

GENERAL EMBRYOLOGY – NOTES

Spermatogenesis

Spermatogenesis – Formation of male gametes(sperms)

Begins at puberty, process by which mature sperms are formed. From spermatogonia to sperm (Spermatogenesis) – time taken is 74 days. When fully formed, spermatozoa enter the lumen of the tubule – to epididymis

Involves two processes

- Cell multiplication including reduction division
- Cellular differentiation

At birth germ cells are found in the sex cords of the testis as large pale cells. They are surrounded by supporting cells called Sertoli Cells. Shortly before puberty, sex cords become seminiferous tubules. At the time of puberty primordial germ cells give rise to spermatogonial stem cells.

These stem cells divide to form **Type A Spermatogonia**. Type A cells undergo mitotic divisions and give rise to more Type A spermatogonia and the last cell division produces **Type B Spermatogonia** (46XY). Type B enlarge and divide to form **Primary spermatocyte** (46XY). Primary spermatocytes enter into a prolonged prophase (22 days) followed by a rapid completion of meiosis and divide into two **Secondary spermatocytes** with haploid chromosomes (23X and 23Y). The secondary spermatocytes further undergo mitotic division to form **Spermatids** (23X and 23Y). Each spermatid then transforms into a **Spermatozoon**, the process called Spermiogenesis.

SPERMIOGENESIS: (SPERMETELEOSIS)

The series of changes resulting in the **transformation** of spermatid into spermatozoa is called **Spermiogenesis**. Spermatid is a circular cell with nucleus, golgi apparatus, centriole and mitochondria. All these take part in the formation of spermatozoa.

Changes:

- 1) Condensation of the nucleus forms the **head**
 - 2) Formation of **acrosome** from Golgi apparatus (this covers half of the nuclear surface and contains enzymes which assist in penetrating the egg)
 - 3) Formation of **neck, middle piece** and **tail**
 - 4) Centrioles divide into two parts. Axial filaments grow out of them. One centriole becomes spherical and lies in the neck. The other forms the annulus.
 - 5) Part of the axial filament between neck and annulus gets surrounded by mitochondria and forms the middle piece
 - 6) Remaining part of axial filament elongates to form the principal piece or tail
 - 7) Shedding of most of the cytoplasm occurs and the Cell membrane persists as a covering
- (Structure of a mature sperm : Refer diagram in text book)**

Oogenesis

Oogenesis is a cyclical process characterized by the growth, maturation and shedding of one or more ova from the ovary. In the early embryonic period germ cells are located in the endoderm of the yolk sac. In the gonad of a genetic female they differentiate into oogonia (44+xx)(comparable to spermatogonia).

Oogonia undergo mitotic divisions. By the end of 3rd month, they are arranged in clusters surrounded by a layer of flat epithelial cells – **follicular cells** derived from surface epithelium. The number of oogonia increase and by 5th month it is maximum. By 7th month majority degenerate and the surviving ones have entered the **prophase of Meiosis 1** and they are covered by **flattened epithelial cells**. Majority of oogonia divide, some are arrested in the **diplotene stage** of prophase and form **primary oocytes**.

Near the time of birth all primary oocytes have started prophase, but instead of completing the prophase they enter into **diplotene stage**. They remain like this till **puberty**. The arrested state is produced by oocyte maturation inhibition factor. Some remain dormant in the diplotene stage for years. Chances of chromosomal abnormalities are more as age advances.

Primary oocyte surrounded by a **layer of flattened cells** is called as **primordial follicle**. Primary oocyte increases in size. Surrounding flattened follicular cells become **cuboidal** in shape. Primordial follicles are now called **primary follicles**. Cells undergo mitotic division to give rise to stratified layer of cells – Late primary follicle. Formation of **zona pellucida** occurs between oocyte and granulosa cells. As the cells increase in number, the follicle becomes oval in shape

Several irregular spaces appear filled with fluid, these join together to form a large cavity – antral cavity. Oocyte becomes eccentric in position. Now it is called the **secondary follicle**. The ovarian stroma surrounding the follicle becomes organised into a sheath. This differentiates into a highly vascular theca interna and fibrous theca externa. A fully developed secondary follicle with theca interna and externa is called as **Graafian follicle (Mature follicle)**.

(Structure of Graafian follicle: Large Antral cavity. Oocyte eccentric in position. Theca interna, theca externa. Zona pellucida, Cumulus oophorus & discus proligerus.) **(Refer Diagram in text book)**

In oogenesis the **Meiosis 1** is completed in the **graafian follicle**. Two cells of unequal size are formed. One cell – **Secondary oocyte** receives most of the cytoplasm and the second cell – **First polar body** receives practically no cytoplasm and it lies between zona pellucida and the cell membrane of the secondary oocyte.

The **Secondary oocyte** enters **Meiosis 2**, but **arrested at Metaphase** (3 hours before ovulation).

The second meiotic division is complete only after ovulation if fertilization takes place.

<u>SPERMATOGENESIS</u>	<u>OOGENESIS</u>
• Process of formation of sperms. • Starts after puberty & continues till death • Completes two meiotic divisions • Creates four equal haploid sperms • Most of the cytoplasm is shed	• Process of formation of Ova • Starts during intrauterine life, continues from puberty till menopause. • Meiosis is not a continuous process & fertilization is required to complete second meiotic division • Creates only one Ova & 3 polar bodies from one Oocyte • Cytoplasm is conserved

Ovulation:

The process by which the follicle ruptures and sets free the ovum enabling it to meet the sperm and become fertilized is called **ovulation**. At the time of ovulation, the oocyte with the surrounding cells becomes free. It occurs under the influence of L.H, follicle bursts releasing the ovum. The secondary oocyte (in the arrested metaphase state) is expelled from the ovary. The ruptured follicle after release of ovum becomes corpus luteum.

Corpus Luteum

After ovulation the wall of the follicle and the theca collapse and becomes folded. Under the influence of LH the walls develop into a **glandular structure** called **Corpus luteum**.

Cells increase in size and cytoplasm is filled with a yellowish pigment called lutein and change into lutein cells which secrete progesterone and oestrogen. These hormones prepare the endometrium for implantation. Corpus luteum become vascularised by the in growth of vessels.

Fate of corpus luteum

Corpus luteum of pregnancy : If ovum is fertilized, the corpus luteum persists for 3-4 months. It is larger. Progesterone secreted by it is essential for maintenance of pregnancy in the first few months after which the placenta takes over the function.

Corpus luteum of menstruation: If ovum is not fertilized, the corpus luteum persists for 14 days. They remain small. Then degenerates into fibrous tissue called **Corpus albicans**

Tests for ovulation:

1. Calendar method – 14 days prior to next menstrual cycle
2. Raise in BBT
3. Spinnbarkeit – Cervical mucus method
4. Hormonal estimation – LH surge
5. USG
6. Mittelschmerz

Infertility

- Male Infertility – Oligospermia, Azoospermia or poor sperm motility
Normal ejaculate – 2-6ml with 100 million sperm per ml
- Female Infertility – Occlusion of fallopian tube, Anovulatory cycles, Uterine anomalies
Hostile cervical mucus, Immunity to sperms

Fertilization

Definition : Sequence of events that begins with the contact between sperm and oocyte and ends with the fusion of its nuclei to form the zygote.

Site : Usually in the **ampulla** of uterine tube within 24 hrs after ovulation

Fertilization is divided into 3 stages:

- 1) Approximation of gametes
- 2) Contact & fusion of gametes
- 3) Effects of Fertilization

Approximation of gametes : This involves transport of Sperms and Ovum to meet in the Uterine tube.

Oocyte transport : Shortly before ovulation fimbriae sweep over the surface of the ovary. Tube begins to contract rhythmically. Oocyte surrounded by granulosa cells is carried into the tube.

Sperm transport : From the storage site in the epididymis the sperms are transported to the urethra by the contractions of the vas deferens. It is mixed with secretions of seminal vesicle, prostate and bulbourethral glands. 200- 600 million sperms pass through uterus and uterine tube mainly due to contraction of walls of the organs. Semen contains fibrinolysin which liquefies semen within 30 minutes after ejaculation. Prostaglandins in the semen help in uterine motility at the time of intercourse and assist in the maturation of sperms. Sexual intercourse stimulates release of oxytocin which also produces uterine contractions. Aspiration of sperms by vacuum generated by repeated uterine contractions. Chemotaxis – Sperm is attracted towards ovum by chemicals secreted by corona radiata. Sperms takes 2-7 hrs to reach fallopian tube from cervix. Sperm is viable for 24-48 hrs in female genital tract. Freshly ejaculated sperms has to undergo capacitation (7hrs) to fertilize the oocyte. During ascent no. of sperms decrease & 300-500 sperms reach the site of fertilization.

Viability : Human oocytes remain viable for a period ²⁴ 12 hrs after extrusion from the ovary. Most of the sperms do not survive for more than 48 hrs in the genital tract.

Contact & fusion of gametes : 3 barriers protect female gamete before penetration by fertilizing sperm

- ✓ Corona Radiata
- ✓ Zona Pellucida
- ✓ Vitelline membrane

Sequence of events sperms has to undergo

Capacitation: It is lasting for 7 hours. Glycoprotein coat and seminal proteins are removed from surface of sperm acrosome. Capacitated sperm show no structural change - become more active.

Significance of Capacitation: Capacitation of sperms is a **pre-requisite for fertilization**. It is now confirmed by experiments that the freshly ejaculated sperm are infertile and must be in the female genital tract for at least 7 hours before they can fertilize a secondary oocyte. During **in vitro fertilization**, the capacitation of sperms is induced artificially by treating the ejaculate with a solution consisting of g globulin, free serum, follicular fluid, dextran serum dialysate, and adrenal gland extract.

Acrosome Reaction: Only capacitated sperms undergo this reaction. Angiotensin converting enzyme (ACE) present in acrosome of sperm involved. Capacitated sperm come in contact with corona radiata and changes undergo - perforation of the acrosome occurs. Multiple points of fusion between plasma membrane and acrosomal membrane and breakdown of membrane at these sites forms Apertures. Enzymes like hyaluronidase and acrosin are released.

Passage of sperm through the corona radiata (**1st Barrier**) caused by hyaluronidase and also by the movement of the tail.

Penetration of Zona Pellucida (**2nd Barrier**) is helped by the enzymes esterase, acrosin and neuraminidase. Sperm head binds with Zp2 & Zp3 receptor proteins. Once penetration occurs there is a biochemical **zona reaction** that alters the permeability of zona that causes the egg to become impermeable to other sperms, (Hydrolyses Zp3).

Disappearance of plasma membrane of acrosome. Spermatozoon touches vitelline membrane (**3rd barrier**) the two plasma membranes fuse. Entry of head and neck of sperm into cytoplasm of ovum.

Calcium Wave: Sperm fusion with vitelline membrane induces a calcium wave & depolarizes vitelline membrane. It is responsible for:

- a) 2nd meiotic division of secondary oocyte
- b) Zona reaction
- c) Alters vitelline membrane - Vitelline block - prevents Polyspermy

Effects of Fertilization:

1. Completion of 2nd meiotic division of the oocyte and formation of the female pronucleus
2. Sperm becomes the male pronucleus
3. Breakdown of nuclear membrane
4. Male and female pronuclei fuse - Diploid no. of chromosomes restored
5. Sex of the embryo determined
6. Variation of human species through biparental inheritance

- Condensation of chromosomes & arrangement of chromosomes for mitotic cell division
8. Initiation of cleavage. This results in the formation of two daughter cells and this division is called as first Cleavage division
 9. One sperm enters egg and nuclei fuse, producing a zygote

Assisted Reproduction Technology

Process of fertilizing ovum outside the body (Test tube baby)

Indications:

- Blockage of fallopian tubes
- Problem with ovulation
- Endometriosis
- Polycystic ovarian syndrome
- Cervical problems

Methods:

Counselling for reproduction

Fertility medications

IVF – In Vitro fertilization

Intracytoplasmic Sperm Injection (ICSI)

GIFT – Gamete intrafallopian transfer

ZIFT – Zygote intrafallopian transfer

Surgical Approach – In obstruction of Fallopian tube or Vas deferens

In vitro fertilization (IVF):

IVF is now a common procedure used in various laboratories to help couples for whom normal pregnancy is not possible due to tubal occlusion.

The following techniques are used in a sequential manner for IVF and embryo transfer:

1. The follicular growth in the ovary is stimulated by administration of gonadotrophins/clomiphene citrate. (Ovulation inducing agents)
2. Several mature oocytes are collected by needle aspiration from ovarian follicles by laparoscopy or with the assistance of ultrasound visualization
3. The ejaculate is collected by masturbation, and the sperms are separated from the seminal fluid and capacitated artificially by exposure to ionic solution.
4. The oocytes are placed in the culture medium and capacitated sperms are added immediately (i.e., oocytes and sperms are cultured together)
5. The cleavage is allowed to proceed in vitro till the 8- cell stage of the embryo.
6. At least three 8-cell embryos are transferred in to the uterus to develop to full term fetus because of the low success rate of implantation. The remaining embryos are frozen for further use in case the first attempt of IVF fails to cause pregnancy.

Intracytoplasmic Sperm Injection (ICSI)

Direct transfer of a single sperm into cytoplasm of oocyte.

Indications:

- Teratospermia
- Poor sperm motility
- Oligospermia
- Obstruction in passage of sperm or Vasectomy
- Anti-sperm antibodies

Artificial Insemination

- Deliberate introduction of sperm into uterus or cervix using a catheter

Gamete Intrafallopian Transfer (GIFT)

- Collected ova and sperms are transferred into the fallopian tube using laparoscope

Zygote Intrafallopian Transfer (ZIFT)

- In vitro fertilized ova is transferred to fallopian tube using laparoscope

Surrogate mothers: Some have normal ovaries and produce mature oocytes but are unable to become pregnant due to agenesis of the uterus or hysterectomy. In such cases, IVF may be performed and the embryos are transferred to another woman's uterus (after pretreatment with progesterone) for development and delivery.

Methods of fertility control (contraception*) are:

Contraception refers to prevention of pregnancy

AIMS:

- Family planning
- Prevent STDs
- Prevent pregnancy in diseased females, Eg: heart disease

Methods:

• **Spacing Methods:**

- Natural methods
- Barrier methods
- Contraceptive pills
- IUCDs

• **Terminal Methods:**

- Tubectomy
- Laproscopic tubal occlusion
- Vasectomy
- No scalpel vas occlusion

In female	In males
• <u>Temporary methods</u> 1. Prevention of union of sperm and oocyte: a) By avoiding coitus during the fertile period of the menstrual cycle (10 – 17 days). The remaining days of the menstrual cycle are safe. b) By using mechanical barriers such as diaphragm/ cervical cap. c) By using spermicidal jellies/ cream (not really useful). 2. Prevention of implantation: By using intrauterine contraception devices (IUCD) such as a copper –T 3. Prevention of ovulation: By using contraceptive pills with or without suppressing normal menstrual cycle. A contraceptive pills is a combination of progesterone analogue – progestin and estrogen. 4. Medical Termination of Pregnancy (MTP): This is done only till 12 weeks of pregnancy. • <u>Permanent methods</u> Tubectomy:The fallopian tube on each side is ligated at two places and the intermediate part is removed.	• <u>Temporary methods</u> 1. Prevention of union of sperm and oocyte: By using mechanical barriers, such as condom, during coitus. 2. Prevention of production of sperm by using the contraceptive pill: A male pill has been developed and tested in clinical trials. It contains synthetic androgen that prevents both LH and FSH secretion that either stop the production of sperm (70% - 90% of men) or reduce it to the level of infertility. • <u>Permanent methods</u> Vasectomy: The vas deferens on each side is ligated at two places and the intermediate part is removed

Cleavage

Rapid succession of mitotic divisions resulting in increase in the number of cells. Presence of zona so the size of the cells decreases with each division. Cells are called blastomeres. Cells divide by mitosis and first form two cells, then followed by four cell stage, eight cell stage and then 16 cell stage in which cells are in contact (compaction) forming a compact mass – **Morula**.

4th day the morula enters the uterus and the cells are segregated into a centrally placed – Inner cell mass (Embryoblast) and an outer large cells – Trophoblast. In the uterine cavity, fluid enters through the zona forming blastocoele. Inner cell mass pushed to an eccentric position. Now it is called **Blastocyst**.

Inner cell mass is Embryonic and trophoblast is Extra-embryonic.

Zona Pellucida:

Zona pellucid is a homogenous membrane that appears between the Follicular cells and the Ovum in an ovarian follicle. The trophoblast has the property to stick, invade and erode into the epithelium or endometrium. As the zygote travels through the uterine tube the Zona pellucid prevents it from sticking to its epithelium. Zona pellucid disappears as soon as the morula reaches the uterine cavity.

Thus the **functions of zona pellucida** is to prevent implantation to abnormal sites. The glycoprotein in zona pellucid is responsible for induction of acrosome reaction. Zona pellucida allows the sperms of same species to fertilize the ovum. It is responsible for zona reaction which prevents additional spermatozoa from entering fertilized ovum. Zona pellucida holds the blastomeres of early embryo together.

Implantation

- Process by which blastocyst gets attached and embedded into the endometrium of the uterus. Implantation occurs at embryonic pole
- Site: Posterior wall of upper segment of uterus in between the openings of uterine glands
- Process starts at the end of first week (6th day) & completes by the end of second week. Zona pellucida degenerates (Day 5) and the blastocyst adheres to endometrial epithelium (Day 6)
- The Trophoblast divides into Syncytiotrophoblast & Cytotrophoblast (Day 7). Syncytiotrophoblast erodes the endometrial tissue by the proteolytic enzymes produced by it. Blastocyst starts to embed in the endometrium. Blood filled lacunae appear in syncytio-trophoblast. Blastocyst sinks beneath the endometrium and defect filled by a closing fibrin plug. This type of implantation is called **Interstitial Implantation**.

Syncytium produces Chorionic gonadotrophin (hCG) which enters lacunae and maintains the hormonal activity of Corpus luteum. This is the basis of pregnancy tests. Positive urine pregnancy test seen at the end of second week.

ABNORMAL SITES OF IMPLANTATION

Abnormal implantation can be within or outside the uterus. When implantation occurs outside the uterus, this is called Ectopic pregnancy or extra uterine pregnancy.

Different sites of **Extra uterine implantation** :

- Tubal pregnancy (95%)—Blastocyst gets implanted in the uterine tube, Ampulla, infundibulum or interstitium. Commonest site—Ampulla. It may result in rupture of uterus.
- Ovary
- Abdominal (1%)—in peritoneum or pouch of Douglas or in the mesentery.

Abnormal Intra uterine implantation:

✓ Implantation in lower segment of uterus – **Placenta previa**. Placenta previa is classified according to the placement of the placenta.

Type I or low lying: The placenta encroaches the lower segment of the uterus but does not infringe on the internal os. **Type II** or marginal: The placenta touches, but does not cover, internal os. **Type III** or partial: The placenta partially covers the internal os, but not when dilated. **Type IV** or complete: The placenta completely covers the internal os and occludes even after dilatation.

Decidua

Uterine endometrium after implantation. Under the influence of HCG secreted by syncytiotrophoblast, the endometrial stromal cells become enlarged & polyhedral, become vacuolated and are filled with glycogen and lipids. This change in the stromal cells is called **Decidual reaction**.

Three regions of deciduas: **Decidua basalis** : The part of decidua deep to the developing blastocyst where placenta is formed. This is also called decidual plate and is firmly adherent to the chorion.

Decidua capsularis: The part of decidua that separates the embryo from the uterine lumen.

Decidua parietalis: The part of decidua lining the rest of uterine cavity.

Bilaminar Embryo:

• Blastocyst completely gets implanted in the endometrium at the end of second week. The inner cell mass of the blastocyst differentiates into two types of cells. The lower, flattened cells are called the Hypoblast and the upper columnar cells, the Epiblast. Basement membrane appears between the two layers. The **Hypoblast & Epiblast** together forms the **Bilaminar germ disc**.

Epiblast get separated from trophoblast forming the Amniotic cavity. In the floor of amniotic cavity is the epiblast and in the roof is flattened amniogenic cells (Amnion). Flattened mesothelial cells appear on inner surface of cytotrophoblast at abembryonic pole which is called the **Heuser's membrane**. It is continuous with hypoblast, enclosing a cavity called the **Primary yolk sac cavity**.

Extraembryonic mesoderm: New set of cells appear between inner surface of cytotrophoblast and outer surface of exocoelomic membrane called the Extra embryonic mesoderm. Cavities appear in extra embryonic mesoderm and fuse to form large cavity, the **Extra embryonic coelom**. It separates the amniotic cavity and yolk sac cavity from the trophoblast except at caudal end of germ disc which is the future connecting stalk. Extra embryonic coelom divides

the extra embryonic mesoderm into – **Somatopleuric & Splanchnopleuric extra embryonic mesoderm.**

Yolk sac cavity: Primary yolk sac cavity is seen between the hypoblast and the Heuser's membrane lining the cytotrophoblast. With the formation of extraembryonic mesoderm and the extraembryonic coelom, the primary yolk sac undergoes reduction in size. A large portion is pinched off. The reduced sac is now called the **Secondary yolk sac**. The lining cells become cubical and the extra embryonic coelom is now called the **Chorionic cavity**.

Gastrulation

The process of formation of three germ layers is called gastrulation. It begins at the end of 1st week by the formation of Hypoblast, continues during 2nd week by the formation of Epiblast and is completed during 3rd week with the formation of Intra Embryonic Mesoderm. The **bilaminar germ disc is converted into trilaminar germ disc**. Whole process controlled by **fibroblast growth factor 8**.

The inner cell mass of the blastocyst differentiates into two types of cells. The lower, flattened cells are called the **Hypoblast** and the upper columnar cells, the **Epiblast**. Basement membrane appears between the two layers. The Hypoblast & Epiblast together form the **Bilaminar germ disc**. Endodermal cells in future anterior region become columnar forming the **Prochordal plate**. No mesoderm passes in between and this forms site of the future buccopharyngeal membrane. A similar area at caudal end of embryonic disc where ectoderm and endoderm remain in contact is called the **Cloacal membrane**.

Two processes occurring during Gastrulation: Formation of primitive streak & notochord. The circular embryonic disc first becomes oval and then converted to pear shaped.

Primitive streak

A vaguely defined elevation appears on the caudal edge of embryonic disc on 14th – 15th day. Elevation has a narrow median groove with slightly raised margins. On the cephalic end, a thickening or an elevation appears called **Hensen's node**. Centre of Hensen's node shows a pit – **Primitive pit** and an opening – **Primitive pore**.

Primitive streak establishes the craniocaudal axis of the embryo and also the bilateral symmetry, dorsal and ventral aspects. Primitive streak is a midline proliferation of epiblast cells which later break free and start migrating to the interior. Epiblast undergoes rapid division. Cells migrate through the streak and occupy the space between the epiblast and hypoblast which forms the 3rd layer called the **Intraembryonic mesoderm**. Intraembryonic mesoderm does not extend into the region of prochordal plate and cloacal membrane. Hereafter epiblast is called ectoderm and hypoblast – **endoderm**.

Fate of primitive streak Normal cells undergo degeneration and disappears by the end of 4th week. It may persist and produce tumours - **Sacrocoecygealteratoma**. Since it is derived from pluripotent cells these tumours contain elements of 3 germ layers

Notochord

Notochord is a cellular rod that develops by transformation of the notochordal process. It defines the primordial axis and forms the basis for development of axial skeleton. (It indicates the future site of vertebral bodies) (IT DOES NOT FORM THE VERTEBRAE)

Development of notochord: Cellular rod of cells move forward from primitive pit of the primitive streak, to the prochordal plate between the ectoderm and endoderm. This is called the **Notochordal process**. Primitive pit extends into the notochordal process as the Notochordal canal. Notochordal process fuses and intermingles with the endoderm and openings appear in the floor of notochordal canal which communicates with yolk sac. Temporarily, amniotic cavity communicates with yolk sac cavity. The openings join and the floor disappears. Notochordal process broadens to form the Notochordal plate in the roof of yolk sac. Notochordal plate infolds and separates from endoderm to form definitive notochord. The endoderm is repaired. By the end of 4th week, Notochord is in its final position. **Notochord functions as primary inducer which induces the formation of neural plate**. Later most of the notochord disappears.

Remnants of Notochord:

- Nucleus pulposus
- Apical ligament of dens

Neural tube

Notochord induces overlying ectoderm to form neural plate. It extends from primitive knot to prochordal plate. Ectoderm lying over the notochord thickens to form Neural plate.

Neurulation is the process of formation of neural plate, neural fold, and closure of the fold to form neural tube. Embryo during this period - Neurula.

Neural plate forms a gutter called the **Neural groove** by 18th day. Laterally the groove is bounded by elevations called the **Neural folds**. Tips of neural folds are lined by special type of neuroectodermal cells called the **Neural crest**. The neural folds gradually meet and fuse to form **Neural tube**. Dorsal surface of closed Neural tube detaches from the surface ectoderm. Fusion occurs first at cervical region and then extends cranially and caudally. Later two openings appear at the cranial and caudal ends - Anterior neuropore and Posterior neuropore.

Fusion of neural plate occurs by the end of 3rd week. Anterior neuropore closes by 25th day and the Posterior neuropore closes by the 27th day. The enlarged cranial end of neural tube gives rise to brain and caudal tubular part to spinal cord.

Neural Tube Defects :Result from a failure of closure of the cranial and/or caudal neuropores during the 4th week of development. Failure of closure of cranial neuropore –**Anencephaly** it is a failure to form the cranial vault and the cerebral hemisphere. Failure of closure of the caudal neuropore –**Spina bifida**.

Neural Crest

Tips of neural folds are lined by special type of neuroectodermal cells called the **Neural crest**. Some neuroectodermal cells lose their epithelial nature forming the Neural crest cells. Neural crest cells migrate ventrolaterally on each side of the neural tube. Cells form an irregular flattened mass – Neural crest. It lies between surface ectoderm and neural tube. They separate into right and left parts and migrate to dorsolateral aspects of neural tube.

Derivatives of neural crest:

- Dorsal root ganglion cells
- Cells of sensory ganglia of cranial nerves V, VII, VIII, IX, X
- Autonomic ganglion cells
- Adrenal medulla
- Schwann cells
- Pigment cells (Melanoblasts) of skin
- Pia and Arachnoid mater (Leptomeninges)
- Several skeletal, connective tissue and muscular components in the head
- Odontoblasts
- C-cells of thyroid gland
- Ganglion cells of GIT
- Chromaffin cells

Derivatives of ectoderm:(Include derivatives of neural crest also)

- Central nervous system & Peripheral nervous system
- Retina, Lens & musculature of iris
- Epithelium of cornea, conjunctiva, ciliary body and iris
- Epidermis of skin and its appendages
- Lower part of anal canal and terminal part of male urethra
- Mucous membrane of lips, cheeks, gums, floor of mouth, part of palate, nasal cavities & paranasal sinuses
- Outer layer of tympanic membrane and epithelium of external acoustic meatus
- Sweat & sebaceous glands, Parotid glands, lacrimal glands, Mammary gland & Pituitary gland
- Enamel of teeth

Intra embryonic mesoderm (Mention about gastrulation)

Formed by proliferation of cells from primitive streak. It separates ectoderm and endoderm except at the buccopharyngeal membrane, cloacal membrane and midline caudal to prochordal plate where it is occupied by notochord.

Divides into – **Paraxial mesoderm**

Intermediate mesoderm

Lateral plate mesoderm

Paraxial mesoderm – Mesoderm on either side of notochord. At first it is homogeneously arranged and later in the 3rd week of development Paraxial mesoderm begins to organize into segments called the **Somitomeres** and **Somites**.

Somitomeres lie in the region of head and form bones and muscles of head and jaw.

Somites first appear in cephalic region and proceed cephalocaudally. 1st pair of somites are seen in the occipital region – 20th day. By the end of 5th week there are 42-44 pairs of somites. Occipital – 4, Cervical – 8, Thoracic – 12, Lumbar – 5, Sacral – 5, Coccygeal – 7. Later 1st occipital & last 5-7 coccygeal disappear. Somites form the axial skeleton.

Fate of Somites: Somites get divided into **Sclerotome** (Ventromedial part). Cells migrate medially and surround the neural tube. It forms cartilage & bones (vertebral column & ribs)

Myotome (Intermediate part). Intermediate part. Gives rise to Segmental muscles **Dermatome** (Lateral part). The cells migrate and come to line the deep surface of ectoderm covering the entire body. These cells give rise to dermis of skin and subcutaneous tissue.

Intermediate mesoderm: Temporarily connects paraxial mesoderm with lateral plate mesoderm. Differentiates into urogenital structures except part of bladder, prostate & urethra.

Lateral plate mesoderm: Lateral plate mesoderm splits into Somatopleuric mesoderm and Splanchnopleuric mesoderm by the formation of intra embryonic coelom. Intra embryonic coelom is a horse shoe shaped cavity. The two halves joined cranial to prochordal plate. First it is a closed cavity but later it communicates with extra-embryonic coelom and gives rise to body cavities.

Somatopleuric or parietal intra-embryonic mesoderm is in contact with ectoderm

Splanchnopleuric or visceral intra-embryonic mesoderm is in contact with endoderm.

Cranial to cardiogenic area both layers are continuous and this area is the Septum transversum

Derivatives of intraembryonic mesoderm:

- Connective tissue proper, Cartilage and Bone
- Teeth – except enamel
- Muscles – except iris and branchial arch
- Cardiovascular and lymphatic system
- Urogenital system with the exception of part of bladder, prostate and urethra
- Serous membranes lining body cavities
- Substance of cornea, sclera, choroid, ciliary body and iris
- Spleen & Cortex of suprarenal gland

Endoderm

A embryonic disc grows, the anterior and posterior ends project into amniotic cavity. Head fold and tail fold and lateral folds are formed and also the umbilicus. Flat trilaminar disc is converted into cylindrical embryo. The folding in median plane – Head and tail folds and Folding in horizontal plane – Lateral folds.

As the head & tail folds are formed the yolk sac is enclosed within the embryo. The tube lined by endoderm is called the Primitive gut. Gut communicates with the yolk sac. Part cranial to communication – Foregut, Part caudal to communication – Hindgut and intervening part – Midgut. Communication with yolk sac becomes narrower and forms the definitive yolk sac. The narrow channel connecting the gut with yolk sac is called the Vitello-intestinal duct. By the formation of lateral folding the whole embryo gets enclosed by ectoderm except at vitello-intestinal duct which forms a circular aperture, the Umbilical opening.

Endodermal derivatives:

- Epithelium of glands like thyroid, parathyroid, thymus, liver, pancreas
- Epithelial lining of alimentary tract, respiratory tract, auditory tube, middle ear, part of urinary bladder, part of ureter, most of urethra and greater part of vagina

Development of Placenta

Placenta develops partly from embryonic structures, the **Chorion frondosum** and partly from **Decidua Basalis**. The essential element of placenta is the Chorionic villi.

Formation of chorionic villi: The Trophoblast differentiates into outer **syncytiotrophoblast** and inner **cytotrophoblast**. It is then lined with **extra embryonic mesoderm**. Extra embryonic mesoderm is divided into somatopleuric and splanchnopleuric mesoderm by **chorionic cavity**. The somatopleuric extra-embryonic mesoderm and overlying trophoblast is called the **Chorion**.

First chorionic villus appears by end of 2nd week. Villi are the essential structure of early placenta. They are formed as off shoots from the surface of trophoblasts. Syncytium erodes into the blood vessels and glands of endometrium. Small spaces appear in the syncytium which get filled with blood and secretions of glands (lacunae). This is called the **Lacunar stage** (9th day) of development of placenta. The Blastocyst sinks beneath the endometrium completely by the 10th day. This marks the beginning of Uteroplacental circulation. **Lacunae** are radially arranged separated by syncytium. The syncytium between the lacunae is called the **Trabeculus**.

Primary villus: Cells of cytotrophoblast grow into the trabeculae. A trabeculus with central core of cytotrophoblast & outer syncytium is called Primary villus. Now the lacunar spaces are known as the **intervillous space**.

Next the mesoderm grows into the primary villus, and it becomes a **secondary villus**.

Mesoderm differentiates into blood vessels in the 3rd week. When blood vessels appear in secondary villus, it becomes a **tertiary villus** or **definitive villus**.

The cytotrophoblast initially growing into the villus does not penetrate the entire length of syncytiotrophoblast. But at a later stage it penetrates and spread out to form an entire layer separating the syncytium from decidua basalis. This is called the **cyto trophoblastic shell**. Foetal blood circulates through villus and Maternal blood through intervillous space. **Anchoring villus** :

The first formed villus extends from chorionic plate to cytotrophoblastic shell. Each anchoring villus has a stem called **Truncus chorii**. **Free villus:** Villi that branch from the stem is called **Rami chorii** and which further branch from them are called **Ramulichorii**.

During early development of placenta, villi are formed all over the chorion. By 3rd month villi degenerate at abembryonic pole and leaves a smooth chorion called **Chorion leave**.

Villi over embryonic pole which grow and gives rise to placenta is called **Chorion frondosum**. Full term placenta is discoid in shape with a diameter of 15 to 20cm (6 – 8 inches), weighing 500–600 gms. It is expelled 30 minutes after birth of child. A mature placenta shows cotyledons on maternal side and smooth chorionic plate on the foetal side.

On the foetal side placenta bordered by chorionic plate (Extraembryonic mesoderm surrounding the trophoblast) and on the maternal side by decidua (decidua basalis).

Placental circulation: Maternal blood in inter villous space and Foetal blood in villi. They do not mix. They are separated by **placental membrane** which is formed by endothelium & Basement membrane, Mesoderm, Cytotrophoblast & Basement membrane and Syncytiotrophoblast. Later stages efficiency of placental membrane is increased by disappearance of cytotrophoblast. Placental membrane is **Haemochorial**. Most hormones do not cross the placental membrane. Viruses such as rubella, cytomegalo virus, coxsackie, variola, varicella, measles, poliomyelitis traverse without difficulty.

Functions of placenta:

- Metabolism
- Transport of oxygen, water, electrolytes and nutrients
- Excretion of CO₂, urea and other wastes
- Maternal antibodies reach fetus – Immunity
- Placental barrier prevents harmful substances from reaching fetus
- Endocrine secretion: Progesterone (for maintenance of pregnancy), Oestrogens (Oestriol) (promotes uterine growth and breast development), Human chorionic gonadotropins (hCG) & Somatomammotropin (hCS)

Annomalies of Placenta:

1. Placenta Previa – Abnormal lower uterine segment implantation
 2. Placenta accreta – Chorionic villi penetrate full thickness of myometrium
 3. Placenta percreta – Chorionic villi reaches perimetrium. Third trimester bleeding.
4. Types of placenta –

- Bilobed or Bidiscoidal
- Lobed (divided into lobes)
- Diffuse (chorionic villi all over)
- Placenta succenturiata (a small part separated)
- Fenestrated (hole)
- Circumvallate (peripheral edge covered by fold of decidua)

Abnormal attachment of umbilical cord

- Marginal – Cord attached to the margin of placenta (Battledore placenta)
- Furcate – Blood vessels divide before reaching placenta
- Velamentous insertion – Blood vessels attached to amnion.

Foetal membranes

Any tissue developed from zygote, that does not form part of embryo proper are called **Foetal Membranes**. Foetal membranes develop from the zygote but do not participate in the formation of foetus except for the parts of yolk sac and allantois. Yolk sac takes part in formation of primordium of gut and Allantois remains as Urachus.

They are:

1. Chorion: The Trophoblast differentiates into outer syncytiotrophoblast and inner cytotrophoblast. It is then lined with extra embryonic mesoderm. Extra embryonic mesoderm is divided into somatopleuric and splanchnopleuric mesoderm by extraembryonic coelom or chorionic cavity. The somatopleuric extra-embryonic mesoderm and overlying trophoblast is called the **Chorion**. The chorionic cavity becomes larger and by

19th or 20th day The embryo is attached to the trophoblast by the connecting stalk which later develops into umbilical cord. The extra embryonic mesoderm lining the inside of cytotrophoblast is now known as **chorionic plate**. During early development placental villi are formed all over the chorion. By 3rd month villi degenerate at abembryonic pole and leaves a smooth chorion called **Chorion leave**. Villi over embryonic pole which grow and together with decidua basalis, gives rise to placenta is called **Chorion frondosum (Describe chorionic villi formation – Refer placenta)**. Amniotic sac enlarges faster than chorionic sac. The amnion and smooth chorion fuse to form amniochorionic membrane. This fuses with decidua capsularis and later with decidua parietalis. It is this membrane which ruptures during labour.

2. Umbilical cord/Connecting stalk: Trophoblast attached to the embryo by the undivided part of extra embryonic mesoderm forms the connecting stalk. This undivided mesoderm forms the connecting stalk. As the embryo grows the area of attachment becomes smaller and with the formation of tail fold, the area of attachment comes to the ventral aspect at the region of umbilical opening where the line of reflection of amnion and ectoderm is seen which is the primitive umbilical ring. Blood vessels develop in the embryo and placenta which communicate through the connecting stalk. There are two arteries and two veins, but later right vein disappears.

At about 5th week the following structures passing through umbilical opening:

- Vitello-intestinal duct & remnants of yolk sac
- Mesoderm of connecting stalk (Wharton's jelly)
- Blood vessels in Umbilical cord
- Allantois
- Canal connecting extra and intra embryonic cavities

Umbilical cord: Amniotic cavity now enlarges at the expense of chorionic cavity and the amnion begins to envelop connecting stalk and yolk sac crowding them together and give rise to primitive umbilical cord. Physiological hernia is withdrawn and cavity in the cord is obliterated. The allantois and the vitelline duct with the vessels are obliterated. Only the umbilical vessels remain in the cord surrounded by jelly of warton. Which is a protective layer for blood vessels. The walls of the arteries are muscular and contains elastic fibers also, which helps in the constriction and contraction of the vessels when the cord is cut. Umbilical cord increases in length to allow for the free movement of the embryo in the amniotic cavity. Length at the time of birth – 50cms and diameter – 2 cms. Short or long cord can cause problems at the time of delivery

Abnormalities of umbilical cord:

False and true knots can appear in the cord.

Normally attached to centre of placenta. Anomalies can be due to marginal attachment, velamentous, furcated. (Refer placenta)

3. **Allantois:** When the cloacal membrane is formed, the posterior wall of the yolk sac forms a diverticulum that extends to the connecting stalk. It appears around 16th day of development. After the tail fold formation part of this diverticulum is absorbed into the hind gut. Vesicourethral canal is connected to umbilicus through allantois. Allantois is small in human beings. It is involved in early blood formation and in the development of bladder. Intraembryonic part of allantois runs from umbilicus to urinary bladder. Later this degenerates and involutes into **urachus** and after birth becomes **Median umbilical ligament**.

4. **Amnion:** Epiblast get separated from trophoblast forming the Amniotic cavity. In the floor of amniotic cavity is the epiblast and in the roof is flattened **amniogenic cells (Amnion)**. The thin but tough amnion is a foetal membrane that forms the amniotic sac. As it enlarges, the chorionic cavity is obliterated. Later the amnio-chorionic membrane fuses with decidua capsularis & later with decidua parietalis. Amniotic cavity is filled with a watery fluid amniotic fluid, produced by amniotic cells and from maternal blood.

Amniotic fluid: Cavity is filled with a watery fluid, produced by amniotic cells and from maternal blood called amniotic fluid. At 10 weeks it is 30ml, 20 weeks – 450 ml, 37 weeks – 800 to 1000 ml and at Full term – 1500-2000ml. Functions of amniotic fluid are that it serves as a protective cushion, absorbs jolts, prevents adherence of embryo to amnion, allows foetal movements, permits symmetrical external growth of fetus, acts as barrier to infection and helps to control the embryo's body temp.

From 5th month foetus drinks its own amniotic fluid. Foetal urine is added from 5th month.

During childbirth chorio-amniotic membrane breaks.

Composition of Amniotic fluid: 98-99% is water, fetal epithelial cells, organic constituents are proteins, carbohydrates, fats, enzymes, hormones and pigments and Inorganic salts.

Applied aspects:

Oligohydramnios: Low volumes of fluid (400 ml). Can be due to Placental insufficiency, Reduced placental blood flow or Renal agenesis.

Hydramnios: Too much fluid (>2 lt). Idiopathic causes, Maternal diabetes, CNS malformations like Anencephaly & Hydrocephalus and Gastrointestinal defects (Duodenal atresia)

Amniocentesis: Study of Amniotic fluid. Amniotic fluid is taken by introducing a needle into amniotic cavity. Preferably done – 16th week. Concentration of alpha-feto protein is increased in neural tube defects.

5. **Yolk sac (Umbilical Vesicle):** Primary yolk sac cavity is seen between the hypoblast and the Heuser's membrane lining the cytotrophoblast. With the formation of extraembryonic mesoderm and the extraembryonic coelom, the primary yolk sac undergoes reduction in size. A large portion is pinched off. The reduced sac is now called the **Secondary yolk sac**. 32 days – yolk sac is large. With the formation of head and tail folds, part of yolk sac become enclosed within the embryo. The part of the primitive gut communicating with the yolk sac is the

midgut The communication gradually becomes narrow. The yolk sac becomes very small and is called the definitive yolk sac. By 10 weeks it is a pear shaped remnant 5mm in diameter, connected to midgut by vitello-intestinal duct or the yolk stalk. At 20 weeks it is very small and then invisible. The Yolk stalk usually detaches from midgut loop. In 2% of adults proximal intra-abdominal part persists as **Meckels diverticulum**.*

Multiple pregnancy:

When a mother gives birth to 2 infants at the same time they are called twins. Three infants – triplets, Four – quadruplets and Five – quintuplets. Twins can be Monozygotic and Dizygotic.

Twinning:

Dizygotic twins: (Fraternal Twins) Two ova may be shed simultaneously and each of them fertilized separately. They have independent genetic constitutions. So may be of same or different sex. Do not resemble each other. They have separate chorionic and amniotic cavities

Monozygotic twins: Develop from a single fertilized ovum. They are called Identical twins. Can result from splitting of zygote at various stages.

The cells formed in the first few divisions can separate and form individual fetuses. They will have separate chorionic and amniotic sacs (Dichorionic, Diamniotic).

Next type, when the blastocyst is formed, two inner cell masses develop in it and each develop into a complete fetus. Both share a common chorionic cavity (Diamniotic, Monochorionic).

Lastly, the inner cell mass may split into two. Two embryonic discs are formed within it. They share a common chorionic as well as amniotic cavity (Monochorionic, Monoamniotic).

Incomplete separation of monozygotic twins results in birth of two infants joined in some part of the body. They are called **Conjoined twins or Siamese twins**.

- Pygopagus - back-to-back, joined at the rump
- Ischiopagus - joined sacrum to sacrum
- Cephalopagus - connected at the head
- Dipygus - 1 upper body with 2 lower bodies
- Thoracopagus - joined at the chest
- Omphalopagus - joined at the chest
- Rachipagus - back-to-back, joined along the spine above the sacrum

Parasitic twinning is when one conjoined twin is much smaller and perhaps not as fully formed or developed as the larger twin.

Another even more rare form of parasitic conjoined twinning exists. **Fetus in fetu** is when a malformed twin is discovered inside the body of a host twin

Prenatal diagnosis:

Process undertaken to diagnose genetic abnormalities and structural anomalies of early embryo and fetus in order to undertake timely prenatal counseling and appropriate interventions.

Indications: 1. Advanced maternal age, 2. Previous child with chromosomal abnormalities, 3. Women who are pregnant with twins, 4. Family h/o single gene disorder, 5. Family h/o neural tube defect, 6. Any abnormalities identified during pregnancy / Women who had miscarriages
8. Any other risk factors (consanguinity)

Methods are:

Invasive – 1. Amniocentesis, 2. Cordocentesis, 3. Chorionic villous sampling, 4. Biopsy from foetal tissue,

Non-invasive – 1. USG, 2. MRI, 3. Triple test

Amniocentesis: Amniocentesis is a procedure in which amniotic fluid is removed from the uterus for testing or treatment. Performed in 2nd trimester. Indications: 1. Genetic testing, 2. Fetal lung testing, 3. Diagnosis of fetal infection, 4. Treatment purpose, 5. Paternity testing

Chorionic villous sampling: CVS utilizes either a catheter or needle to biopsy placental cells that are derived from the same fertilized egg as the fetus