Reproduction

Pregnancy

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INTRODUCTION

Pregnancy is the state of carrying and nurturing the developing embryo(s) in the uterus of the mother. In therians (marsupials and eutherians), ovum is devoid of adequate amount of yolk proteins and, therefore, the embryo stays inside the uterus of the mother for deriving nutrients during the development. Pregnancy begins with the fertilization of ovum and ends with the parturition or birth of the offsprings. The period through which a female carries a developing fetus in her body is known as gestation period, and it varies from species to species.

Human pregnancy

In humans, pregnancy lasts for approximately 38 weeks from the date of fertilization or 40 weeks from the date of last menses. The duration of human pregnancy is divided into three equal segments called trimesters. The first trimester extends upto the twelfth week of preganancy and encompases fetal organogenesis. This is the period when all organs, nerve and brain cells develop. The menses ceases to recur during pregnancy. Therefore, the first and most obvious sign of pregnancy is the absence of menses for 10 or more days after the usual length of the menstrual cycle. However, this might not hold true for those women in whom the cycle is irregular. This may be accompanied by tiredness, nausea, frequent urination, constipation, tenderness of breast, and the weight gain by the maternal body. Miscarriages, also called spontaneous abortion, occur most frequently during the first trimester. The second trimester lasts from thirteenth to twenty seventh weeks of gestation and embraces the development of fetal bones, fingernails, toenails and hair. The differentiation of external genitalia occurs in the second trimester. Although nausea and chances of miscarriage decreases in second trimester, a mild swelling in feet, ankles, hands and face occurs due to an increase in blood volume and fluid retention. The breast enlarges in response to female sex steroids, prolactin and placental lactogen. The third trimester from 28 to 40 weeks is a period of rapid fetal growth. During this period, the protective sheath of fat covers the nerve fibers and allows brain impulses to travel faster. Thus, the learning ability of the fetal brain enhances. Air sacs are developed in fetal lungs during the third trimester. In the last four weeks of pregnancy, the baby puts on a lot of weight and develops a thick layer of fat called ‘baby fat’. The increased size, weight, and activity of baby causes heartburn, shortness of breath, heart palpitations, and leg cramps in mother. The fetal movement can be felt by the mother in the third trimester though movement of fetus is initiated around 18-22 weeks of pregnancy.

EVOLUTION OF VIVIPARITY

Viviparity in mammals is believed to have originated from oviparous reptilian ancestors. It has evolved many times in squamates (lizards and snakes), so that around 20% of existing species exhibit viviparity (Andrews, 1997). In lacertilians, oviparity to different degrees of viviparity such as simple placenta with little nutrient uptake from mother (lecithotrophic viviparity), intermediate placental complexities to the highly developed placenta (obligate placentotrophy) are reported. This transition from oviparity to viviparity is seen associated with the reduction in egg size and shell, increased vascularization of uterus for nutrient transfer and respiratory gas exchange, prolonged retention of egg in oviduct, and development of extraembryonic membranes for the protection of developing embryo (Thompson et al., 2004). The reptiles, birds and mammals share the common extraembryonic membranes namely, chorion, amnion, allantois and yolk sac, and hence, are kept in the same group, amniota. However, extraembryonic membranes of viviparous reptiles, unlike mammals, develop into two structurally and spatially separated placentome, one at the embryonic pole (choriallantoic placenta) which is most likely the site of gaseous exchange,

and other at the abembryonic pole (omphaloplacenta) that is involved in istotrophic nutrition by apocrine secretion (Thompson and Speake, 2006).

In egg laying mammals (prototherians), a temporary skin pouch develops on the ventral side of the female body in which eggs (eg. Echidna) or newly hatched young ones (eg. Platypus) are incubated for further development. The pouch contains teat-less rudimentary mammary glands that exude their secretion near the large tufts of hair. This secretory substance is licked by the young ones. However, in marsupials that belongs to the primitive group of theria (eg. Kangaroo, wallaby, Opossum), the skin pouch called marsupium contains well developed mammary glands. The marsupial young ones have a very short gestation time (12-13 days in Opossum, 42 days in Kangaroo) and are born at a very premature stage. After parturition, the mother transfers the young ones into the marsupium for further growth and development (Anderson, 1999). In case of eutherians, ovum is devoid of adequate yolk proteins (alecithal type) and hence, incapable to complete the embryonic development. The eutherians adapt a strategy for nurturing their embryo inside the uterus during pregnancy. The length of pregnancy or gestation is species specific. In human, it is calculated as the interval between conception and labor (280 days). The gestation period is measured from the first day of the mother's last menstrual period. Pregnancy is initiated at fertilization followed by sequential developmental events such as pre-implantation, implantation, maternal recognition and maintenance of pregnancy, placentation, maternal adaptations, and is terminated by parturition.

FERTILIZATION

In mammals, the ovulated oocytes (ova) are surrounded by cellular and non-cellular layers, cumulus and zona pellucida, respectively. After passing through these layers, sperm fuses with the egg plasma membrane to introduce the paternal genes during fertilization. Subsequently, a zygote is formed. Although many spermatozoa infiltrate the cumulus, only one sperm can penetrate through the zona pellucida. The zona pellucida is composed of three glycosylated proteins designated as ZP1, ZP2 and ZP3. While ZP1 and ZP3 are glycosylated forms, ZP2 is little or non-glycosylated. ZP3 acts as the primary sperm receptor, mediating both initial binding of spermatozoon to the egg and activation of acrosome reaction. These saccharides are removed following fertilization. ZP2 acts as the secondary sperm receptor. It binds to the acrosome- reacted spermatozoa (Bauskin et al., 1999). The oligosaccharides linked to ZP3/ZP2 helps in binding with the sperm. ZP1 is not required for sperm binding and fertilization. However, it provides the structural stability to zona matrix and helps along with specific O- and N-glycans of ZP3/ZP2 in sperm recognition in a taxon-specific manner (Hoodhboy and Dean, 2004). Also, zona pellucida is involved in polyspermy block. After fertilization, the proteolytic cleavage of ZP2 changes the zona pellucida matrix and thus rendering it unable to support the sperm binding. This is called zona reaction and is attributed to slow block to polyspermy (Yanagimachi, 1994; Abbott and Ducibella, 2001). In addition, fast block to polyspermy is observed in sea urchins, star fish and anuran amphibians. The post-fertilization membrane depolarization occurs in these species within a few seconds of fertilization so that the sperm penetration of egg is not favored (Jaffe and Gould, 1985). Although marked membrane depolarization has not been observed in mouse or hamster egg, the membrane block to prevent polyspermy is reported in mammals (Gardner and Evans, 2006). Shortly after fusion, an intracellular transient increase in calcium leads to the fusion of cortical granules with the egg plasma membrane (cortical reaction). Also, Ca2+ activates the resumption of cell-cycle arrested at metaphase II. These changes are collectively known as egg activation (Swann et al., 2004). It results in the extrusion of second polar body and the formation of egg pronuclei. The first obvious change in the sperm nucleus after its incorporation into the egg is the breakdown of the nuclear membrane. This is followed by nuclear decondensation and formation of large pronucleus. Finally, the fusion of male and

female pronuclei occurs to form the zygote. The entire process is known as syngamy. The fertilization in all eutherian mammals takes place in the ampullary region of the oviduct.

CLEAVAGE AND BLASTULATION

Zygote formation is followed by a series of quick cell divisions called cleavage. It involves rapid cell division without the cell growth**.** Thus, total cellular volume of embryo remains similar to the zygote. Unlike mitosis, cleaving cell shows a modified cell cycle with completely omitted G1 and G2 phases. Therefore, the cells cycle rapidly between S and M phases. After third cleavage (8-cell stage), blastomeres begin to flatten. As a result of this, cleaving embryo changes into a smooth ball of cells with indistinguishable cell boundaries. This developmental phenomenon is known as compaction (Watson et al., 2004). It is stabilized by expression of tight and gap junctions that enable the smooth exchange of ions and small regulatory molecules between the blastomeres. Further cleavage of compacted blastomeres forms a ball of solid cell mass (16-32 blastomeres) known as morula. It consists of a small group of internal cells known as inner cell mass (ICM) (Barlow et al., 1972). This gives rise to the embryo and its associated yolk sac, allantois and amnion. The cells of the outer ring are known as trophoblast. This group of cells forms the tissue of the chorion, the embryonic portion of the placenta. Thus, trophoblasts provide protective covering and helps in supplying nutrients to the embryo from the mother. Initially, the morula does not have an internal cavity. Later, a cavity is formed in which the fluid secreted by the trophoblast is accumulated. This cavity is called blastocoel and the process is known as cavitation. Now, the resulting structure is called the blastocyst. While traveling through the oviduct en route to the uterus, trophoblast secretes a trypsin like proteases that lyse the zona pellucida which prevents the adherence of blastocyst to oviduct. However, if such adherence takes place, it is called as ectopic or tubal pregnancy.

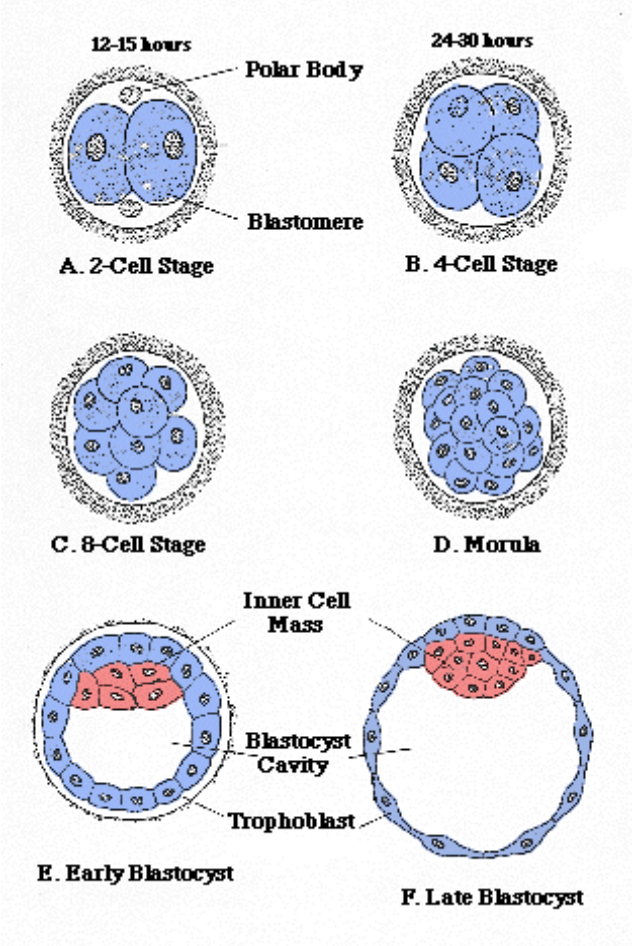

Fig. 1. Stages of early embryonic development in mammalian model (departments.weber.edu/.../blastocyst.html)

During pre-implantation, different genes are implicated in regulation of various developmental processes such as compaction, trophectoderm differentiation and blastocoel formation (Watson et al., 2004). The predominant expression of IQGAP (IQ GTPase-activating protein), rac1 (ras-related C3 botulin toxin substrate 1) and cdc42 (cell division cycle 42 homolog) in cytoplasm of outer blastomeres of early 8-cell embryo facilitate the cell-cell adhesion and thus, regulate the compaction. In addition, p38 MAPK (p 38 mitogen-activating protein kinase) may be involved in compaction since it upregulates the formation of actin filaments. The trophectoderm differentiation is reported to be associated with down regulation of Oct-4 (Octamer-4) transcription factor. In case of blastocoel formation, Na/K-ATPase generates the trans-trophectoderm ionic gradients that promote the accumulation of water across the epithelium. This, along with the formation of tight junction which block the paracellular movement of water between adjacent trophectoderm cells, results in the formation of a fluid- filled cavity. In recent years, the role of aquaporins or water channels is demonstrated as physiological mediators of fluid movement across the trophectoderm.

IMPLANTATION

At the blastocyst stage, the trophectoderm acquires the competence to attach to the receptive uterine endometrium. The duration required for implantation differs among species (hours in rodents to days in humans) primarily due to variation in degree of endometrial invasion by

the trophoblast. The implantation has been classified broadly into three phases: apposition, attachment, penetration and decidualization (Dey and Lim, 2006)

Apposition: This is the stage when embryonic trophectoderm cells become closely apposed to the endometrial luminal epithelium (LE). At the site of blastocyst apposition, the permeability of uterine stromal capillaries increases. This is the first discernible sign of implantation and can be visualized as distinct blue bands along the uterus after an intravenous injection of blue dye solution (Carson et al., 2000). In mice, the attachment reaction occurs around mid-night on day 4 of pregnancy. The vaginal plug formation can be considered as day 1 of the pregnancy. The attachment is preceded by uterine edema and consequently, luminal closure. This leads to close apposition of blastocyst trophectoderm with the LE. However, the blastocyst can be flushed out despite its apposition to the LE (Carson et al., 2000).

Adhesion: In this stage, the trophectoderm cells come in such intimate association with LE that dissociation of blastocyst become difficult by flushing out the uterine lumen. The up- and down- regulation of various surface molecules are implicated in adhesion of blastocyst with the LE. The expression of mucin-1 (MUC-1) that is known to inhibit the cell-cell / cell-extracellular matrix adhesion is decreased at the time of attachment in mice. The other adhesion molecules such as integrins, heparan sulphate, lacto-N-fucopentose 1, trophonin and tastin, which help in attachment, are seen upregulated in blastocyst and uterine cells (Carson et al., 2000).

Penetration: The embryo invades or penetrates through the uterine epithelium and basal lamina into the stroma to establish a definitive vascular relationship with maternal blood vessels. It is mediated by proteinases such as matrix metalloproteinases, serine proteinase and plasmins expressed on the cell surface of trophoblasts (Duc-Goiran et al., 1999). In rodents, penetration of trophoblast is usually followed by stromal cell hypertrophy, repeated polyploidy, formation of intercellular gap junctions, and increased vascularization of endometrial spiral arteries round the implantation site. This process is known as decidualization (Dey and Lim, 2006). The decidua starts producing a large amount of secretary proteins, importantly prolactin, rennin, insulin-like growth factor binding protein-1 (IGFBP-1), and extracellular matrix proteins (ECM) such as laminin and fibronectin, around the mesenchymal cells. These are concerned with the cell-cell interaction, adhesion and embryo nourishment. Also, decidua secretes tissue inhibitors of metalloproteinases (TIMP-1 and -2), leukemia inhibiting factor (LIF) and transforming growth factors-β (TGF-β) (Duc-Goiran et al., 1999). These are involved in homeostasis of invasion control. TIMPs and LIF hinder the trophoblastic invasion by inhibiting the proteinase activity. TGF-β induces the expression of TIMP-1 in both decidua and the trophoblast. In addition, TGF-β inhibits the trophoblast proliferation.

HORMONAL REGULATION OF IMPLANTATION

The ovarian steroid hormones, estrogen (E2) and progesterone (P4) are primarily involved in controlling uterine changes during implantation. The uterine sensitivity with respect to implantation has been classified into pre-receptive, receptive, and non-receptive (refractory) phases (Dey and Lim, 2006). On days 1 and 2 of pregnancy in mice, preovulatory ovarian E2 increases the epithelial cell proliferation. From the third day of pregnancy, luteal P4 is also involved in increasing the pre-receptivity of the uterus by inducing the stromal cell proliferation. The luteal phase E2 is implicated on day 4 of pregnancy i.e. the day of implantation. It synergizes the effect of P4 on stromal cells. However, in response to luteal E2 and P4 uterine epithelial cells stop proliferating and become differentiated on day 4 (Dey and Lim, 2006). Thus, the prereceptive uterus on day 3 of pregnancy/pseudopregnancy becomes receptive on day 4. The luteal phase E2 is critical for embryo implantation since

ovareictomy prior to luteal E2 secretion (on the morning of day 4) results in inhibition of implantation. The embryos at blastocyst stage become dormant. This condition is termed as delayed implantation (DI) and can be maintained for certain period by daily P4 treatment. A single injection of E2 can terminate the state of blastocyst dormancy and make the uterus receptive for implantation. Thus, the time period in receptive phase during which the uterus is most conducive for blastocyst attachment is referred to as "window of implantation" (Dey and Lim, 2006). Subsequent to receptive phase, the uterus become refractory, as it fails to respond to the presence of blastocysts.

The primate model baboon has been extensively studied to understand the hormonal control of blastocyst implantation in humans. In baboon, the changes in uterine receptivity during implantation can be divided into three distinct phases (Kim et al., 2004). The first phase extends from days 8 to 10 postovulation. During this phase, the blastocyst implantation usually occurs. E2 and P4 are primarily involved in regulating the luminal and glandular epithelial cells in preparation for blastocyst apposition and attachment. The second phase of uterine receptivity is induced by blastocyst-derived 'signals', importantly, chorionic gonadotropin (CG) that superimposes its effect on the E2/P4-primed receptive endometrium. The third phase of uterine receptivity is initiated after attachment of blastocyst. On day 23 of the menstrual cycle in human, stromal edema is observed irrespective of whether implantation occurs or not. This is followed 3 to 4 days later by pre-decidual reaction. In case of implantation, the reaction is intensified and becomes the decidua of pregnancy. In baboon, the pre-decidual reaction does not occur during menstrual cycle though the stromal fibroblasts undergo extensive modification following implantation to form the decidua (Kim et al., 2004). Decidualization is the major change that occurs in the primate endometrium after conception. E2 and P4 fail to induce decidualization in the absence of conceptus. In decidualization, the fibroblast-like stromal cells express specific decidual proteins, importantly, insulin-like growth factor binding protein-1 (IGFBP-1). Apart from sex steroids, 3':5'cyclic adenosine monophosphate (cAMP) is also needed to complete the decidualization in baboon.

TYPES OF IMPLANTATION BASED ON:

(A) Implantation mechanism

The interaction between trophoblasts and uterine epithelial cells is the common event of implantation in all the species. However, the degree of fusion of their apices and invasion by trophoblasts in uterine wall is species specific. In pig, though the apices of trophoblasts and LE cells closely interdigitate with each other, their fusion does not occur. In this species, trophoblast never penetrates through the LE cells. Therefore, the conceptus remains superficial in uterine lumen for the growth and development (Carson et al., 2000). The placenta also remains superficial in ruminants, but there is a fusion of trophoblasts with LE cells (Carson et al., 2000). In case of superficial implantation, blastocyst occupies the central position in the uterine lumen, and hence, implantation is known as centric implantation.

In rabbit, an intricate phenomenon is involved in implantation. The trophoblasts after fusion with each other form the syncytiotrophoblast which further fuses with LE cells. After that, syncytiotrophoblast invade through LE cell to reach to the basal lamina and stroma (Carson et al., 2000).

A distinct type of implantation is observed in rodents wherein uterine epithelium adjacent to the decidual area undergoes apoptosis. The trophoblast cells phagocytose the sloughed tissues. During this shedding of uterine epithelium, decidual cells migrate to the site of implantation and are reported to induce the shedding phenomenon. The rodent blastocyst has been described to lack invasive behavior until its surrounding epithelium is removed away.

Further, the basal lamina is denuded so that the stromal invasion can occur for reorganization of stromal tissue surrounding the blastocyst. In essence, trophoblast penetration through the epithelium is not involved in rodents, since LE is removed away before the blastocyst commences stromal cells migration and rearrangement to from the implantation chamber (Carson et al., 2000). Such type of implantation is called displacement implantation.

In primates, implantation is intrusive. Invasion proceeds between epithelial cells without destroying them. In humans, thin folds of trophoblast cells form invadopodia that help in intrusive invasion to reach the underlying basement membrane (fig. 2). After destroying basement membrane, trophoblast cells reach the stromal compartment. During progression, some trophoblast cells fuse to form a syncytium that proliferates and invades the endometrial stroma. Thereafter, the blastocyst is completely embedded in the uterine stroma and the site of entry is covered by fibrin, over which the uterine epithelial cells grow and hide the blastocyst (Bischoff and Campana, 1996).

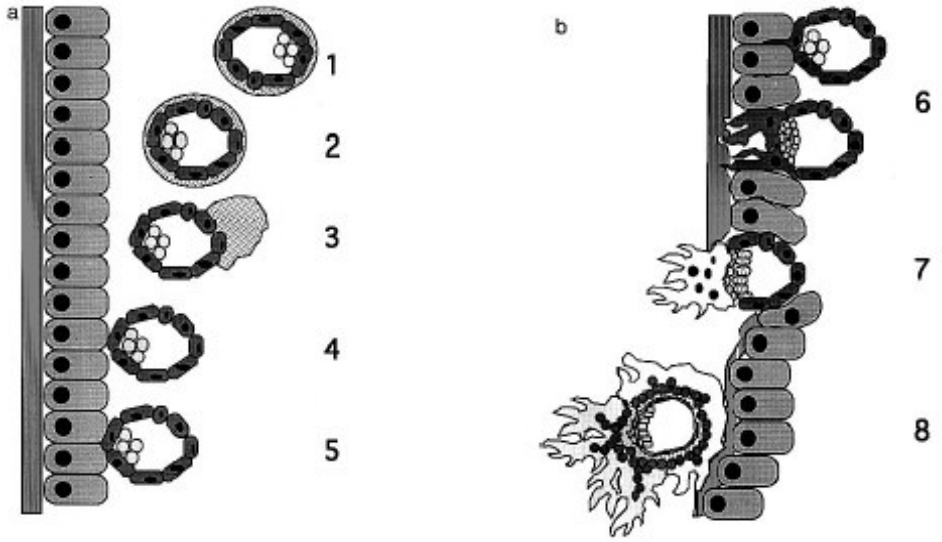

Fig. 2. (a) Implantation of the human blastocyst step by step.

Step 1: transport. The blastocyst arrives in the uterus 132–144 h after fertilization (Findlay, 1984). Step 2: orientation. The inner cell mass is orientated towards the endometrial epithelial lining. Step 3: hatching. The zona pellucida dissolves perhaps because of the secretion of proteases by trophectodermal cells. Step 4: apposition. The blastocyst is now in close contact with the endometrial lining but no connections have been established. The embryo can still be dislodged by washing. Step 5: adhesion. Connections of an unknown nature are established between the embryo and the endometrial epithelium. The embryo can no longer be dislodged. **(b) Implantation of the human blastocyst step by step.** Step 6: invasion. Thin folds of trophectodermal cells intrude between the endometrial epithelial cells. Step 7: syncytialization. Some trophectodermal cells fuse to form syncytia. These syncytia proliferate and invade the endometrial extracellular matrix. Step 8: villous formation. The former trophectodermal cells, now called cytotrophoblastic cells, migrate between the syncytia followed by the fetal stroma. This will lead to the formation of the placental villi (Bischof and Campana, 1996).

(B) Embryo position

Based on the position of embryo in the uterus, implantation can be classified into three categories: centric, eccentric and interstitial (fig. 3). In centric type, blastocyst grows to a larger size and remains in the centre of uterine lumen for further growth and development (e.g. ruminants, and pigs). The eccentric type of implantation is observed in rodents. In these

animals, blastocyst is small in size and implants in the implantation chamber on the side of the uterus. However, in interstitial type, blastocyst is completely embedded in uterine stromal tissues, and encircled by LE cells at the entry site. Such implantation is often called nidation ("nest making") and is observed in primates and guinea pigs.

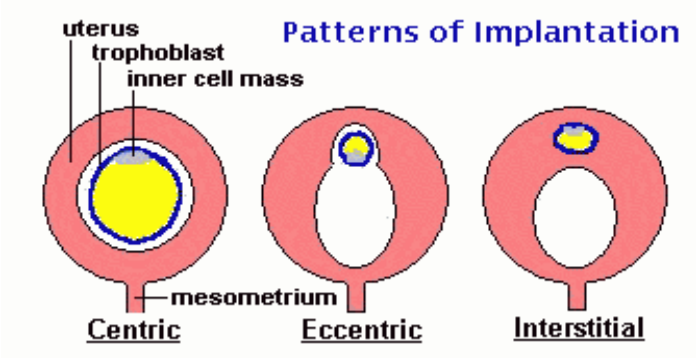

Fig. 3. Types of implantation depending on the position of embryo in uterus

PLACENTATION

In mammals, the growth and development of embryo solely depend on mother for their nutrients and oxygen supply. To accomplish this, embryo develops a transient organ called placenta that also facilitate the excretion of metabolic wastes and CO2 in the maternal system. In other words, placenta is an interface between fetus and mother for the transfer of nutrients and exchange of respiratory gases.

Placentation in mice starts with the separation of trophoblasts from the ICM on 3.5 days post coitum (dpc). The trophoblast cells adjacent to the ICM, called polar trophoblast, differentiate into extraembryonic ectoderm and ectoplacental cone (Watson and Cross, 2005). The extraembryonic ectoderm gives rise to the chorion. It is supported by the ectoplacental cone-derived spongiotrophoblast. The trophoblast cells away from ICM are known as mural trophoblasts. They cease to divide. However, the DNA replicates and results in formation of polyploid cells, the giant trophoblasts. Meanwhile, ICM gives rise to the endodermal cells which migrate to an area inner to the giant trophoblasts. The endodermal cells participate in the formation of basement membrane that separates the endodermal cells from the giant trophoblasts. On day 8 of gestation, the mesoderm derived from ICM lines the inner surface of the endoderm and gives rise to the first vascular cells and primitive vitelline vessels (visceral yolk sac) (Malassine et al., 2003). The mesoderm also gives rise to the allantois. On embryonic day 8.5, the chorion fuses with the allantois to form the chorioallantoic attachment. Immediately after the chorioallantoic fusion, the chorion folds to form villi. Further, blood vessels derived from allantoic mesoderm penetrate the villous. This process, known as vascular invasion, occurs on 9 dpc. The chorion along with the fetal blood vessels referred as labyrinth differentiate into three layers, two layers of multinucleated syncytiotrophoblasts (STB) and a single layer of mononuclear cytotrophoblasts. While STB surrounds the endothelium of fetal capillaries, mononuclear cytotrophoblasts make a close association with maternal blood (Malassine et al., 2003). The maternal blood enters the labyrinth, bathes the fetal trophoblast and allows the transfer of nutrients and respiratory gas exchange with fetal blood.

In humans, fluid-filled spaces called trabeculae appear in the syncytiotrophoblasts (STB) and transforming it into a sponge like material (Bischoff and Campana, 1996). Thereafter, the cytotrophoblasts (CTB) invade and proliferate in these trabeculae to form the primary

chorionic villi. These villi further branch extensively and by day 21 a definite structure of placenta becomes apparent. Each villus is composed of outer STB covering the monolayer of CTB. Gradually, STB disappears from the tip of villus following apoptosis and thus, allowing the underlying CTB to proliferate. The CTB invade the decidua and remodel the spiral arteries so that fresh blood from mother begins to flow into the intervillous space and bath the embryo (Bischoff and Campana, 1996). Further, extraembryonic connective tissue underlying the trophoblast penetrates the cytotrophoblasts strands. This is closely followed by the invasion of blood vessels that are extension and ramifications of the allantoic blood vessels.

TYPES OF PLACENTA

Although the placenta of all eutherian mammals shares common structural and functional features, the contact sites between allantochorion and endometrium vary considerably. In horse and pig, the entire surface of the allantochorion is in contact with endometrium to form the placenta. This type of placenta is known as diffuse placenta. However, in ruminants, instead of entire surface of allantochorion, some discrete sites referred as cotyledon make contact with the patches of the endometrium to form the cotyledonary placenta. In case of zonary placenta in dog, cat, bear, and elephant, the contact sites between allantochorion and endometrium is in the form of a distinct band or ring. Placenta in primates and rodents is discoidal, as only some parts of allantochorion in the form of a disk interact with the endometrium to form the placenta (fig. 4).

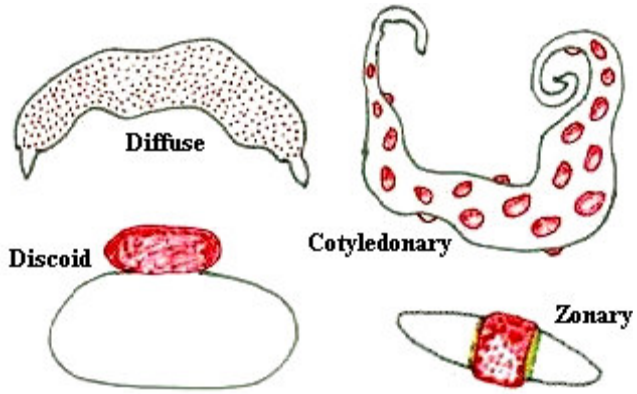

Fig. 4. Types of placenta depending on contact sites between allantochorion and endometrium

The other way of classifying placenta is the number of layers separating the fetal and maternal blood supply. In fact, there are three maternal tissue layers (endothelium, connective tissue and endometrial epithelium) and equal number of fetal tissue layers (fetal chorionic epithelium, connective and endothelial tissue) that separate the fetal blood from the maternal blood (fig. 5). In horses and ruminants, all the six layers are present. Here, uterine endometrial epithelium remains in close association with fetal chorion, and therefore, the placenta is referred as epitheliochorial type. In contrast, in case of humans, chorionic villi have eroded through maternal endothelium, resulting in the direct contact of maternal blood with fetal chorion. This type of placenta is called as hemochorial placenta. In third type of placenta, the endothelium of uterine blood vessels is retained. It prevents the direct contact of maternal blood with chorioallantois. This category of placenta, endotheliochorial, is observed in dog and cat.

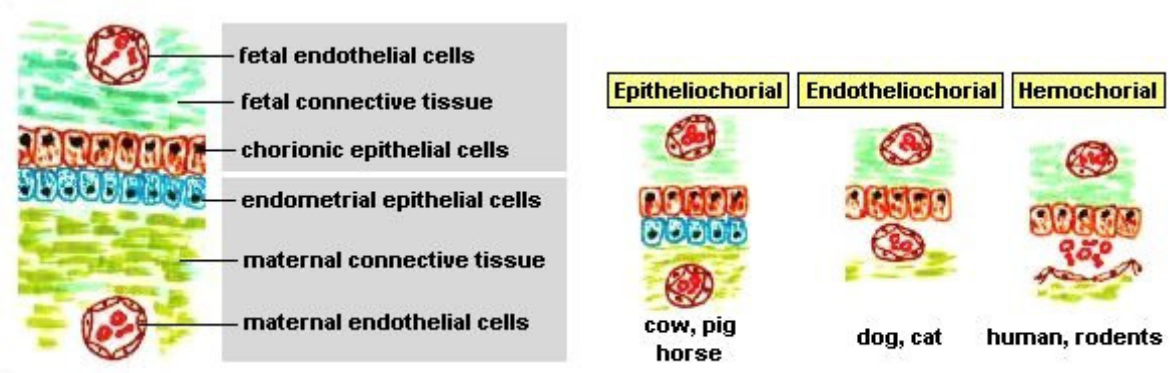

Fig. 5. Types of placenta depending on the number of layers separating the fetal and maternal blood supply

PREGNANCY RECOGNITION AND MAINTENANCE

The maternal recognition of pregnancy reflects the various ways in which the mother responds to the presence of conceptus within her reproductive tract. The conceptus acquires some measures to regulate the function of corpus luteum, uterine blood supply and maternal physiology including immune responses. The embryo signals its presence to maternal system and thereby prolongs the life span of corpus luteum (CL) (Niswender et al., 2000). CL majorly produces the pregnancy maintenance hormone, progesterone, which makes the uterine environment conducive for embryonic development. However, if embryo fails to signal its presence to the mother, CL regresses. In most of the mammals, CL regression and degeneration (luteolysis) is mediated by prostaglandin F2α (PGF2α) (Roberts et al., 1996).

In man and anthropoid apes, the chorionic gonadotropin (CG) which is functionally comparable to LH, is primarily involved in maintenance of pregnancy. The immunization of marmoset monkeys against the CG results in luteolysis and termination of pregnancy. CG abrogates the luteolytic effect of PGF2α of ovarian origin (Roberts et al., 1996). During first eight week of pregnancy, CG regulates the progesterone production from CL (Niswender et al., 2000). Thereafter, progesterone from STB takes over the function of CL since ovariectomy after eight week of gestation fails to influence the pregnancy (Niswender et al., 2000). In mice, instead of CG, prolactin secreted from the pituitary in response to neural-reflex activated by mating maintains the early phase of pregnancy upto 10-11 dpc by keeping the CL functional. After that, trophoblastic giant cells start producing prolactin-like lactogens known as placental lactogens. This takes over the function of pituitary prolactin because hypophysectomy after embryonic day 11 does not terminate the pregnancy (Malassine et al., 2003). In cattle and sheep, CL regression is mediated by the pulsatile release of PGF2α, secreted from the uterine endometrium. To maintain the prolonged functionality of CL, the secretion of PGF2α is inhibited by interferon-tau (IFN-τ) (Roberts et al., 1996). This antiluteolytic factor IFN-τ is secreted by mononucleated trophoblasts.

MATERNAL ADAPTATIONS DURING PREGNANCY

A plethora of changes occur in maternal respiratory, cardiovascular and renal systems during pregnancy to facilitate the fetal growth (Norwitz et al., 2005). Placental progesterone stimulates the respiratory centre in brain. This causes hyperventilation to rapidly remove the CO2 from the maternal blood. Consequently, maternal plasma CO2 is lowered down to less than the fetus. This difference facilitates the effective diffusion of CO2 from fetus to maternal circulation. In addition, differences between the oxyhemoglobin dissociation curves of fetal hemoglobin and adult hemoglobin helps the fetus to effectively extract the oxygen from the

maternal blood. Further, progesterone increases the glomerular filtration rate and renal blood flow to eliminate the nitrogenous wastes. This increase in GFR induces secretion of aldosterone that in turn enhances the renal sodium reabsorption and water retention. Thus, the maternal blood plasma volume increases. In addition, progesterone increases the number of red blood corpuscles (RBC) by affecting the secretion of erythropoietin. The increase in plasma volume and erythrocyte mass enhances the efficient transfer of nutrients and gaseous exchange between fetus and mother.

The early gestation period is highly anabolic. In this phase, energy is reserved in the form of fats in maternal adipose tissue. These reserves facilitate the rapid fetal growth in later gestation. During early pregnancy, peripheral tissues of mother develop resistance for insulin, and thereby, glucose consumption by peripheral tissues decreases and plasma glucose level increases. This condition is known as gestational diabetes. Further, the increased glucose plasma level during pregnancy induces the amplified secretion of pancreatic insulin referred as relative hyperinsulinemia.

PARTURITION

The ultimate event of pregnancy is parturition which is mutually controlled by maternal and fetal systems. In goat, parturition is initiated with the demise of CL and consequently sharp decrease of progesterone. In case of sheep and primates, a decrease in progesterone of placental origin initiates the parturition. Throughout the pregnancy high level of progesterone maintains the uterine quiescence and cervical integrity by decreasing myometrial contractility, inhibition of myometrial gap junction formation, down regulation of prostaglandins synthesis and oxytocin receptor expression (Graham and Clarke, 1997). In contrast, the quiescent uterus must be converted to an active and coordinately contracting organ for parturition. In addition, cervical connective tissue and smooth muscle tend to become capable to dilate the cervix for the smooth passage of fetus.

Fetus also actively participates in parturition. Towards term end, an inadequate supply of nutrients and respiratory gases to developing fetus generates a state of stress that in turn activates the fetal hypothalamo-pituitary-adrenal axis (HPA). This results in increased secretion of cortisol and dehydroepiandrosterone (DHEA) from fetal adrenal cortex zone (Weiss, 2000). DHEA is converted into the estrogen by the placenta. Estrogen reduces the negative feedback effects of cortisol on the fetal hypothalamic-pituitary centers by facilitating the conversion of cortisol into inactive cortisone. A decrease in negative feedback effect leads to increase of fetal ACTH secretion and eventually estrogen production. The estrogen dominant environment stimulates the synthesis of contractile proteins, regulatory enzymes necessary for uterine contractility, expression of connexin 43, and gap junction in the myometrium to increase the intracellular communication (Weiss, 2000). Also, estrogen enhances the expression of oxytocin and _- adrenergic receptors in the myometrium. Oxytocin through activation of phospholipase C increases the myometrial contraction by inducing the release of PGE2, PGF2_ from fetal membranes. The last step of parturition includes cervical ripening wherein rearrangement and realignment of collagen, elastin, and glycosaminoglycans occurs in cervix in response to the estrogen. At the same time, relaxin of CL origin relaxes the pubic symphysis for smooth passage of fetus through cervix.

ABNORMALITIES IN PREGNANCY

(A) Ectopic pregnancy

The uterine cavity is the potential site for embryo implantation. In certain pathological conditions, the implantation of embryo occurs at a site away from the uterine cavity. Such type of implantation is known as ectopic implantation and the pregnancy is referred as

ectopic pregnancy. It might lead to haemorrhage in pregnancy and also maternal deaths during first trimester. Ectopic pregnancy can be categorized into two types:

1. **Tubal pregnancy:** In this case, the fertilized ovum fails to enter into the uterine cavity. herefore, the implantation of embryo occurs in fimbriae, ampulla or isthumus rather than the uterine cavity.

2. **Abdominal pregnancy:** It is characterized by the development of zygote in the peritoneal cavity. The fertilized ovum either enters the peritoneal cavity and becomes attached to the mesentery or abdominal viscera, or expelled in the peritoneal cavity due to rupturing of oviduct or uterus.

(B) Molar pregnancy

It is a kind of genetic disorder due to chromosomal imbalance at the time of conception. The normal fertilized egg contains 23 chromosomes from the father and 23 from the mother. Any chromosomal imbalance such as the loss of maternal chromosomes or an excess copy of paternal chromosomes in the fertilized egg leads to the development of a malformed placenta known as "mole". Such type of placenta is clinically known as hydatidiform mole. The mole is further classified in two types:

1. **Complete Mole:** It is a pathological condition in which the loss or inactivation of ovum's nuclei occur. The fertilized egg has none of the mother's chromosomes. In order to compensate, the father's chromosomes are doubled. This causes the development of an abnormal placenta which looks like a cluster of grapes that occupy the entire uterine cavity and prevents the formation of the fetus.

2. **Partial Mole:** A partial mole is formed when an egg is fertilized by two sperms. As a result, the zygote contains 69 chromosomes (23 from mother and 46 from father) instead of 46. In this case along with cluster of abnormal cells, partial development of normal placental tissue occurs. Also, the fetus development does occur in the uterus. However, due to extra chromosomes of father, the embryo suffers severe birth defects and dies in the uterus.

PREGNANCY TEST

(A) hCG assay: The chorionic gonadotropin (hCG) assay in urine sample is the most common test used for detecting human pregnancy. hCG is the heterodimeric glycoprotein hormone. It is composed of dissimilar α and β -subunits that are held noncovalently. It is the unique β-subunit that confers the biological and immunological specificity to the hCG molecule**.** hCG produced by syncytiotrophoblast is detectable as early as seven days after ovulation and is considered the earliest hormonal signal of pregnancy. The level of hCG rises very rapidly after implantation. Peak levels occur at approximately 9-10 weeks of gestation. After that the levels fall slowly, reach to a nadir by 17-18th week of gestation, and remain low for the remainder of pregnancy. The β-hCG ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay (Ozturk et al., 1987). The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the β -subunit of the hCG molecule. Mouse monoclonal anti-β -hCG antibody is used for solid phase immobilization on microtiter wells. The test samples of urine are added to the monoclonal anti-β -hCG coated microtiter wells and incubated at 37°C. The hCG present in sample binds to the antibody coated on wells. After 30 min, the wells are repeatedly washed to remove unbound hCG in sample. Thereafter, rabbit anti-β -hCG antibody conjugated with enzyme horseradish peroxidase is supplemented to microtiter

wells. As a result, β -hCG molecule is sandwiched between the solid phase and enzyme-linked antibodies. The wells are washed with deionized water to remove unbound antibody. A solution containing 3, 3', 5, 5' tetramethylbenzidine (TMB) and hydrogen peroxide is added and incubated for 20 min to develop the blue color. The color development is stopped with the addition of stop solution (1N HCl) changing the color to yellow. Absorbance is measured spectrophotometrically at 450 nm. The concentration of β -hCG is directly proportional to the color intensity of the test sample.

(B) Ultrasound

The high frequency sound waves are extensively used in medical field to localize the size and shape of any organ in the body, and also for imaging the fetus and female pelvic organs during pregnancy. Obstetrical ultrasound can be performed trans-vaginally or trans-abdominally. Trans- vaginal ultrasound is used commonly in the first trimester, while trans-abdominal ultrasound is most useful in the second and third trimesters. In addition to pregnancy test, healthcare provider uses ultrasound to look for growth of baby, rule out any pathological condition, evaluate the volume of amniotic fluid, diagnose multiple pregnancy, and to confirm the expected date of baby's birth. It is to be noted that identification of sex following ultrasound is illegal and amounts to punishment.

CONCLUSION

Pregnancy is a complex sequential process that begins with fertilization and ends with parturition. Fertilization occurs in the ampullary region of the oviduct. Fusion of male and female gametes leads to the formation of zygote that undergoes cleavage to form the morula. With the formation of a fluid-filled cavity, morula is transformed into the blastula. The various developmental processes leading to blastula formation is controlled by up/down regulation of different gene products such as IQGAP, rac-1, Cdc 42, MAPK, actin filaments and Oct-4. The trophoectodermal cells of blastula acquire the competence to attach to the receptive endometrium. The process of implantation completes in three steps, namely, apposition, adhesion and penetration. During apposition, trophoblasts come close to the endometrium. The decrease of MUC-1 and increase of integrins, heparan sulphate, lacto-N-fucopentose 1, trophonin and tastin facilitate the adherence of blastocyst to the endometrium. After that, blastocyst penetration takes place. The degree of penetration varies from species to species. The penetration is facilitated by the expression of matrix metalloproteinases, serine proteases and plasmins on the surface of trophoblasts. The blastocyst penetration is accompanied by decidualization of stromal cells. The decidual cells secrete a number of factors that helps in nourishing the developing embryo and regulates the invasion of trophoblasts. The preovulatory E2 and luteal P4 primarily control the implantation. Moreover, the E2 of luteal phase is responsible for the receptivity of endometrium. However, the blastocyst-derived CG is reported to be mandatory in baboon to induce the uterine receptivity and decidualization. In the absence of uterine receptivity due to lack of luteal phase E2, the implantation does not occur and the blastocyst remain in dormant stage for a certain period. This is called the delayed implantation. Embryo develops a transient organ called placenta for deriving nutrients and oxygen from the mother. It also helps in excreting metabolic wastes in the maternal vascular system during placentation. The ICM-derived extraembryonic mesoderm together with trophoectodermal cells forms the allantochorion that associate with endometrium or uterine blood vessels. The different types of placenta are described based on the intensity of contact between allantochorion and endometrium or number of maternal tissue layers separating the fetus from the maternal blood. Progesterone is the key player in maintenance of pregnancy. The high level of P4 down regulates the

expression of oxytocin receptors and PGF2α. This reduces the myometrial contraction and decreases the chances of pregnancy termination. In other words, P4 keeps the uterine environment favorable for embryonic development. Further, P4 modulates the cardiovascular, respiratory and renal physiology of mother to support the fetal growth and development. At the time of parturition, a sharp decrease in P4 results in marked increase of prostaglandin production and oxytocin receptor expression that induces the myometrial contraction and consequently parturition. In addition to P4, relaxin also contributes in parturition by relaxing the pubic symphysis.

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