the protein into a STOP codon (TGA). Thus no complete estrogen receptor could be synthesized. This man was extra tall, had osteoporosis and "knock-knees", but was otherwise well. His genetic defect confirms the important role that estrogen has in both sexes for normal bone development. It is not known whether this man (or any of the few other men who have been found with the same disorder) is fertile or not. But male mice whose **estrogen receptor gene** has been "**knocked out**" are sterile.

### 9.2.2. Anabolic Steroids

A number of **synthetic androgens** are used for therapeutic purposes. These drugs promote an increase in muscle size with resulting increases in strength and speed. This has made them popular with some athletes, e.g., weight lifters, cyclists, runners, swimmers, professional football players. Usually these athletes (females as well as males) take doses far greater than those used in standard therapy. Such illicit use carries dangers (besides being banned from an event because of a positive drug test): acne, a decrease in libido, and — in males — testicle size and sperm counts to name a few.

### **9.3. Genetic Abnormalities of Gonadal Function**

Many things can go wrong with sexual development in both males and females; fortunately rarely. Let's look only at a few that clearly result from the inheritance of single-gene mutations. Inherited mutations in both copies of the gene encoding the **GnRH receptor** result in failure to develop at puberty. Mutations in the gene encoding the **LH receptor** prevent normal sexual development in both sexes. Mutations in the gene encoding the **FSH receptor** block development of the gonads in both males and females. Mutations in any of the genes encoding the enzymes for synthesis and metabolism of **testosterone** interfere with normal sexual function in males. A similar spectrum of disorders in males can be caused by mutations in the genes encoding the **androgen receptor**.

### **9.4. Birth Control**

#### **9.4.1. Mechanical and/or Chemical Barriers**

##### **(a) Condom**

It is a sheath of thin, flexible material (e.g., latex) worn over the penis which is highly-effective and also protects against sexually-transmitted disease (STD) agents such as HIV, the cause of AIDs, **herpes virus**, **human papilloma virus (HPV)**, chlamydiae and *Neisseria gonorrhoeae*, the cause of gonorrhea

##### **(b) Diaphragm**

This is a rubber dome placed at the upper end of the vagina which may be used along with spermicidal chemicals

##### **(c) Cervical Cap**

This is an impermeable cap fitted over the cervix which may be left in place until menstruation

##### **(d) Spermicides**

These are chemicals, such as nonoxynol 9, that inactivate sperm. Inserted into the vagina - often incorporated in sponge - prior to intercourse.

#### **9.4.2. Hormonal Contraception**

##### **(a) Oral Contraceptives; the "Pill"**

These are many formulations combining varying amounts of a synthetic **estrogen** and a synthetic **progestin (progesterone-like steroid)**. They are taken for 3 weeks; then stopped to allow **menstruation** and are most widely-used method. They are associated with a small increased risk of cardiovascular disease

##### **(b) Skin Patch**

The Ortho Evra® patch releases hormones through the skin and lasts one week

##### **(c) Vaginal Rings**

These are small plastic ring inserted into the vagina. NuvaRing® releases both an estrogen and a progestin and lasts for 3 weeks. A progestin-only ring that blocks the menstrual cycle for 3 months

##### **(d) Injectable Preparations**

These are injections containing: both an estrogen and a progestin (Lunelle®); given once a month. The progestin only (lasts for 12 weeks)

##### **(e) The Norplant System**

These are capsules of a synthetic **progestin** are inserted under the skin (requiring a local anesthetic), prevents pregnancy for up to 5 years. If pregnancy is desired sooner,

is easily removed (again requiring a small incision and a local anesthetic) and normal fertility quickly returns.

### **(f) "Morning After" Pill**

The most popular formulation in the U.S., called **Plan B**, contains a high dose of a **progestin**. If taken within 72 hours after unprotected intercourse, the drug interferes with **ovulation** and, if ovulation has occurred, with **fertilization**. Even if fertilization should occur, Plan B may also block **implantation**. If so many days have elapsed that implantation has occurred, RU-486 may be used. **RU-486** is a synthetic steroid related to progesterone. Unlike the progestins discussed above, that mimic the action of progesterone, RU-486 blocks the action of progesterone. (Synthetic molecules that mimic the action of a natural molecule are called **agonists**; those that oppose it are **antagonists**.) RU-486 (also known as mifepristone) is a progesterone **antagonist**. It binds to the **progesterone receptor**, and in so doing prevents progesterone itself from occupying its receptor. Thus the gene transcription normally turned on by progesterone is blocked, and the proteins necessary to begin and maintain pregnancy are not synthesized. Because RU-486 is used after implantation, it is causing an early **abortion** and thus has been subjected to controversy.

### **9.4.3. Intrauterine Devices (IUD)**

For centuries camel drivers in northern Africa inserted a stone in the uterus of their female camels before starting on a long trek. This prevented the animal from becoming pregnant on the journey. The intrauterine device (IUD) used by some 2% of U.S. women accomplishes the same purpose. It must be inserted by a physician. A variety of materials (usually containing some copper) and shapes work, leading one to suspect that it is simply the presence of a foreign body within the uterus that does the job. However, some IUDs have caused such bad side effects (e.g., infections of the uterus and fallopian tubes) that only two types remain on the U.S. market. One that does is Mirena® which also releases a **progestin** and can be left in place for up to 5 years.

### **9.4.4. Natural Family Planning - Rhythm Methods**

An egg can be fertilized only during the day or so after **ovulation**. Sperm can live in the female reproductive tract for up to 6 days. So copulation that takes place more than 5 days before or 2 days after ovulation is unlikely to lead to pregnancy. Abstinence during this period is called natural family planning or the rhythm method. Its success (which is low) depends upon being able to determine accurately just when ovulation occurs. Highly-motivated women can do this by monitoring their body temperature (which rises slightly at ovulation) the amount and consistency of the mucus secreted by their uterus, and - more recently measuring the concentration of estrogen and/or progesterone in the urine (which mirrors the level in the blood). It is favoured by those who do not currently want a baby, but do not wish to use contraceptive devices.

### **9.4.5. Abortion**

This is a deliberate removal of the embryo or foetus before it is ready for birth. It is done mechanically using a suction device (during the first 3 months of pregnancy), using surgery (later in pregnancy) or chemically (using RU-486 and prostaglandins) during the first 7 weeks of pregnancy. All methods of birth control have been the subject of controversy (except for **natural family planning**). In general, the controversy over a given method is proportional to the lateness of the stage of the reproductive process. So not surprisingly, abortion is a particularly controversial

procedure, especially when it is induced in the later stages of pregnancy. Nevertheless, worldwide it is the most common method of birth control.

### 9.4.6. Sterilization

Roughly one-third of U.S. couples still in their reproductive years have chosen for one or the other to be sterilized.

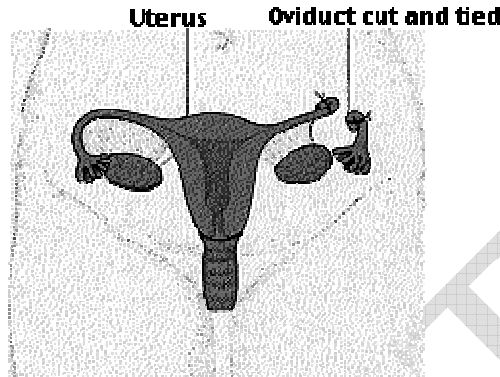


Fig.9.4. Female Sterilization

#### (a) Tubal Ligation

The fallopian tubes (both of them!) are cut and tied so that no egg can be fertilized. This requires incision(s) and so must be done under anaesthesia.

#### (b) Vasectomy

Each **vas deferens** is cut near the top of the scrotum. This can be done in the doctor's office, with a local anesthetic, in 30-40 minutes. Testosterone secretion by the testes is not inhibited and does not stop the production of the various glandular secretions that make up the bulk of the semen so copulation and ejaculation proceed normally. Sometimes the operation can be reversed, but don't count on it.

#### (c) Quinacrine Sterilization (QS)

Pellets of the antimalarial drug quinacrine are placed (by a physician) in the uterus done twice, a month apart, causes scarring of the fallopian tubes.

<i>Popularity and relative effectiveness of several methods of birth control</i>		
<b>Method</b>	<b>Popularity</b>	<b>Pregnancy Rate</b>
Natural family planning (rhythm)	2.3%	8-19
Condom	13.3%	1-10
Diaphragm	< 3.6%	6-14
Oral contraceptives ("the pill")	15.6%	1-2.5
Intrauterine devices (IUD)	0.7%	1.5-5
Sterilization	37%	0
None	24%	85-90*

\*assuming normal fertility

### 9.4.7. Future Prospects

Some research is proceeding on contraceptive **vaccines**; that is, using the immune system to block one or another step in the process (e.g. fertilization). Examples: a vaccine to raise antibodies against **gonadotropin-releasing hormone, GnRH** (for males) against **human chorionic gonadotropin, HCG** (for females) to immobilize sperm (also for females). But, what risks such antibodies might present is not at all clear.