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Developmental Genetics and Preimplantation Genetic Diagnosis

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Additional information is available at the end of the chapter

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Abstract

The genetic code information programs the embryogenesis. In this chapter the three phases of prenatal development the pre-embryonic, the embryonic and the fetal are elaborated. The role played by transforming growth factor beta related genes in the growth and differentiation of early embryo will be described. The implications of Fibroblast growth factor gene in binding heparin its effect on cell survival and mitogenic pathways is discussed. The orchestrated symphony of Sonic Hedgehog genes in early human embryo development is elaborated. The Homeobox genes (Hox gene) and their role in early human embryogenesis are described. The different tests available for prenatal genetic testing are briefly described. Preimplantation genetic diagnostic procedures are indispensable in clinical embryology. The microsurgical techniques of the Polar body biopsy, Blastomere biopsy and Blastocyst biopsy are discussed at the end of the chapter. A diagrammatic representation of individuals and relationships in clinical genetics is described. A brief description of procedures of invasive fetal tests for collecting the fetal tissue is also discussed.

Keywords: preimplantation genetic diagnosis, clinical genetics, developmental genetics

1. Introduction

A diploid embryo is formed by the fusion of two haploid gametes. All cells are totipotent till the embryo is 17 days. After 17 days there is specification that is followed by determination. Specification is the first stage of initial commitment which is labile the cells are adaptable. During specification if a few cells are moved to another location, they can acquire a different fate and develop according to the area to which they are shifted. Following specification the commitment is irreversible. The cells are not adaptable and changing the location of cells cannot change their fate. Congenital birth defects are often caused by errors in embryogenesis.

This is important because an embryonic injury when the cells are pluripotent may be lethal or may not have any effect at all. But when the cells cross the phase of specification and determination an injury invariably results in a structural anomaly. For example, exposure to rubella virus causes loss of cells in fetal lens and results in congenital cataract and microphthalmia.

2. Phases of prenatal life

Prenatal life can be divided into three main stages, i.e., pre-embryonic, embryonic and fetal. The pre-embryonic phase is the period during which the small collection of cells gets differentiated to form three germ layers, i.e., ectoderm, mesoderm and endoderm by the process of gastrulation. The body axes, i.e., anteroposterior, dorsoventral, left and right also established during gastrulation. The embryonic phase lasts from 5 to 8 weeks.

The pre-embryonic and embryonic phases are the times during which a single cell progresses to form the organ primordia which is the first 8 weeks of human development.

The final fetal stage after 8 weeks leads to rapid overall growth and maturation of the embryo into a viable human fetus. The integration of complex phenomena, which leads to the formation of a viable infant, has generated interest in understanding the molecular and structural aspect of this process [1].

Various comparative and evolutionary studies have been done to understand this fascinating developmental phenomenon. Many experiments were done to trace cells during their development. Transparent embryos were observed and even living cells stained with vital dyes were used to observe their fate. Later radiographic labels and autoradiography techniques were used.

Grafting experiments using quail cells into chick embryos at early stages of development were some of the pioneering efforts in monitoring cell fates that lead to origin of different organs and tissues. Other grafting experiments were grafting the primitive node from its normal position on the body axis to another site could induce formation of a second body axis.

It was also concluded that a piece of tissue from posterior axial border of one limb if grafted to the anterior border of another limb then digits on the host limb are duplicated. This posterior signaling was called zone of polarizing activity and now the signaling molecule is identified as Sonic Hedgehog. Thus the advent and progression in the field of molecular biology has led to better understanding of embryology of normal and abnormal developmental processes.

3. Regulation of gene expression

The cell fates can be mapped by identifying cells using reporter genes, fluorescent probes and other markers. The gene expression can be regulated at several levels

1. How different genes may be transcribed?
2. How DNA transcribed from a single gene may be selectively processed to regulate which RNA s will reach the cytoplasm to become mRNAs?
3. How mRNAs are selectively translated?
4. How the proteins from mRNA can be modified?

Fertilization is the process of union of male and female gametes and it occurs in the ampullary part of the uterine tube. Out of 100–200 million spermatozoa deposited in the female genital tract only a single spermatozoon penetrates through the corona radiates and zona pellucida. Sperm cell membrane fuses with the oocyte membrane.

The penetration of sperm induces cortical and zona reactions, which prevents further sperm binding and penetration. This also leads to the resumption and completion of second meiotic activation of oocyte. The results of fertilization lead to restoration of diploid number of chromosomes, determination of gender of the zygote and initiation of cleavage (**Figure 1**).

The zygote undergoes repeated mitotic divisions through the process of cleavage. As it attains an eight-cell stage it undergoes compaction and segregates into an inner cell mass and outer cell mass. Inner cell mass, which is the embryoblast, forms the embryo proper and outer cells mass forms the trophoblast. With accumulation of fluid in the embryo the embryoblast and trophoblast separate from each other and a blastocyst is formed (cyst = fluid filled cavity). The zona pellucida disintegrates and the embryo is implanted.

By Day 8 of fertilization the blastocyst gets partially embedded into the endometrial stroma (**Figure 2**). The inner cell mass differentiates into hypoblast layer and a layer of tall columnar

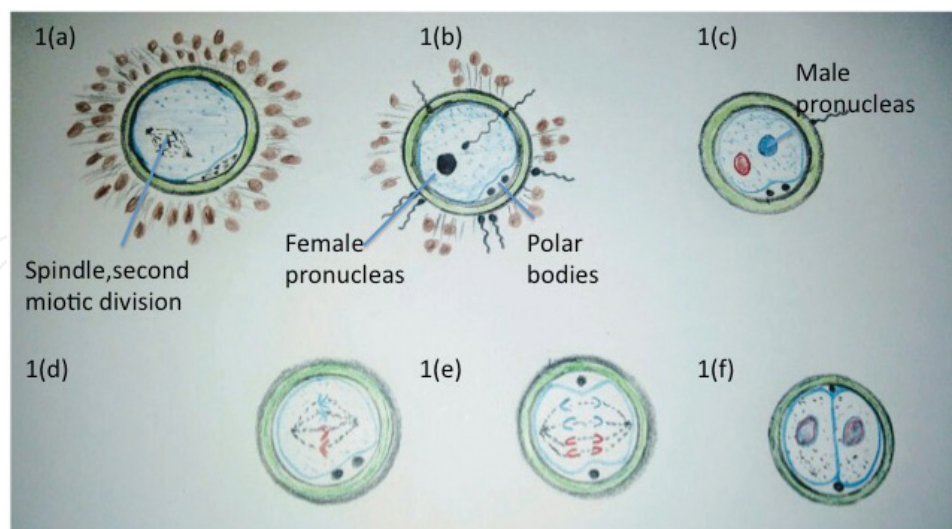


Figure 1. (a) Oocyte after ovulation. (b) Spermatozoa penetrate the oolemma. (c) Syngamy with male and female pronuclei seen. (d) Chromosomes are arranged in the equatorial plane. (e) Chromosomes are migrating towards poles. (f) Two-cell stage.

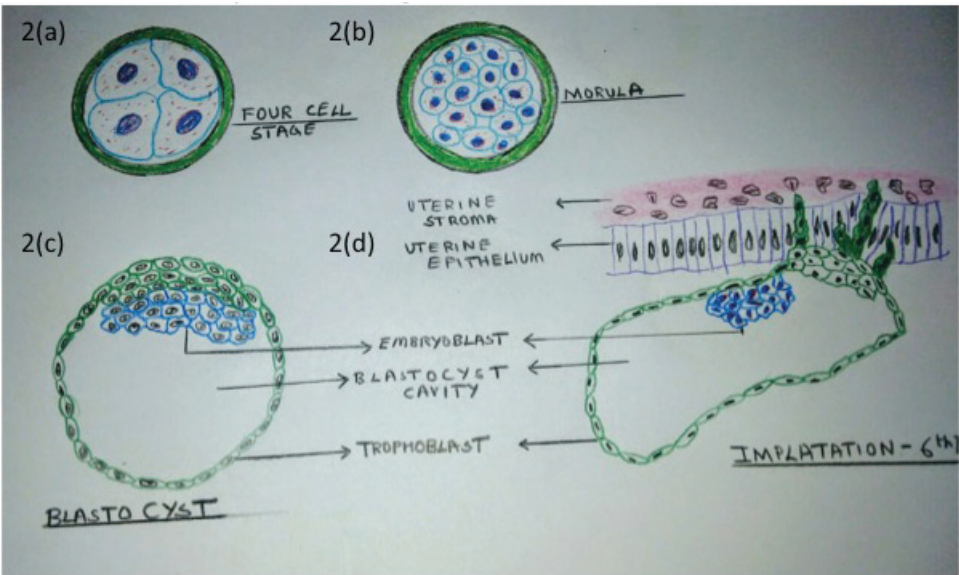


Figure 2. (a) Blastocyst stage. (b) Morula formation. (c) Blastocyst formation. (d) Blastocyst on 6th day of development showing trophoblast cells of blastocyst penetrating the uterine mucosa.

cell which is epiblast. This also leads to formation of two fluid filled cavities in the blastocyst, the primitive yolk sac and the amniotic cavity (**Figures 3 and 4**).

The most important event during the third week of gestation is gastrulation by which all three germ layers are formed, i.e., ectoderm, mesoderm, and endoderm. Gastrulation starts with the formation of primitive streak on the epiblast (**Figure 5**).

At the cephalic end of the streak a small primitive pit develops which the primitive node surrounds. The epiblast cells of the primitive streak undergo proliferation and migration by

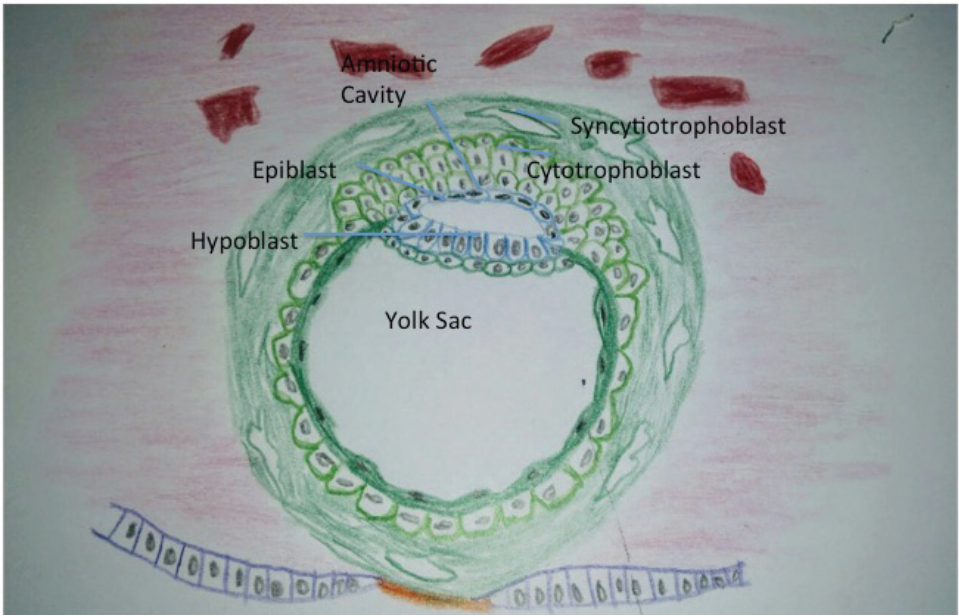


Figure 3. Blastocyst in the endometrial cavity at 9 days.

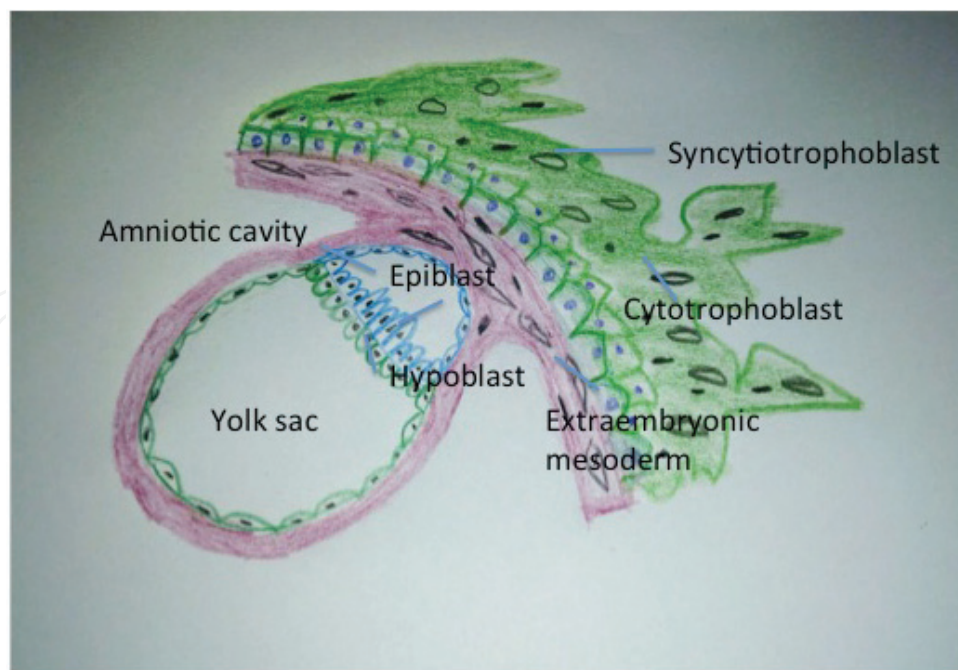


Figure 4. Bilaminar embryonic disc at 14 days in the implantation site in endometrium.

inward movement called invagination. This process is controlled by Fibroblast growth factor 8 (FGF 8) synthesized by the cells of primitive streak.

Fibroblast Growth Factor 8 down regulates E cadherin, which is the binding protein of epiblast cells thus helping in cell migration and invasion into underlying layer. Cells that are specified by regulation of Brachyury T expression form the mesoderm layer. FGF 8 also controls this expression. Some of the epiblast cells will even replace the hypoblast Layer and proliferate to form the embryonic endoderm (**Figures 6 and 7**). Cells, which continue to remain in epiblast,

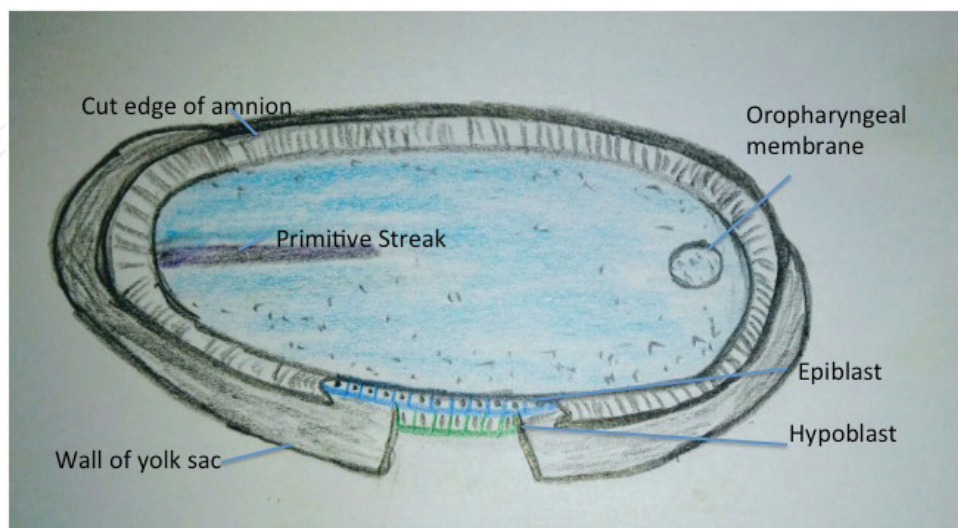


Figure 5. Dorsal view of germ disc at 14 days showing epiblast and hypoblast. A primitive node is formed at the cephalic end and a primitive streak is seen at the caudal end.

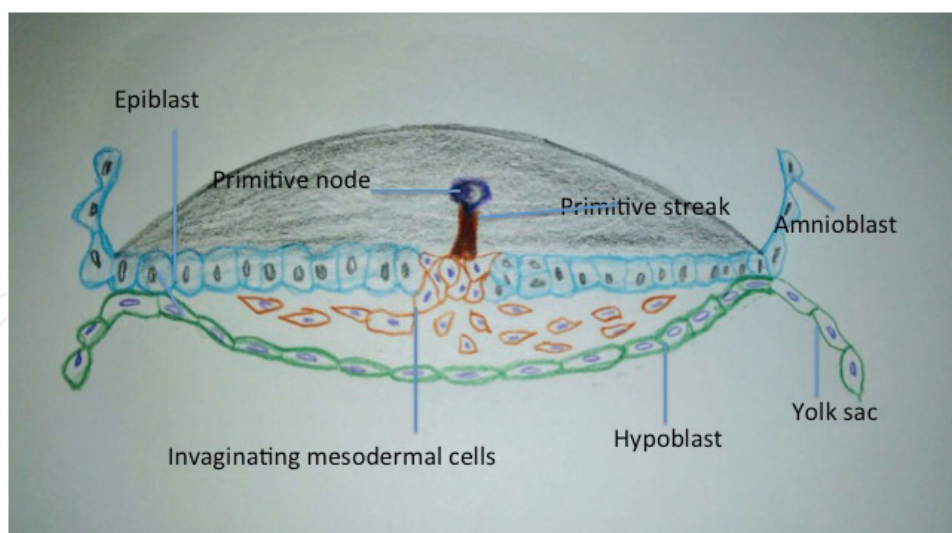


Figure 6. Cross section through the cranial region of primitive streak at 15 days showing invagination of epiblast cells.

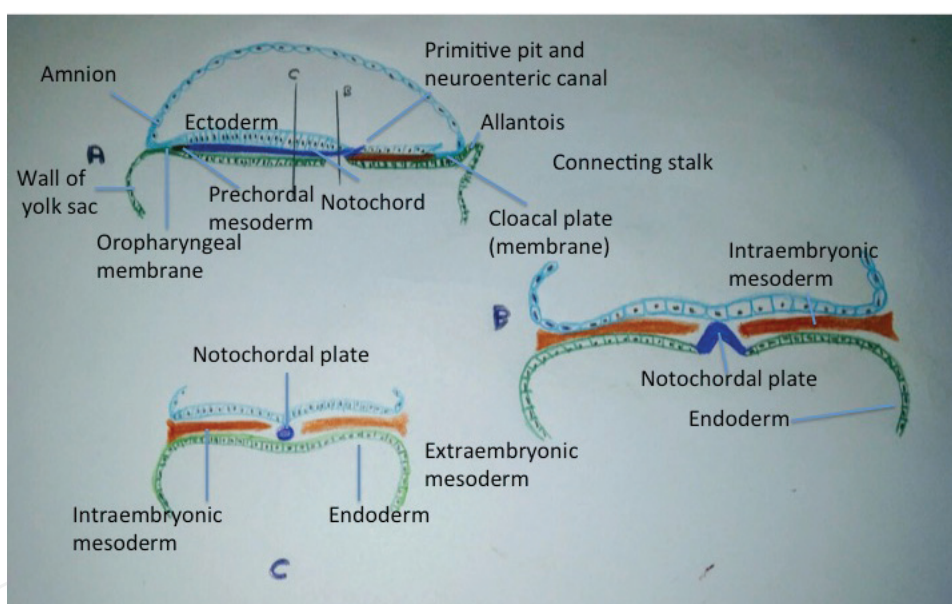


Figure 7. (a) Sagittal section of a day 17 embryo showing most cranial part of notochord. (b) Schematic cross section through the region of notochord plate. (c) Schematic view showing definitive notochord.

give rise to the embryonic ectoderm. Thus all the three germ layers are gradually formed in the epiblast and hypoblast and the process of gastrulation is complete [2].

During and before gastrulation another important event, which occurs, is the establishment of body axes—anteroposterior, dorsoventral and left—right (**Figure 8**). As already discussed the primitive streak with the primitive node at its head end determines the anteroposterior axis of the developing human embryo [3]. The anterior visceral endodermal cells at the anterior end of embryonic disc expresses genes and transcription factors like OTX2, LIM1 and HESX1 and also signaling proteins like Cerberus and Lefty which decreases the primitive node activity at head

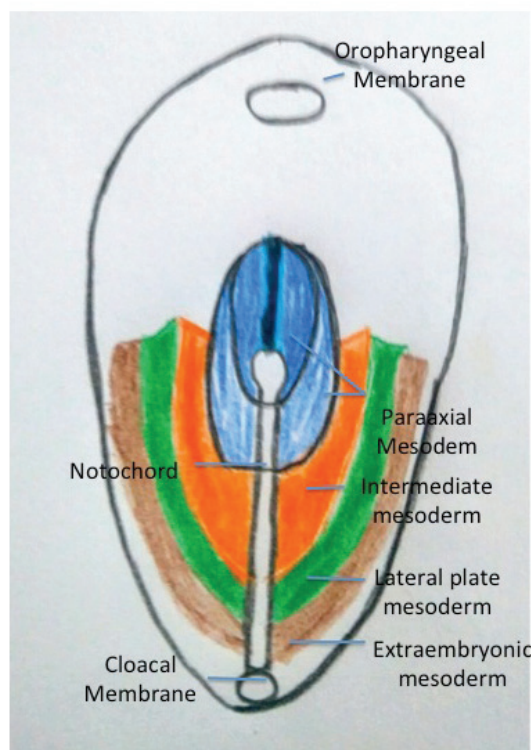
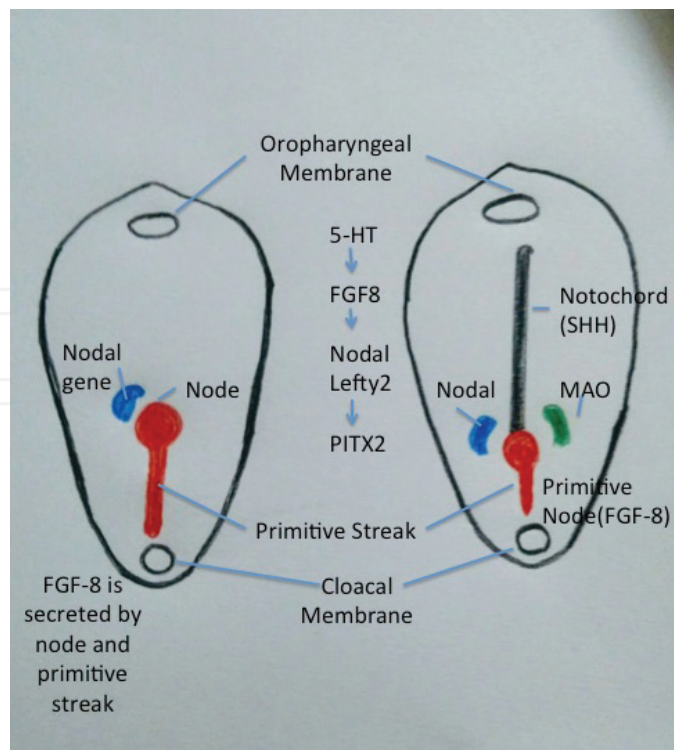


Figure 8. Dorsal view of the embryonic disc showing gene expression patterns responsible the laterality in the embryo (om: oropharyngeal membrane, cm: cloacal membrane, N: notochord, pm: paraaxial mesoderm, im: intermediate mesoderm, lpm: lateral plate mesoderm, eem: extraembryonic mesoderm). (a) FGF8 secreted by the node and primitive streak establishes expression of nodal gene which leads to TGF α family on the left side. (b) Expression of nodal and LEFTY on the left side of the ventral aspect of neural tube and this is mediated by serotonin monoamine oxidase (MAO) on right side breaks down 5-hydroxy traptamine (5HT). (c) Dorsal view of the primitive streak and fate map of epiblast cells.

end of the embryo. Nodal which is a protein of transforming growth factor beta family establishes and maintains the primitive streak.

Another important protein of Transforming growth factor beta is Bone Morphogenic Protein 4, which is secreted throughout the embryonic disc. This is important for ventralising mesoderm for kidney, blood and body wall development through the formation of lateral plate and intermediate mesoderm. The primitive node acts as an organizer and inhibits action of Bone Morphogenic Protein 4 by promoting other genes. Chordin, noggin and follistatin antagonize the action of BMP4. This results in dorsalisation of cranial mesoderm to form notochord, somites and somatomes.

Brachyury T gene regulates the dorsal mesoderm formation in middle and caudal regions of the embryo. Laterality, i.e., left-right sidedness is development by the interaction of signal molecules and genes. Genes regulating left side development are well known like LEFTY-2, Nodal that up regulates PITX2, which is a transcription factor. A neurotransmitter serotonin (5HT) also plays an important role in establishing left sidedness in the embryo. Defects of Lefty-2 and PITX2 and 5 HT can lead to defects like dextrocardia, situs inversus and cardiac abnormalities.

The cells of the epiblast, the regions of streak and node from they migrate determines the fate and the type of mesoderm they will eventually form. The cells which migrate from cranial end of node form the notochord and prochordal plate. The cells that proliferate and migrate from lateral edges of the node and cranial part of primitive streak form the paraxial mesoderm. The cells that proliferate and migrate from middle of primitive streak form the intermediate mesoderm. The cells migrating from caudal part of the streak form the lateral plate mesoderm. The cells from the caudal most part of the streak contribute to extra embryonic mesoderm. Thus the fate map of development of various organ systems is established at the time of gastrulation itself [2].

4. Genes as carriers of information

Genes are not only carriers of inherited information but also they are extremely important instructors for embryological development. In the last decade there is a tremendous progress in identifying specific genes, which are responsible for control of development in human embryo. The earlier studies were initially performed in *Drosophila* and other laboratory animals have immensely contributed in understanding the pathways and genes involved in human development.

There are genes, which are involved in development of code for proteins, which include signaling molecules and receptors, DNA transcription factors, enzymes, transport systems and components of extracellular matrix. Thus mutations and defects in all of the mentioned factors above can lead to birth defects [3].

The genetic factors which initiate, maintain and establish embryonic development is not clearly determined yet. There are some key developmental pathways, which are identified

after exhaustive experiments in genetic expression studies, but still the processes are not clear. The developmental gene families identified in vertebrates and mammals show sequence homology with developmental regulators of fruit fly *Drosophila*. The developmental genes produce proteins called transcription factors, which regulate RNA transcription from DNA template, by binding to regulatory DNA sequences to form complexes, which induces transcription by RNA polymerase. Thus gene expressions can be controlled by transcription factors thus in other words they can switch genes on and off. The variety of processes taking part during development include induction during which extracellular signals change fate of one type of cells to another, segmentation, migration, differentiation and apoptosis or programmed cell death. The protein signaling molecules identified to be involved in the above processes during development across specific belong to Transforming growth factor family, (TGF-beta), Wingless (Wnt) family and the Hedgehog (HH) family.

5. Cell signaling

Cell signaling plays a very important role in in normal embryogenesis. The receptor tyrosine kinase (RTK) signaling phosphorylates tyrosine residues and is activated by ligands and thus brings about diverse functions during development. Certain ligands, which activate RTK, are fibroblast growth factor (FGF), epidermal growth factor, TGF, Platelet derived growth factor, etc. The intracellular cascade of reactions that follows once binding of ligands activates the receptors has been studied in vitro experiments and is believed to bring about multiple changes though not fully understood [4].

The TGF-beta family of signaling molecules constitutes at least 33 members of this cytokine family. They are involved in cross talk between cells thus establish cellular communication. These extracellular signaling polypeptides are transduced to regulate other gene expression. These signaling molecules bind to the receptors on the cell surface brings about a cascade of reactions and activation of receptor kinases which leads to translocation of complexes into nucleus of cells and thus either activate or suppress the target gene expression.

The TGF-beta family can be grouped into (1) the BMPs and (2) TGF-beta s, activin, nodal, and myostatin. These groups of signaling molecules are actively involved in various cellular and developmental processes like cell cycle, cell migration, cell size, gastrulation and axis specification and various metabolic processes. BMP signaling is also involved in dendritogenesis and axonal transport.

6. Segmentation

The end of third week also marks the beginning of segmentation of the paraxial presomitic mesoderm into somatomeres proceeding rostrocaudally. By the end of fifth week there are about 42–44 pairs of somites divided into occipital, cervical, thoracic lumbar and coccygeal pairs. The Wnt, Notch and FGF signal molecules play a vital role in the formation of somites.

The notch delta pathway is responsible for the somite formation and it is expressed in oscillating pattern. The Notch accumulates in that paraxial mesoderm which is destined to form the somite and then once the somite is formed it reduces in concentration [1].

The Sonic Hedgehog -Patched Gli pathway promotes cell proliferation in the notochord, floor plate of neural tube, brain and developing limbs and gut. This Sonic Hedgehog Gli pathway malfunctioning can lead to a variety of birth defects and cancers which includes holoprosencephaly to a basal cell carcinoma, medulloblastoma, etc. The Sonic Hedgehog is first broken down to form an active N-terminal form to which a cholesterol moiety gets added which binds to the Patched 1 and Patched 2 trans membrane proteins in the target cells.

The normal action of Patched is to inhibit a transmembrane protein Smoothed but Sonic Hedgehog-Patch combination the inhibition is released. Smoothed thus activated. Further activates a cascade within the cell involving the GLI family of transcription factors, i.e., activation of GLI 1 and GLI 2 and Inhibition of GLI 3. Disruption of hedgehog signaling during development either due to mutation or teratogens can lead to developmental anomalies. Excessive activation of Hedgehog pathway does promote increase in angiogenic factors and lead to development of cancers of brain, lungs, mammary gland, prostate and skin [5].

Sonic Hedgehog functioning is essential for human development and its absence or mal expression is associated with certain specific defects as holoprosencephaly, limb defects and ventral defects of neural tube. This sonic hedgehog which is a morphogen and its signaling is dependent on its concentration and duration. The fundamental cellular processes like proliferation, survival, cell fate determination, migration, apoptosis during development are controlled by key developmental signaling pathways like Receptor tyrosine Kinase, Hedgehog, BMP, WNT, Notch, Retinoic acid signaling pathways [4].

7. Transcription factors

The transcription factors are the genes whose products will activate or suppress other genes and thus mutations in the transcription factors can lead to a variety of birth defects. These include the Homeobox (HOX) genes, Paired box Genes (PAX), T Box (TBX), SRY type HMG Box (SOX) genes, Zinc Finger Genes. The transcription factors as mentioned above leads to an orderly distribution of already differentiated cells to form specific tissues and organs by spatial arrangement and this process are called pattern formation. Studies in *Drosophila* the homeotic or HOX genes determine segment identity and faulty expression of these genes result in major structural abnormalities.

Drosophila has eight HOX genes arranged in single cluster whereas in humans there are 39 HOX genes arranged in four homeobox cluster genes on chromosomes 7p, 17q, 12q, 7q as HOX A, HOX B, HOX C, HOX D respectively numbered as 1–13. These genes regulate the cell fate and thus establish a pattern along the cephalocaudal axis and also limb bud axis and genital axis. Thus these genes play important role in development of CNS, axial skeletons, limbs gastrointestinal and urogenital systems.

The Hox genes are numbered and always expressed in cephalocaudal manner and there is a direct linear relation between the position of a particular gene and its spatial expression so playing a major role in early morphogenesis. Thus it results in a coordinated patterning of the derivatives of all the three germ layers. There are 39 HOX genes in humans but it is very astonishing that very few syndromes and malformation is attributed to HOX gene mutations. This can be explained by assuming that mutation in HOX genes can be so devastating that the embryo cannot survive. There can also be another explanation that the paralogous group of HoX genes like HOXA4, HOXB4, HOXC4 and HOXD4 though situated in different chromosome segments can compensate for loss of function or mutation in paralogous genes.

The paired box genes (PAX-genes) are a highly conserved DNA sequence and 9 PAX genes have been identified in humans. Loss of function of 5 PAX genes has been associated with developmental abnormalities in humans. PAX2 mutations causes renal coloboma syndrome in which renal malformations occur along with defects in eye. PAX 6 mutations also lead to eye defects as absence of iris. The SRY is a Y linked gene and plays a major role in male sex determination. This SRY genes show homology with the SOX genes and in humans it is seen that mutations in SOX2 have shown to cause anophthalmia or microphthalmia, esophageal atresia, and genital hypoplasia in males.

The T-box genes or TBX genes are dispersed throughout the human genome. Loss of function or mutation of TBX3 causes ulnar-mammary syndrome, which includes developmental abnormality of upper limb and mammary gland hypoplasia. Mutations in TBX-5 cause Holt Oram syndrome, which is an autosomal dominant disorder, characterized by congenital heart abnormalities and upper limb abnormalities.

The zinc finger genes are the genes, which have a zinc finger motif, and it acts as a transcription factor, which binds to DNA, and thus they can result in single gene developmental disorders. GLI3 is a zinc finger motif containing gene and large deletion or translocations involving GLI3 results in Grieg cephalopolydactyly and on the other hand frame shift mutation of the same gene result in Pallister-Hall syndrome which is characterized with polydactyl imperforate anus hypothalamic hamartomata. Mutations in WT1 another zinc finger motif gene causes Wilms tumor. Mutations in other zinc finger genes such as ZIC2 and ZIC3 results in holoprosencephaly and lateral polarity defects, which are essential for development of left-right axis.

The testis determining factor or the SRY gene, which is now determined to be located in the sex-determining region of the Y chromosome and evidence, shows that this is the primary factor, which determines maleness. Expression of SRY gene leads to activation of other genes like SOX9 which leads to the differentiation of the medulla of the undifferentiated gonad to develop into a testis in which the Leydig cells are formed which starts producing testosterone which leads to Wolffian duct stimulation and formation of male external genitalia.

The Sertoli cells on the contrary start producing the Mullerian Inhibitory hormone, which regresses the Mullerian duct. On the absence of SRY gene the medulla of the undifferentiated gonads develop into ovary the Mullerian duct forms the internal genitalia and the external

genitalia does not fuse and develop into normal female genitals. Normal sexual differentiation is completed by 14 weeks though the testis descends at a much later part of pregnancy [1].

8. Gene expression during development

Different cells in an embryo express different sets of genes at different times. A common set of genes is present in all cells at all times and is called as the “House-keeping genes.” These genes carry out the normal functions of a cell. Speciality genes are the special genes that enable a cell to carry out special functions. The genes responsible for embryonic body plan Homeobox genes, proto-oncogenes and PAX genes.

The gene expression is regulated by three sets of proteins called as the promoter, silencer and enhancer proteins. These proteins can modify the gene expression and define a particular cell type. There are also signaling proteins that facilitate the development of adjacent areas in a particular organ.

The SRY gene on the Y chromosome produces testis determining factor, which in turn activate the SOX9, and steroidogenesis factor (SF1) and it stimulates development of Sertoli and Leydig cells. WNT4 is the master gene for ovarian development. It inhibits SOX9 and up regulates DAX1. WNT4 along with other genes promotes formation of cortical cords and causes regression of medullary cords and prevents the tunica albuginea to develop [2].

The extracellular growth factors regulate cell division and differentiation by the process of signal transduction and thus mutations in genes, which produce these factors and receptors, have been implicated in cancer development and also in developmental anomalies. The Fibroblast growth factor receptors (FGR) play an important role in embryogenesis. Mutations in FGR have been associated with two groups of disorders of skeletal system, which broadly include the craniosynostosis syndromes and achondroplasias. Genes such as Fibrillin-I and Elastin code for proteins that are needed for arranging microfibrils in the matrix and thus the mutations are associated with Marfans’ syndrome [3].

9. Limb development

The process of development of limbs is well understood as the genes controlling the growth, patterning and signaling pathways for its development is well conserved from *Drosophila* to mammals. The developing limb is divided into a proximal stylopod, middle zeugopod and distal autopod. In humans the stylopod becomes the arm or thigh, the zeugopod becomes the forearm or leg and the autopod changes into the hand or foot. The limb buds appear on Day 26 for upper limb and 1 or 2 days later for lower limb. The limb bud to begin with consists of a mesenchyme core as an extension from the parietal layer of lateral plate mesoderm which leads to the formation of bones and connective tissues and it is covered with a layer of cubical ectoderm. The positioning of the limb buds depend on the correct functioning of HOX genes, which control the expression of FGF8, and the latter determines the limb type, i.e., fore or hind limb. The apical

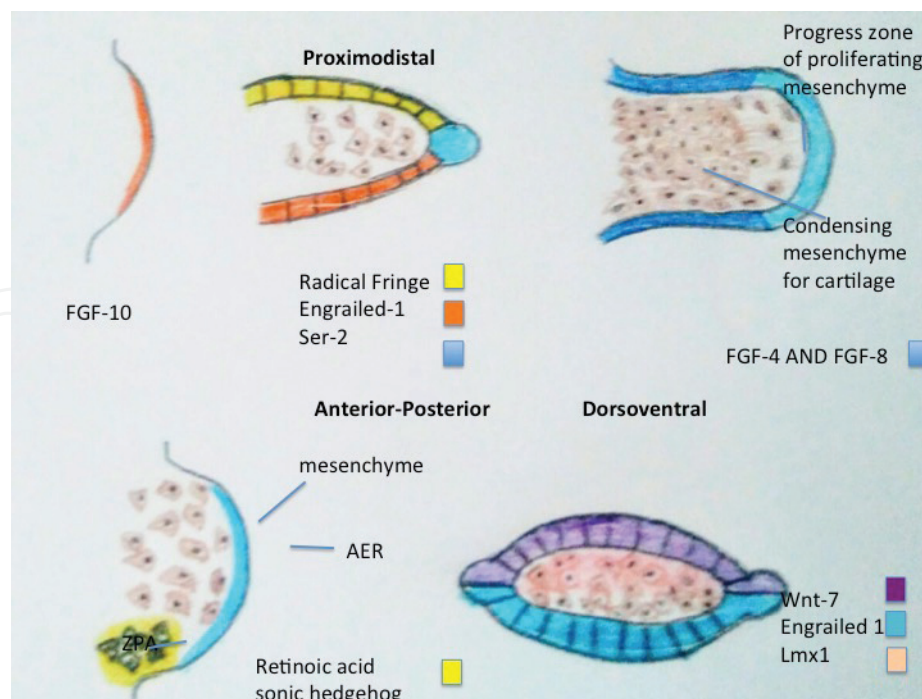


Figure 9. Molecular regulation of limb development. Fibroblast growth factor 10 and AER initiate limb growth. These are induced by bone morphogenetic protein S (RA is retinoic acid, ZPA is zone of polarizing activity, AER is apical endodermal ridge).

ectodermal ridge (AER), which is a localized area of thickening on the surface ectoderm covering the limb bud, produces growth signals such as FGF4 and FGF8, which result in growth in proximal-distal axis. A collection of cells at the posterior margin of limb bud near body wall also undergoes proliferation to form the zone of polarizing activity which produce morphogen retinoic acid which in turn expresses SHH (Sonic Hedge Hog) which plays a huge role in determining the anteroposterior axis for limb development (**Figure 9**). Bones of the limb are derived by endochondral ossification of the parietal layer of the lateral plate mesoderm which is determined by HOX genes (HOXA and HOXD), which regulate the type, and shape of the bones. Digits are formed due to organized apoptosis of the AER into separate ridges and ultimately by interdigital apoptosis. HOXD13 mutations result in synpolydactyly, which is a dominantly inherited condition. Hand foot genital syndromes results from mutations of HOXA13 and presents hypospadias in males and bilateral thumb and great toe hypoplasia [6]. Dorsalization of the limb bud is under the control of genes like radical fringe and Wnt7a. Holt Oram syndrome an autosomal dominant inheritance is due to mutation of T-box 5 gene and is characterized by forelimb abnormalities and cardiac defects thus T-box genes determine the limb identity by activating FGFs. Another gene is the PitX, which contribute in forming hind limb characteristics [6].

10. Head and neck formation

The most important feature in development of head and neck is the formation of pharyngeal arches resembling the gills of the fish. These arches are bars of mesenchyme tissue appearing at fourth or fifth week of development with an outer covering of ectoderm and inner layer of

endoderm at the cranial most part of the primitive gut. The arches are separated from each other by ectodermal clefts and form out pouching in the lateral wall of developing pharynx as endodermal pouches. The mesoderm which lies in the center of the arches form the arch arteries and musculature and the skeletal elements are formed from the neural crest cells, which migrate early from hindbrain region. The FGF signaling plays an important role in determining the migration and fate of neural crest cells in the arches, endodermal pouch formation and differentiation [7]. Though it was previously thought that the neural crest cells are important for pharyngeal arch development but nowadays it is shown that the endoderm of the pharyngeal apparatus acts as the principal organizer and thus it is proved that neural crest cells are not necessary for pharyngeal arch development. The out pocketing of the endoderm is significant for pharyngeal arch development and regulated by TBX1 and RA signaling [8]. The most well-known condition due to faulty development of third and fourth pouches is the DiGeorge syndrome which results due to submicroscopic chromosome deletion 22q11 and loss of approximately 30 genes. Treacher Collins is hypoplasia of the maxilla and mandible with coloboma of the lower eyelid, cleft palate, hearing impairment has an autosomal dominant inheritance affecting the first arch and results due to mutation in TCOF1 gene [1].

11. Ciliary development

The cilia on the apical surface of the cells have an important role in human embryological development deciding cell specification, axis determination and pathology of human congenital disorders. A variety of congenital defects like polycystic kidney, laterality defects, nervous system defects, and retinal degeneration are associated with ciliopathies thus proving that cilia can serve as signaling factor in embryonic development. Cilia plays an important role in Hedgehog patch smoothened Gli pathway and thus it regulates the Gli protein activity. The cilia transition zone a short segment just above the basal body acts as an essential gatekeeper for movement of protein in and out of the cilia. Cilia also play an important role in movement of fluid in various confined embryonic spaces, which may act as a developmental shear force [9]. The congenital polycystic kidney disease, which results in numerous cyst formations, can be inherited as autosomal dominant or recessive. In autosomal recessive type the cysts are formed in the collecting tubules and it is a progressive disorder though less common but highly progressive and leads to kidney failure early in infancy and childhood. The autosomal dominant type is more common (1/500 to 1/1000) results in cyst formation in all segments of the nephron is less progressive and does not result in renal failure till adulthood, The above two types of conditions are related to the mutation of genes that code ciliary protein and thus fall under the group of disorders the ciliopathies which are creating a lot of interest in recent times. Bardet-Biedl syndrome and Meckel-Gruber syndrome are characterized with renal cysts and other features are also some of the ciliopathies recently identified [2].

12. Reciprocal interactions

Organogenesis results due to reciprocal interaction between the differentiated epithelial cells and the underlying mesenchyme, which is brought about by signaling molecules. The

transcription factor NKX2 which is a homolog of the gene tinman that regulates heart development in *Drosophila*, is induced in the endoderm overlying the splanchnopleuric mesoderm for human heart development. NKX2 is considered as the master gene for human heart development and its expression is brought about by BMP activity and WNT inhibition. The venous portion of the heart is specified and formed under influence of retinoic acid and cardiac looping is due to expression of PITX2 in lateral plate mesoderm.

The cephalocaudal and lateral folding of the embryo gives rise to formation of primitive gut tube which is endoderm lined yolk sac cavity incorporated into the embryo by fourth week of development. The retinoic acid (RA) concentration varies in this primitive gut and it is the highest in colon region and least in that part of this gut tube which give rise to the pharynx. This variation of RA concentrations causes expression of certain transcription factors, which leads to the specification of the gut tube to develop the parts. SOX2 is transcription factor for development of esophagus and stomach, PDX1 is for duodenum development, CDXC is for small intestine, and CDXA is for large intestine and rectum. The interaction of the epithelial and underlying mesenchyme components of the gut follows the initial patterning and brought about by sonic hedgehog expression throughout the gut. The transition of the foregut to midgut and thus hind gut derivatives and the change in the epithelial characteristics are brought about by nested expressions of HOX genes which are activated by sonic hedgehog to bring about an orchestrated development of the gut. The secretion of FGFs from cardiac mesoderm and BMPs from the septum transversum leads to the hepatic development. The FGFs inhibits the activity of inhibitors and thus specifies the development of hepatic endoderm by a negative feedback mechanism as suppressing the inhibitors brings about the induction. The sonic hedgehog expression in gut endoderm, which is destined to form dorsal pancreatic bud, is suppressed in the gut endoderm by FGF2 and activin. The ventral bud is induced by visceral mesoderm, which up regulates expression of pancreatic and duodenal homeobox gene (PDX). Two types of PAX genes specify the endocrine cells. PAX4 and PAX6 genes specify the insulin, somatostatin, and pancreatic polypeptide secreting cells whereas PAX 6 genes specify the glucagon secreting cells [5].

13. Urogenital system

The urogenital system develops from a common mesodermal ridge, i.e., the intermediate mesoderm. The intermediate mesoderm gives rise to three overlapping kidney systems the pronephros, the mesonephros and the metanephros. The metanephros, which appears by fifth week, gives rise to the definitive kidney in humans. The mesonephric duct of the intermediate mesoderm gives rise to an ureteric bud which forms the collecting part of the kidneys the renal pelvis, calyces, 1–3 million collecting tubules. The interaction of this ureteric bud from the mesonephric duct with the mesenchyme of the metanephric blastema is the key factor in determining the initiation of kidney development. WT1 is the transcription factor expressed by the mesenchyme of metanephros, which increases the sensitivity of the metanephros to interact with the ureteric bud and respond to it. The branching and growth of ureteric bud is under control of production of Glial derived neurotrophic factor (GDNF) and Hepatocyte growth factor produced by the mesenchyme of metanephros which bind to the receptor on

ureteric bud. The ureteric bud too produces FGF and BMP7, which induce the metanephric mesenchyme to undergo proliferation, and also blocks apoptosis. Epithelial conversion of the mesenchyme of metanephros occurs under the influence of WNT9B and WNT6 from the ureteric buds, which in turn activate PAX2 and WNT4 in the metanephric mesenchyme. All these interactions lead to the modifications in extracellular matrix protein to form the characteristic epithelial basal lamina rich in laminin and type IV collagen. Wilms tumor, a malignant neoplasm of the kidney usually affecting children of age group 5–10 years, is due to mutations in WT1 gene on chromosome 11p13 and can be associated with other abnormalities. Mutations in GDNF gene, which causes branching of the ureteric bud and interaction of the ureteric bud and metanephros, can result in renal agenesis [2].

14. Molar pregnancy

Hydatiform mole can be either partial or complete in which the placenta may proliferate abnormally. This can be partial with 69 chromosomes or triploid in which 46 chromosomes are derived from father and 23 from mother, which can be either due to dispermy or endoduplication of haploid sperm. In complete hydatiform molar pregnancy there are only 46 chromosomes and solely paternal in origin. It's due to fertilization of an empty ovum by two sperms or duplication of single sperm. The complete mole can undergo malignant change into invasive choriocarcinoma and outcome can be fatal if untreated [1, 3].

15. Multifetal gestation

Multiple births result from simultaneous nurturing two or more embryos in the uterus and can be of monozygotic or dizygotic types. In monozygotic twins a very early division of the zygote before separation of the cells which form chorion result in dichorionic twins, twinning at blastocyst stage results in monochorionic diamniotic twins which constitutes about 70% of monozygotic twins. Twinning after first week leads to monoamniotic twins. There is 2–5 times increase in monozygotic twins in babies born by IVF. Very late divisions after 14 days can lead to conjoined twins which is common in females about 75% and thus late twinning and X inactivation can be interrelated. Dizygotic twins result from fertilization of two ova by two sperms thus they are also called fraternal twins. Dizygotic twins are diamniotic and dichorionic. Increased maternal age, family history and intake of ovulation inducing drugs can increase risks for dizygotic twins [1, 3].

In 1961 Dr. Mary Lyon experiments on mice led to the development of Lyon's hypothesis regarding X chromosome deactivation. Later this was recognized and the term lyonization is used for the process of X chromosome, which occurs around 15th, or 16th day of development in female embryos. Either the maternal or paternal X chromosomes can be deactivated in any particular cell and thereafter the same X chromosome is inactivated in daughter cells. Barr bodies are the inactivated darkly stained mass of chromatin seen during interphase. During

cell division this inactive X chromosome is late replicating. But not all of the X chromosome is inactivated. Genes at tip of Xp (short arm) and other genes at other loci of Xp also escape deactivation. There are more genes which get deactivated in Xq thus resulting in less severe phenotypic effects in people with Xq deletions compared to Xp deletions. In men and women with more than one X chromosomes the number of Barr bodies seen during interphase is one less than the total number of X chromosomes. Dosage compensation mechanisms are evolved which lead to a balance in X linked gene products between sexes. This can be achieved by two fold increase in expression of X linked genes in males, a two fold down regulation of X linked genes in females and lastly complete inactivation of one of the two X chromosomes in females. A complex of above mentioned strategies are adapted in mammals [10].

16. Pathogenetic mechanisms of birth defects

The mechanism of birth defects can be broadly grouped as deformation, disruption, dysplasia and malformation. Major congenital anomalies are those that are life threatening but minor abnormalities are those that do not pose a threat to extra uterine survival. Examples of structural anomalies are cleft lip and palate, diaphragmatic hernia, hydrops, congenital heart diseases, choroid plexus cysts and short femur.

17. Markers of structural anomalies

In the first trimester, nuchal translucency is a marker of chromosomal anomalies. In the second trimester there may be echogenic bowel, echogenic chordae or absent nasal bone. Presence of these biophysical markers in early ultrasound becomes an indication to test for chromosomal anomalies in the fetus. Chromosomal anomalies can be detected by performing direct tests (fetal blood sampling, amniocentesis, cordocentesis) on the fetus or chorionic villi (chorionic villus sampling). Indirect screening tests can be done in mother (fetal DNA in maternal serum) to obtain indirect evidence.

18. Genetic testing

There are about 25–30 trillion cells (1 trillion = 1,000,000,000 000) in the human body. They are of two types: somatic cells and gametes. The somatic cells are diploid with 23 pairs of chromosome. The gametes are haploid with 23 chromosomes [11].

Each chromosome has 100–1000 genes. The genetic information contained in Chromosomes is in the form of base pairs. The total base pairs in a haploid set of 23 chromosomes are around 3000 million. Direct testing can be karyotype, FISH or QF PCR, Micro deletion detection, microarray, mutation specific testing or exon sequencing. Single nucleotide polymorphism testing is also important [12–14].

19. Karyotyping

Karyotyping detects the number of chromosomes (Aneuploidies or the gross structure of chromosomes). A karyotype detects any chromosomal anomaly greater than 5 Million base pairs. This could be monosomy, trisomy, tri and tetra ploidy, deletions, duplications, etc. Karyotyping is done by culture that may take 3–4 weeks. An example is detection of Down's syndrome by karyotyping of fetal cells obtained after amniocentesis). Any abnormality lesser than 5000 million base pairs cannot be detected by karyotyping. For example a small micro deletion or a mutation responsible for a genetic disease like CAH (Congenital genital hyperplasia) or spinal muscular atrophy.

20. FISH

FISH will detect a specific chromosomal anomaly that has been previously suspected. In prenatal diagnosis typically abnormality of chromosome 21, 13, 18, X, Y. It gives results between 72 h. For example if the triple markers in maternal serum and first trimester are suggesting Down's syndrome, a specific FISH for chromosome 21 can be performed. But if there is no specific suspicion it is better to do karyotyping.

A FISH may detect micro deletions also but it has to be planned which micro deletion we are looking for. For example 22q micro deletions responsible for Di George Syndrome in a cardiac anomaly like tetralogy of Fallot. Laboratories usually have a panel for common micro deletions. 22q micro deletion is the second most common chromosomal anomaly after Trisomy 21.

21. Microarray

Almost all mutations are very small with only 100–1000 pairs. For these we have to depend on cytogenetic tests like Mutation testing (Sanger Sequence), microarray, Exome sequencing and genome sequencing. If we do not know what to test for than we have to order a microarray. It will scan the entire genome if some few hundreds of base pairs are missing or not. For example, microarray gene testing of uterine endometrium in cases of recurrent implantation failure following in vitro fertilization and embryo transfer.

22. Exom and genome sequencing

These are the most precise tests telling us abnormalities in the minute base pairs. These are expensive with last resorts.

An analogy is a library with 23 bookshelves. Each shelf has 10 rows and each row has 25 books. A karyotype is to find if a bookshelf is in excess or missing. Also it can also tell if a major part of bookshelf is broken. A micro deletion is like a missing row in a bookshelf.

A microarray can identify if a book or two is missing in the library but then sometimes it tells a book which is not important. A mutation specific test is like checking if a particular book is missing. For example a book on Indian cooking in a row of cookery books.

An Exom sequencing is like checking if a few pages are missing in any book in any shelf of the whole library. Genome sequencing is like checking if any paragraph or sentence is missing in any book in any shelf in the whole library. Majority of the fetal birth defects are due to single gene/multiple gene mutations and cannot be identified in karyotyping.

23. Preimplantation genetic testing

Preimplantation genetic testing is defined as a procedure to remove one or more nuclei from oocytes (polar bodies) or embryos for genetic testing before transfer [15, 16]. Preimplantation genetic diagnosis PGD is a term used to determine whether a certain mutation or an unbalanced chromosomal complement has been transmitted to the oocyte or embryo when one or both genetic parents carry a genetic mutation or a balanced chromosomal rearrangement. Preimplantation genetic diagnosis is done to avoid transfer of embryos with mutation and identify healthy embryos for transfer [17].

Preimplantation genetic screening is term used when both genetic parents are chromosomally normal and their embryos are screened for any genetic defects before implantation to improve the success rate of embryo transfer [18, 19].

In preimplantation genetic screening and 24 chromosome copy number analysis (CGH, array comparative genomic hybridization, real-time quantitative PCR, SNP microarray) the aim is

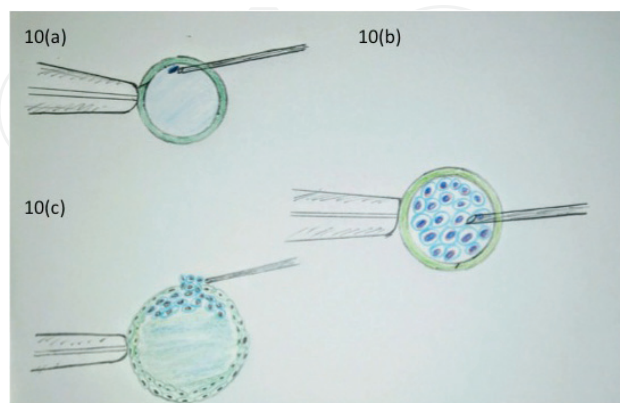


Figure 10. (a) Diagrammatic illustration of the method for polar body biopsy. (b) Blastomere biopsy. (c) Multiple cell biopsies from blastocyst.

only to improve the IVF rates. So the requirements of accuracy are less strict and high false positive results may be acceptable. The test should more importantly be less costly, rapid and non-invasive.

In preimplantation genetic diagnosis diagnostic accuracy is most important. The test should be highly sensitive and specific with very low false negative results. There are three procedures polar body biopsy, blastomere biopsy and blastocyst biopsy [20] (**Figure 10**).

24. Counseling for genetic testing of embryos

The risks associated with ovarian stimulation should be empathically communicated. The couple should be given the option of not proceeding with IVF and PGD/PGS and the choice should be voluntary. The risks associated with embryo biopsy and extended cultures while waiting for the result of biopsy should be explained. The possibility that in case of a false positive result a healthy embryo will be discarded should be explained. The possibility that in case of a false negative result an abnormal embryo may be transferred should be explained. The couple should also be explained about the options of prenatal testing like maternal serum markers, fetal ultrasound, amniocentesis, chorionic villous sampling and fetal blood sampling. The couple should also be explained about the possibility that if all embryos are found affected embryo transfer will not be carried out. Finally the issue of embryos not transferred (discard, cryopreservation, donation, research) should be discussed.

The couple should also be made aware of alternative methods of avoiding genetic defects (like use of donor gametes).

25. Polar body biopsy

Laser or mechanical dissection can be done for opening the zona pellucida and retrieval of the polar body [21, 22]. Laser assisted biopsy are less time consuming. Acid Tyrode solution used for chemical denudation of blastocysts is not tolerated by ovum and is not used for ovum biopsy.

The removal of both polar bodies is done in the time window of 8–14 h. After fertilization. Simultaneous removal of both polar bodies is preferred over sequential removal of polar bodies as there are fewer traumas to the oocyte.

Even with the biopsy of both polar bodies, around 26% of errors are missed. Even in the presence of an abnormal polar body screening 90% of embryos will still be euploid [23]. This is because copy number analysis only determines a relative loss or gain of genetic material-not an absolute copy number. Nondisjunction represents only 10% of errors; others are due to premature segregation of sister chromatins. When reciprocal errors occur for a given chromosome in first and second polar body, 90% of embryos will be euploid. So, with 55% of abnormal

polar bodies 10% will be due to nondisjunction and the remaining 45% from half of the premature separation of sister chromatids.

26. Embryo biopsy

Blastomere biopsy can be obtained at day 3 embryos. Blastocyst biopsy can be done on Day 5 or Day 6 embryos.

Blastomere biopsy is done by zona drilling with acid Tyrode solution or mechanical aspiration. Alternatively laser denudation can be done. If removal of two cells is considered it should be done only after an embryo has six or more cells. Blastomere biopsy is associated with low implantation rates [24].

Blastocyst biopsy on Day 5 or Day 6 embryos is done with noncontact infrared lasers. Laser is used to create an opening in the zona pellucida and the herniating trophectoderm is excised. Around 10 trophectoderm cells are removed and studied. Blastocyst biopsy of trophectoderm does not lead to reduced implantation and delivery rates. It should be remembered that the RCTs that demonstrated a beneficial effect of embryo biopsy were only done on blastocyst stage with trophectoderm biopsy.

27. Blastocyst comprehensive screening and single-embryo transfer

After a comprehensive chromosome testing a single best embryo can be transferred in women. The initial data collected has revealed that frozen embryo transfer after comprehensive gene testing leads to a better on going pregnancy rates as compared to a fresh blastocyst transfer that was screened only morphologically [25, 26]. With the development of newer modalities like comprehensive chromosome screening and single embryo transfer older women will benefit and risks of multiple embryo transfer will be minimized.

28. Direct testing

Direct testing on the fetus is offered when there is an ultrasound marker of anomaly; serum screening (triple markers, quadruple markers) is positive or when one of the parents or siblings is a carrier of chromosomal abnormality. Individuals and relationships are described as a diagram when clinical history is obtained (**Figures 11 and 12**).

28.1. Clinical indications for prenatal diagnosis

Diagnostic interventions in obstetrics are mainly directed at some form of fetal tissue sampling for genetic, biochemical, hematological and histological processing. Samples of fetal tissues like amniotic fluid, chorionic villi and blood can be obtained by a variety of tissue sampling

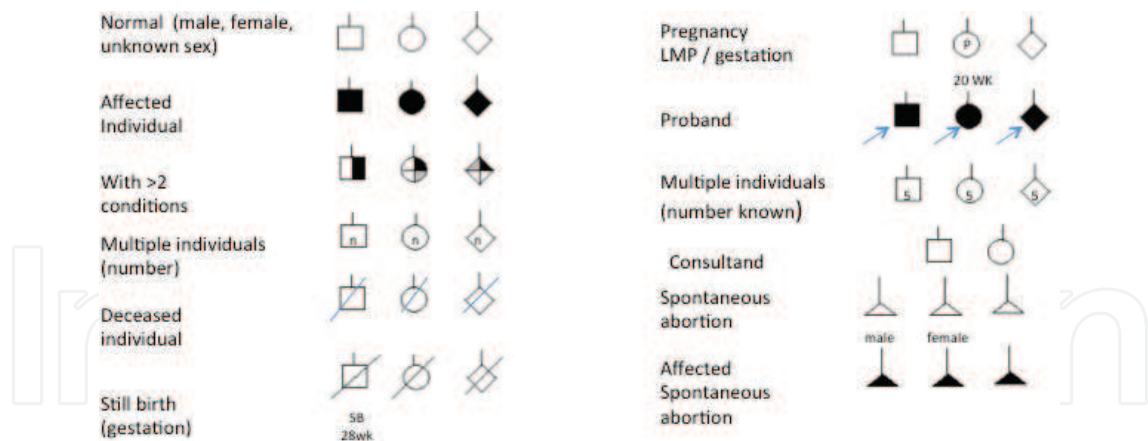


Figure 11. Diagrammatic representation of individuals in clinical genetics.

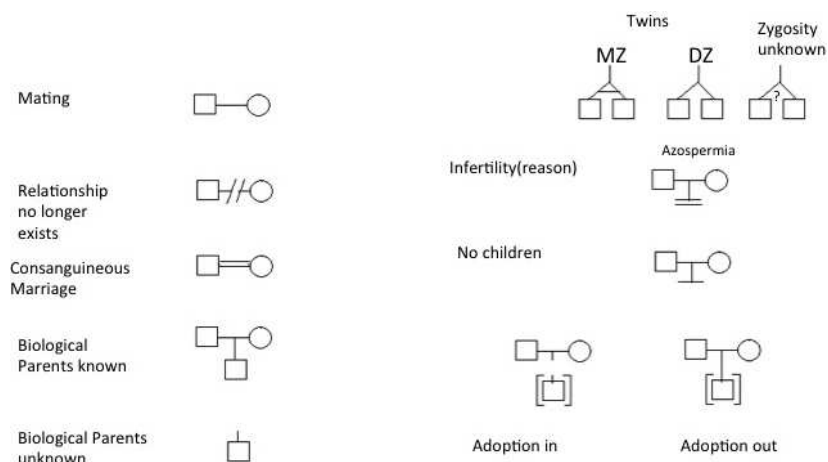


Figure 12. Diagrammatic representation of relationships in clinical genetics.

methods under ultrasound guidance. Other fetal tissues that can be sampled for prenatal diagnosis are fetal urine, skin, liver and brain.

28.2. Indications of prenatal diagnosis

1. Advanced maternal age
2. History of chromosomal abnormality or single gene disorder in a sibling
3. Chromosomal translocation in one parent
4. Rh isoimmunization
5. Congenital infections
6. Fetal growth disorders
7. Increased nuchal translucency in first trimester ultrasound scan

8. Multiple soft tissue markers seen on ultrasound in the second trimester
9. Positive maternal serum biochemistry

The fetal tissue sample obtained by these invasive procedures can further be processed.

1. Karyotyping
2. DNA analysis
3. Hematological parameters
4. Biochemical analysis
5. Enzyme assays
6. Microbial assays
7. Histological studies by light and electron microscopy.

29. Amniocentesis

Amniocentesis is the oldest procedure performed for prenatal diagnosis [27]. As early as 1952, it was found that cells in the amniotic fluid represented fetal tissue and could be used for gender determination. This procedure was performed initially without any guidance. Now, with the help of real time ultrasound and color flow mapping, amniocentesis is performed easily with accuracy. There are minimal complications and the procedure is safe.

Amniocentesis is performed in the second trimester when the uterus has become an abdominal organ and the amniotic fluid is about 200 ml. At any gestation, 10% of amniotic fluid volume can be aspirated for diagnostic procedures. A prerequisite is that there should be an adequate amniotic fluid volume for that gestational age.

29.1. Early amniocentesis

Improved laboratory techniques have made it possible to culture amniotic fluid cells from as little as 10 ml of amniotic fluid at 12 weeks. However, the volume of 10 ml will account for almost a third of the amniotic fluid volume at this gestational period [28]. This may lead to problems in the fetus like lung hypoplasia and limb deformities like congenital talipes equinovarus. To overcome the loss of fluid volume, a technique of entrapping the cells in a filter and returning the amniotic fluid into the cavity has been tried. Randomized controlled trials are required to establish the safety of this procedure.

29.2. Technique of amniocentesis

Under continuous ultrasound guidance and strict aseptic precautions, a 20–22 gauge spinal needle is introduced in a pool of amniotic fluid, which is devoid of fetal parts. It is preferable to avoid the placenta. If the placenta is anterior, the needle is introduced laterally or above the

placental margin. The amnion is pierced with a sudden controlled force, a jab of needle to prevent tenting of the membranes. The first few ml of fluid is discarded to minimize the contamination with maternal blood. About 20 ml of fluid is withdrawn for analysis. Not more than two insertions should be performed at one sitting.

29.3. Amniocentesis in twins

Individual sampling of both sacs using two different needles under direct ultrasound guidance can perform amniocentesis in twins. A single insertion technique in which needle is advanced to the second sac after aspirating the first sac has been advocated by Jeanty.

29.4. Complications

A blood stained aspirate can occur in 1–2% of the procedures. If the color is red, it indicates fresh blood possibly due to maternal blood contamination. Dark red or dark brown color of the fluid indicates prior intraamniotic bleed that is associated with poor fetal outcome. A brown color amniotic fluid can also indicate fetal aneuploidy. The overall pregnancy loss following amniocentesis is estimated as 0.5%.

The incidence of fetomaternal hemorrhage is 63% in anterior placentas wherein the needle has traversed through the placenta. It reduces to 18% in posterior placentas. Hence, it is essential to determine Rh type of the mother prior to the procedure. Anti D immunoglobulin must always be given in rhesus negative women after any prenatal procedure. Fetal trauma is unlikely if the procedure is done continuous ultrasound monitoring.

30. Chorionic villous sampling

Chorionic villous sampling was initiated in clinical practice in 1980s [29]. It is performed between 10 and 14 weeks. Placental biopsies are also done in second and third trimesters for rapid karyotyping.

30.1. Technique

Prior to the procedure the crown rump length is measured to confirm the gestational age. Fetal heart activity is documented and a sample of villi can be obtained by the transcervical or transabdominal route [30].

30.2. Trans cervical route

A special transcervical cannula developed by Rodeck with a malleable obturator is used. With a partially filled bladder, the gravid uterus is imaged transabdominally and the chorionic frondosum is identified. With the patient in lithotomy position a cannula is introduced through the cervical canal. The cannula guided into the chorionic frondosum under continuous ultrasound guidance. A 10 ml syringe with 2 ml of culture media is then attached to the

proximal end of cannula. Suction is applied and a gentle to and fro motion of the cannula will ensure aspiration of villi into the medium in the syringe. The cannula is then withdrawn with continuous suction being maintained. The sample is then examined under a microscope to ensure the presence of branching villi. A minimum of 15 mg of tissue will be required for culture.

30.3. Trans abdominal approach

Rodeck transabdominal chorionic villous biopsy forceps can perform transabdominal collection of fetal villi. A 20 Gauge spinal needle can also be used for sampling the villi transabdominally. The needle is advanced under ultrasound guidance and suction is applied as via the transcervical technique. A double lumen technique can also be used. In this, a large lumen outer needle is introduced into the uterus and a smaller gauge needle is passed through the outer needle to sample the villi. The advantage of this technique is that resampling can be done easily if the sample is insufficient in the first passage of needle. Transabdominal approach has been found safer with few complications than trans cervical route.

30.4. Complications of chorionic villous sampling

Fetal loss can occur in 0.6–2% of cases. Loss rates are greater than 10% if more than two needle insertions are made to collect the chorionic villi. A sub chorionic hematoma may form in 4% cases, which usually resolves spontaneously. Chorioamnionitis is a rare complication occurring in less than 0.3% cases. A delayed rupture of membranes can happen weeks to days after chorionic villous sampling in about 0.3% of cases.

The risk of fetomaternal hemorrhage is dependent on the amount of tissue aspirated and is detected by a rise in maternal serum alpha feto protein [31]. All Rh Negative non-sensitized mothers should receive a prophylactic Anti D immunoglobulin [32]. Perinatal complications like premature rupture of membranes, small for gestational age or intrauterine growth restriction have not been noticed after chorionic villous sampling. Chorionic villous sampling performed prior to 9 weeks of gestation is known to be associated with specific fetal malformations. Oromandibular limb hypo genesis syndrome and terminal transverse limb reduction anomalies have been documented when chorionic villous sampling was performed prior to 9 weeks of gestation. Chorionic villous sampling should not be performed prior to 9 weeks of gestation [33, 34].

31. Fetal blood sampling

Fetal blood can be sampled after 18 weeks of gestation. Fetal blood sampling rules out the possibilities of pseudomosaicisms that are more common in chorionic villi and amniotic fluid cultures. Fetal blood can also be used for hematological estimations and enzyme and hormone assays. Direct access to fetal circulation is also used to give intra uterine exchange transfusions in cases of Rh isoimmunization.

31.1. Technique of fetal blood sampling

Fetal blood can be obtained from the umbilical vein or artery in the umbilical cord near its insertion into placenta. Fetal blood can also be obtained from the intrahepatic umbilical vein or a free loop of umbilical cord. A 22 Gauge spinal needle is used for the procedure. Ultrasound with color Doppler is used to image the site of cord insertion in the placenta. The needle is introduced under ultrasound guidance about 1 cm from the insertion site of the umbilical cord into the placenta. If the cord is punctured close to the placental insertion there are increased chances of contamination with maternal blood. After obtaining the fetal blood sample it is tested for contamination with maternal blood. Kleihauer-Betke test of acid elution with hydrochloric acid can distinguish between fetal and maternal cells. Fetal red blood cells do not get eluted with hydrochloric acid due to presence of fetal hemoglobin. Fetal Red blood cells have a larger Mean Corpuscular Volume compared to maternal red blood cells.

Intrahepatic portion of the umbilical vein is an alternative site, which yields pure blood samples. Direct fetal cardiac sampling has been resorted to in few centers. When blood is obtained directly from the fetal heart or intrahepatic portion of umbilical vein testing for contamination with maternal blood is not necessary.

31.2. Complications

When a needle is inserted into the umbilical vein the most common complication is fetal bradycardia. However it should be monitored and it quickly reverts back to normal after the needle is withdrawn. Sometimes a brisk spurt of blood from the puncture site into the amniotic fluid is seen lasting for 2 min. However this bleeding invariably stops and the fetus is unaffected. In cases of continued hemorrhage a bleeding disorder in the fetus should be considered. The overall fetal loss rate following umbilical blood sampling is 1–2%. There is no increased risk of preterm labor, intrauterine growth restriction or fetal congenital malformations.

32. Fetal skin biopsy

Fetal skin biopsy is done between 18 and 20 weeks of gestation [35, 36]. This is indicated for the detection of genodermatoses like epidermolysis bullosa, epidermolytic hyperkeratosis, harlequin ichthyosis and Sjogren-Larsson syndrome [37, 38]. Another indication is oculo cutaneous albinism.

32.1. Technique of fetal skin biopsy

Fetal skin sampling is done using a special biopsy forceps, which is introduced through a trocar needle. The site of sampling is chosen according to the indication. The site of sampling is different in different indications. In suspected genodermatosis the site of biopsy is gluteal region. When oculo cutaneous albinism is suspected the skin biopsy is taken from the eye-brows and scalp. An electron microscope study of the fetal skin sample is required to confirm

the diagnosis. The procedure is safe and in a study of 52-skin biopsy by Rodeck no complications were recorded [39].

33. Fetal liver biopsy

Fetal liver biopsy has been used for the prenatal detection of urea cycle disorders and G6PD deficiency. The procedure is performed with a double lumen fetal liver biopsy needle [40].

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References

- [1] Turnpenny P, Ellard S. Emery's elements of medical genetics. In: Section A, Principles of Genetics, Chapter 6 Developmental Genetics. 14th ed. Elsevier's Churchill Livingstone. pp. 83-103
- [2] Sadler TW. Langman's Medical Embryology. 12th ed. Wolters Kluwer Lippincott Williams & Wilkins; 2012
- [3] Kothari M, Mehta LA, Roychoudhury SS. Essentials of Human Genetics. 5th ed. Universities Press India Limited; 2009
- [4] Albert Bisson M. Signaling in cell differentiation and morphogenesis. Cold Spring Harbor Perspectives in Biology. 2012;4(6):a008151
- [5] Elisabeth H, Villavvicencio, David O, Walterhouse, Philip M, Iannaccone. The sonic hedgehog-patched-Gli pathway in human development and disease. American Journal of Human Genetics. 2000;67(5):1047-1054
- [6] Barham G, Clarke NMP. Genetic regulation of embryological limb development with relation to congenital limb deformity in humans. Journal of Children's Orthopaedics. 2008;2(1):1-9
- [7] Graham A, Jo R. Developmental and evolutionary origins of the pharyngeal apparatus. Evolutionary Developmental Biology. 2012;3:24

- [8] Frisdal A, Trainor PA. Development and evolution of pharyngeal apparatus. *Wiley Interdisciplinary Reviews: Developmental Biology*. 2014;**3**(6):403-418
- [9] Drummond IA. Cilia functions in development. *Current Opinion in Cell Biology*. 2012;**24**(1):24-30
- [10] Brockdorff N, Bryan M. Turner dosage compensation in mammals. *Cold Spring Harbor Perspectives in Biology*. 2015;**7**(3):a019406
- [11] Geraedts JP, De Wert GM. Preimplantation genetic diagnosis. *Clinical Genetics*. 2009;**76**(4):315-325. DOI: 10.1111/j.1399-0004.2009.01273.x Review. PMID: 19793305
- [12] Johnson AD. SNP bioinformatics: A comprehensive review of resources. *Circulation. Cardiovascular Genetics*. 2009;**2**(5):530-536. DOI: 10.1161/CIRCGENETICS.109.872010
- [13] Thorisson GA, Stein LD. The SNP consortium website: Past, present and future. *Nucleic Acids Research*. 2003;**31**(1):124-127
- [14] Shastri BS. SNPs: Impact on gene function and phenotype. *Methods in Molecular Biology*. 2009;**578**:3-22. DOI: 10.1007/978-1-60327-411-1_1. PMID: 19768584
- [15] Handyside AH. 24-chromosome copy number analysis: A comparison of available technologies. *Fertility and Sterility*. 2013;**100**(3):595-602
- [16] Scott KL, Hong KH, Scott RT Jr. Selecting the optimal time to perform biopsy for preimplantation genetic testing. *Fertility and Sterility*. 2013;**100**:608-614
- [17] Capalbo A, Ubaldi FM, Cimadomo D, et al. Consistent and reproducible outcomes of blastocyst biopsy and aneuploidy screening across different biopsy practitioners: A multicentre study involving 2586 embryo biopsies. *Human Reproduction (Oxford, England)*. 2016;**31**(1):199-208. DOI: 10.1093/humrep/dev294
- [18] Cimadomo D, Capalbo A, Ubaldi FM, et al. The impact of biopsy on human embryo developmental potential during preimplantation genetic diagnosis. *BioMed Research International*. 2016;**2016**:7193075. DOI: 10.1155/2016/7193075
- [19] Handyside A, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryo sexed by Y-specific DNA amplification. *Nature*. 1990;**344**:768-770
- [20] Preimplantation genetic testing: A practice committee opinion. *Fertility and Sterility*. 2008;**90**:S136-S143
- [21] Cieslak J, Ivakhenko V, Wolf G, Sheleg S, Verlinsky Y. Three dimensional partial zona dissection for preimplantation genetic diagnosis and assisted hatching. *Fertility and Sterility*. 1998;**71**:308-313
- [22] Montag M, Van der Van K, Delacretaz G, Rink K, Van der Van H. Laser assisted micro dissection of zona pellucida facilitates polar body biopsy. *Fertility and Sterility*. 1998;**69**: 539-542

- [23] Forman EJ, Treff NR, Stevens JM, Gansey HM, MG K-J, Scott RT Jr, et al. Embryos whose polar bodies contain reciprocal chromosome aneuploidy are almost always euploid. *Human Reproduction*. 2013;**28**:502-508
- [24] Van de Velde H, DeV os A, Sermon K, et al. Embryo implantation after biopsy of one or two cells from cleavage stage embryos with a view to preimplantation genetic diagnosis. *Prenatal Diagnosis*. 2000;**20**:1030-1037
- [25] Northrop LE, Treff NR, Levy B, Scott RT. SNP microarray based 24 chromosome aneuploidy screening demonstrates that cleavage stage FISH poorly predicts aneuploidy in embryos that develop to morphologically normal blastocysts. *Molecular Human Reproduction*. 2010;**6**:590-600
- [26] Scott RT, Ferry KM, Forman EJ, Hong KH, Scott KL, Taylor D, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases IVF implantation and delivery rates: A randomized controlled trial. *Fertility and Sterility*. 2013. DOI: 10.1016/j.fertnstert.2013.04.035
- [27] Gray KJ, Wilkins-Haug LE. Have we done our last amniocentesis? Updates on cell-free DNA for down syndrome screening. *Pediatric Radiology*. 2018;**48**(4):461-470. DOI: 10.1007/s00247-017-3958-y. Epub 2018 Mar 17. Review. PMID: 29550862
- [28] Alfievic Z, Navaratnam K, Mujezinovic F. Amniocentesis and chorionic villus sampling for prenatal diagnosis. *Cochrane Database of Systematic Reviews*. 2017;**9**:CD003252. DOI: 10.1002/14651858.CD003252. pub2. Review. PMID: 28869276
- [29] Young C, von Dadelszen P, Alfievic Z. Instruments for chorionic villus sampling for prenatal diagnosis. *Cochrane Database of Systematic Reviews*. 2013;**1**:CD000114. DOI: 10.1002/14651858.CD000114. pub2. Review. PubMed PMID: 23440775
- [30] Von Dadelszen P, Sermer M, Hillier J, Allen LC, Fernandes BJ, Johnson JA, Shime J, Winsor EJ, Ryan G. A randomised controlled trial of biopsy forceps and cannula aspiration for transcervical chorionic villus sampling. *BJOG: An International Journal of Obstetrics & Gynaecology*. 2005;**112**(5):559-566. PMID: 15842277
- [31] Yang YH, Lee MS, Park YW, Kim SK, Sung HR, Lee CH, Kim IK. Studies on the prenatal chromosomal analysis and the changes of maternal serum alpha-fetoprotein following chorionic villus sampling. *Yonsei Medical Journal*. 1991;**32**(4):292-302
- [32] Katiyar R, Kriplani A, Agarwal N, Bhatla N, Kabra M. Detection of fetomaternal hemorrhage following chorionic villus sampling by Kleihauer Betke test and rise in maternal serum alpha fetoprotein. *Prenatal Diagnosis*. 2007;**27**(2):139-142. PMID: 17191260
- [33] Başaran A, Başaran M, Topatan B, Martin JN Jr. Effect of chorionic villus sampling on the occurrence of preeclampsia and gestational hypertension: An updated systematic review and meta-analysis. *Journal of the Turkish German Gynecological Association*. 2016;**17**(2): 65-72. DOI: 10.5152/jtgga.2016.16026. eCollection 2016. PMID: 27403071

- [34] Mujezinovic F, Alfirovic Z. Procedure-related complications of amniocentesis and chorionic villous sampling: A systematic review. *Obstetrics and Gynecology*. 2007;**110**(3):687-694. Review. Erratum in: *Obstet Gynecol*. 2008 Mar;**111**(3):779. PMID: 17766619
- [35] Cadrin C, Golbus MS. Fetal tissue sampling—Indications, techniques, complications, and experience with sampling of fetal skin, liver, and muscle. *Western Journal of Medicine*. 1993;**159**(3):269-272
- [36] Holbrook KA, Smith LT, Elias S. Prenatal diagnosis of genetic skin disease using fetal skin biopsy samples. *Archives of Dermatology*. 1993;**129**(11):1437-1454. PMID: 8239702
- [37] Sybert VP, Holbrook KA, Levy M. Prenatal diagnosis of severe dermatologic diseases. *Advances in Dermatology*. 1992;**7**:179-209; discussion 210. PMID: 173958
- [38] Christiano AM, Uitto J. DNA-based prenatal diagnosis of heritable skin diseases. *Archives of Dermatology*. 1993;**129**(11):1455-1459. Review. PMID: 7902070
- [39] Rodeck CH, Nicolaides KH. Fetal tissue biopsy: Techniques and indications. *Fetal Therapy*. 1986;**1**:46-58. DOI: 10.1159/000262232
- [40] Murotsuki J, Uehara S, Okamura K, Yajima A, Oura T, Miyabayashi S. Fetal liver biopsy for prenatal diagnosis of carbamoyl phosphate synthetase deficiency. *American Journal of Perinatology*. 1994;**11**(2):160-162. PMID: 8198660