**CLEAVAGE**

Once the zygote has reached the two-cell stage, it undergoes a series of mitotic divisions, increasing the numbers of cells. These cells, which become smaller with each cleavage division, are known as blastomeres .Until the eight-cell stage, they form a loosely arranged clump . After the third cleavage, however, blastomeres maximize their contact with each other, forming a compact ball of cells held together by tight junctions . This process, compaction, segregates inner cells, which communicate extensively by gap junctions, from outer cells. Approximately 3 days after fertilization, cells of the compacted embryo divide again to form a 16-cell morula (mulberry). Inner cells of the morula constitute the inner cell mass, and surrounding cells compose the outer cell mass. The inner cell mass gives rise to tissues of the embryo proper, and the outer cell mass forms the trophoblast, which later contributes to the placenta.

**BLASTOCYST FORMATION**

About the time the morula enters the uterine cavity, fluid begins to penetrate through the zona pellucida into the intercellular spaces of the inner cell mass. Gradually, the intercellular spaces become confluent, and finally, a single cavity, the blastocele, forms at this time, the embryo is a blastocyst. Cells of the inner cell mass, now called the embryoblast, are at one pole, and those of the outer cell mass, or trophoblast, flatten and form the epithelial wall of the blastocyst.The zona pellucida has disappeared, allowing implantation to begin. In the human, trophoblastic cells over the embryoblast pole begin to penetrate between the epithelial cells of the uterine mucosa on about the sixth day. New studies suggest that L-selectin on trophoblast cells and its carbohydrate receptors on the uterine epithelium mediate initial attachment of the blastocyst to the uterus. Selectins are carbohydrate-binding proteins involved in interactions between leukocytes

and endothelial cells that allow leukocyte “capture” from flowing blood. A similar mechanism is now proposed for “capture” of the blastocyst from the uterine cavity by the uterine epithelium. Following capture by selectins, further attachment and invasion by the trophoblast involve integrins, expressed by the trophoblast and the extracellular matrix molecules laminin and fibronectin. Integrin receptors for laminin promote attachment, while those for fibronectin stimulate migration. These molecules also interact along signal transduction pathways to regulate trophoblast differentiation, so that implantation is the result of mutual trophoblastic and endometrial action. Hence, by the end of the first week of development, the human zygote has passed through the morula and blastocyst stages and has begun implantation in the uterine mucosa.

**UTERUS AT TIME OF IMPLANTATION**

The wall of the uterus consists of three layers: (1) endometrium or mucosa lining the inside wall; (2) myometrium, a thick layer of smooth muscle; and (3) perimetrium, the peritoneal covering lining the outside wall. From puberty (11 to 13 years) until menopause  (45 to 50 years), the endometrium undergoes changes in a cycle of approximately 28 days under hormonal control by the ovaries. During this menstrual cycle, the uterine endometrium passes through three stages, the follicular or proliferative phase, the secretory or progestational phase, and the menstrual phase. The proliferative phase begins at the end of the menstrual phase, is under the influence of estrogen, and parallels growth of the ovarian follicles. The secretory phase begins approximately 2 to 3 days after ovulation in response to progesterone produced by the corpus luteum. If fertilization does not occur, shedding of the endometrium (compact and spongy layers) marks the beginning of the menstrual phase. If fertilization does occur, the endometrium assists in implantation and contributes to formation of the placenta. Later in gestation, the placenta assumes the role of hormone production, and the corpus luteum degenerates.

**Clinical Correlates**

**Embryonic Stem Cells**

Embryonic stem cells (ES cells) are derived from the inner cell mass of the embryo. Because these cells are pluripotent and can form virtually any cell or tissue type, they have the potential for curing a variety of diseases, including diabetes, Alzheimer's and Parkinson's diseases, anemias, spinal cord injuries, and many others. Using animal model research with stem cells has been encouraging. For example, mouse ES cells in culture have been induced to form insulin-secreting cells, muscle and nerve stem cells, and glial cells. In whole animals, ES cells have been used to alleviate the symptoms of Parkinson's disease and to improve motor ability in rats with spinal cord injuries.

ES cells may be obtained from embryos after in vitro fertilization, a process called reproductive cloning. This approach has the disadvantage that the cells may cause immune rejection, because they would not be genetically identical to their hosts. The cells could be modified to circumvent this problem, however. Another issue with this approach is based on ethical considerations, as the cells are derived from fertilized viable embryos.

As the field of stem cell research progresses, scientific advances will provide more genetically compatible cells, and the approaches will be less controversial. Most recently, techniques have been devised to take nuclei from adult cells (e.g., skin) and introduce them into enucleated oocytes. This approach is called therapeutic cloning or somatic nuclear transfer. Oocytes are stimulated to differentiate into blastocysts, and ES cells are harvested. Because the cells are derived from the host, they are compatible genetically and because fertilization is not involved, the technique is less controversial.

**Adult Stem Cells**

Adult tissues contain stem cells that may also prove valuable in treating diseases. These cells are restricted in their ability to form different cell types and, therefore, are multipotent, not pluripotent, although scientists are finding methods to circumvent this disadvantage. Adult stem cells isolated from rat brains have been used to cure Parkinson's disease in rats, suggesting that the approach has promise. Disadvantages of the approach include the slow rates of cell division characteristic of these cells and their scarcity, which makes them difficult to isolate in sufficient numbers for experiments.

**Abnormal Zygotes**

The exact number of abnormal zygotes formed is unknown because they are usually lost within 2 to 3 weeks of fertilization, before the woman realizes she is pregnant, and therefore are not detected. Estimates are that as many as 50% of pregnancies end in spontaneous abortion and that half of these losses are a result of chromosomal abnormalities. These abortions are a natural means of screening embryos for defects, reducing the incidence of congenital malformations. Without this phenomenon, approximately 12% instead of 2% to 3% of infants would have birth defects.

At the time of implantation, the mucosa of the uterus is in the secretory phase , during which time uterine glands and arteries become coiled and the tissue becomes succulent. As a result, three distinct layers can be recognized in the endometrium: a superficial compact layer, an intermediate spongy layer, and a thin basal layer .Normally, the human blastocyst implants in the endometrium along the anterior or posterior wall of the body of the uterus, where it becomes embedded between the openings of the glands.

If the oocyte is not fertilized, venules and sinusoidal spaces gradually become packed with blood cells, and an extensive diapedesis of blood into the tissue is seen. When the menstrual phase begins, blood escapes from superficial arteries, and small pieces of stroma and glands break away. During the following 3 or 4 days, the compact and spongy layers are expelled from the uterus, and the basal layer is the only part of the endometrium that is retained . This layer, which is supplied by its own arteries, the basal arteries, functions as the regenerative layer in the rebuilding of glands and arteries in the proliferative phase .

**Second Week of Development: Bilaminar Germ Disc**

This chapter gives a day-by-day account of the major events of the second week of development; however, embryos of the same fertilization age do not necessarily develop at the same rate. Indeed, considerable differences in rate of growth have been found even at these early stages of development.

**DAY 8**

At the eighth day of development, the blastocyst is partially embedded in the endometrial stroma. In the area over the embryoblast, the trophoblast has differentiated into two layers: (1) an inner layer of mononucleated cells, the cytotrophoblast, and (2) an outer multinucleated zone without distinct cell boundaries, the syncytiotrophoblast. Mitotic figures are found in the cytotrophoblast but not in the syncytiotrophoblast. Thus, cells in the cytotrophoblast divide and migrate into the syncytiotrophoblast, where they fuse and lose their individual cell membranes.

Cells of the inner cell mass or embryoblast also differentiate into two layers: (1) a layer of small cuboidal cells adjacent to the blastocyst cavity, known as the hypoblast layer, and (2) a layer of high columnar cells adjacent to the amniotic cavity, the epiblast layer.

Together, the layers form a flat disc. At the same time, a small cavity appears within the epiblast. This cavity enlarges to become the amniotic cavity. Epiblast cells adjacent to the cytotrophoblast are called amnioblasts; together with the rest of the epiblast, they line the amniotic cavity. The endometrial stroma adjacent to the implantation site is edematous and highly vascular. The large, tortuous glands secrete abundant glycogen and mucus.

**DAY 9**

The blastocyst is more deeply embedded in the endometrium, and the penetration defect in the surface epithelium is closed by a fibrin coagulum. The trophoblast shows considerable progress in development, particularly at the embryonic pole, where vacuoles appear in the syncytium. When these vacuoles fuse, they form large lacunae, and this phase of trophoblast development is thus known as the lacunar stage. At the abembryonic pole, meanwhile, flattened cells probably originating from the hypoblast form a thin membrane, the exocoelomic (Heuser's) membrane that lines the inner surface of the cytotrophoblast. This membrane, together with the hypoblast, forms the lining of the exocoelomic cavity, or primitive yolk sac.

**DAYS 11 AND 12**

By the 11th to 12th day of development, the blastocyst is completely embedded in the endometrial stroma, and the surface epithelium almost entirely covers the original defect in the uterine wall. The blastocyst now produces a slight protrusion into the lumen of the uterus. The trophoblast is characterized by lacunar spaces in the syncytium that form an intercommunicating network. This network is particularly evident at the embryonic pole; at the abembryonic pole, the trophoblast still consists mainly of cytotrophoblastic cells

Concurrently, cells of the syncytiotrophoblast penetrate deeper into the stroma and erode the endothelial lining of the maternal capillaries. These capillaries, which are congested and dilated, are known as sinusoids. The syncytial lacunae become continuous with the sinusoids, and maternal blood enters the lacunar system. As the trophoblast continues to erode more and more sinusoids, maternal blood begins to flow through the trophoblastic system, establishing the uteroplacental circulation.

In the meantime, a new population of cells appears between the inner surface of the cytotrophoblast and the outer surface of the exocoelomic cavity. These cells, derived from yolk sac cells, form a fine, loose connective tissue, the extraembryonic mesoderm, which eventually fills all of the space between the trophoblast externally and the amnion and exocoelomic membrane internally. Soon, large cavities develop in the extraembryonic mesoderm, and when these become confluent, they form a new space known as the extraembryonic coelom, or chorionic cavity. This space surrounds the primitive yolk sac and amniotic cavity, except where the germ disc is connected to the trophoblast by the connecting stalk. The extraembryonic mesoderm lining the cytotrophoblast and amnion is called the extraembryonic somatopleuric mesoderm; the lining covering the yolk sac is known as the extraembryonic splanchnopleuric mesoderm.

Growth of the bilaminar disc is relatively slow compared with that of the trophoblast; consequently, the disc remains very small (0.1 to 0.2 mm). Cells of the endometrium, meanwhile, become polyhedral and loaded with glycogen and lipids; intercellular spaces are filled with extravasate, and the tissue is edematous. These changes, known as the decidua reaction, at first are confined to the area immediately surrounding the implantation site but soon occur throughout the endometrium.

**DAY 13**

By the 13th day of development, the surface defect in the endometrium has usually healed. Occasionally, however, bleeding occurs at the implantation site as a result of increased blood flow into the lacunar spaces. Because this bleeding occurs near the 28th day of the menstrual cycle, it may be confused with normal menstrual bleeding and, therefore, may cause inaccuracy in determining the expected delivery date.

The trophoblast is characterized by villous structures. Cells of the cytotrophoblast proliferate locally and penetrate into the syncytiotrophoblast, forming cellular columns surrounded by syncytium. Cellular columns with the syncytial covering are known as primary villi.

In the meantime, the hypoblast produces additional cells that migrate along the inside of the exocoelomic membrane. These cells proliferate and gradually form a new cavity within the exocoelomic cavity. This new cavity is known as the secondary yolk sac or definitive yolk sac. This yolk sac is much smaller than the original exocoelomic cavity, or primitive yolk sac. During its formation, large portions of the exocoelomic cavity are pinched off. These portions are represented by exocoelomic cysts, which are often found in the extraembryonic coelom or chorionic cavity.

Meanwhile, the extraembryonic coelom expands and forms a large cavity, the chorionic cavity. The extraembryonic mesoderm lining the inside of the cytotrophoblast is then known as the chorionic plate. The only place where extraembryonic mesoderm traverses the chorionic cavity is in the connecting stalk. With development of blood vessels, the stalk becomes the umbilical cord.

**Clinical Correlates**

**Abnormal Implantation**

The syncytiotrophoblast is responsible for hormone production including human chorionic gonadotropin (hCG). By the end of the second week, quantities of this hormone are sufficient to be detected by radioimmunoassays, which serve as the basis for pregnancy testing.

Because 50% of the implanting embryo's genome is derived from the father, it is a foreign body that potentially should be rejected by the maternal system. Recent evidence suggests that a combination of factors protects the conceptus, including production of immunosuppressive cytokines and proteins and the expression of an unusual major histocompatibility complex class IB molecule (HLA-G) that blocks recognition of the conceptus as foreign tissue. If the mother has an autoimmune disease, such as systemic lupus erythematosus, antibodies generated by the disease may attack the conceptus and reject it.

Abnormal implantation sites sometimes occur even within the uterus. Normally, the human blastocyst implants along the anterior or posterior wall of the body of the uterus. Occasionally, the blastocyst implants close to the internal os (opening) of the cervix, so that later in development, the placenta bridges the opening (placenta previa) and causes severe, even life-threatening bleeding in the second part of pregnancy and during delivery.

Occasionally, implantation takes place outside the uterus, resulting in extrauterine pregnancy, or ectopic pregnancy. Ectopic pregnancies may occur at any place in the abdominal cavity, ovary, or uterine tube. Ninety-five percent of ectopic pregnancies occur in the uterine tube, however, and most of these are in the ampulla. In the abdominal cavity, the blastocyst most frequently attaches itself to the peritoneal lining of the rectouterine cavity, or pouch of Douglas . The blastocyst may also attach itself to the peritoneal covering of the intestinal tract or to the omentum. Sometimes, the blastocyst develops in the ovary proper, causing a primary ovarian pregnancy. In most ectopic pregnancies, the embryo dies about the second month of gestation, causing severe hemorrhaging and abdominal pain in the mother.

Abnormal blastocysts are common. For example, in a series of 26 implanted blastocysts varying in age from 7.5 to 17 days recovered from patients of normal fertility, nine (34.6%) were abnormal. Some consisted of syncytium only; others showed varying degrees of trophoblastic hypoplasia. In two, the embryoblast was absent, and in some, the germ disc showed an abnormal orientation.

It is likely that most abnormal blastocysts would not have produced any sign of pregnancy because their trophoblast was so inferior that the corpus luteum could not have persisted. These embryos probably would have been aborted with the next menstrual flow and, therefore, pregnancy would not have been detected. In some cases, however, the trophoblast develops and forms placental membranes, although little or no embryonic tissue is present. Such a condition is known as a hydatidiform mole. Moles secrete high levels of human chorionic gonadotropin and may produce benign or malignant (invasive mole, choriocarcinoma) tumors.

Genetic analysis of hydatidiform moles indicates that although male and female pronuclei may be genetically equivalent, they may be different functionally. This evidence is derived from the fact that although cells of moles are diploid, their entire genome is paternal. Thus, most moles arise from fertilization of an oocyte lacking a nucleus followed by duplication of the male chromosomes to restore the diploid number. These results also suggest that paternal genes regulate most of the development of the trophoblast, because in moles this tissue differentiates even in the absence of a female pronucleus.

Other examples of functional differences in maternal and paternal genes are provided by the observation that certain genetic diseases depend on whether the defective or missing gene is inherited from the father or the mother. For example, inheritance of a microdeletion on chromosome 15 from a father produces Prader-Willi syndrome, whereas inheritance of the same defect from the mother results in Angelman syndrome. This phenomenon, in which there is differential modification and/or expression of homologous alleles or chromosome regions depending on the parent from whom the genetic material is derived, is known as genomic imprinting. Imprinting involves autosomes and sex chromosomes (in all female mammals, one X chromosome is inactivated in somatic cells and forms a chromatinpositive body [Barr body]) and is modulated by DNA methylation. Certain diseases, such as Huntington's chorea, neurofibromatosis, familial cancer disorders (Wilms' tumors, familial retinoblastoma), and myotonic dystrophy, also involve imprinting. Fragile X syndrome, the leading cause of inherited mental retardation, may be another example of a condition based on imprinting

**Third Week of Development: Trilaminar Germ Disc**

**GASTRULATION: FORMATION OF EMBRYONIC MESODERM AND ENDODERM**

The most characteristic event occurring during the third week of gestation is gastrulation, the process that establishes all three germ layers (ectoderm, mesoderm, and endoderm) in the embryo. Gastrulation begins with formation of the primitive streak on the surface of the epiblast. Initially, the streak is vaguely defined, but in a 15- to 16-day embryo, it is clearly visible as a narrow groove with slightly bulging regions on either side. The cephalic end of the streak, the primitive node, consists of a slightly elevated area surrounding the small primitive pit. Cells of the epiblast migrate toward the primitive streak. Upon arrival in the region of the streak, they become flask-shaped, detach from the epiblast, and slip beneath it. This inward movement is known as invagination. Cell migration and specification are controlled by fibroblast growth factor 8 (FGF8), which is synthesized by streak cells themselves. This growth factor controls cell movement by downregulating E-cadherin, a protein that normally binds epiblast cells together. FGF8 then controls cell specification into the mesoderm by regulating Brachyury (T) expression. Once the cells have invaginated, some displace the hypoblast, creating the embryonic endoderm, and others come to lie between the epiblast and newly created endoderm to form mesoderm. Cells remaining in the epiblast then form ectoderm. Thus, the epiblast, through the process of gastrulation, is the source of all of the germ layers and cells in these layers will give rise to all of the tissues and organs in the embryo.

As more and more cells move between the epiblast and hypoblast layers, they begin to spread laterally and cranially. Gradually, they migrate beyond the margin of the disc and establish contact with the extraembryonic mesoderm covering the yolk sac and amnion. In the cephalic direction, they pass on each side of the prechordal plate. The prechordal plate itself forms between the tip of the notochord and the oropharyngeal membrane and is derived from some of the first cells that migrate through the node in the midline and move in a cephalic direction. Later, the prechordal plate will be important for induction of the forebrain. The oropharyngeal membrane at the cranial end of the disc consists of a small region of tightly adherent ectoderm and endoderm cells that represents the future opening of the oral cavity.

**FORMATION OF THE NOTOCHORD**

Prenotochordal cells invaginating in the primitive node move forward cranially in the midline until they reach the prechordal plate. These prenotochordal cells become intercalated in the hypoblast so that for a short time, the midline of the embryo consists of two cell layers that form the notochordal plate. As the hypoblast is replaced by endoderm cells moving in at the streak, cells of the notochordal plate proliferate and detach from the endoderm. They then form a solid cord of cells, the definitive notochord, which underlies the neural tube and serves as the basis for the axial skeleton. Because elongation of the notochord is a dynamic process, the cranial end forms first, and caudal regions are added as the primitive streak assumes a more caudal position. The notochord and prenotochordal cells extend cranially to the prechordal plate (an area just caudal to the oropharyngeal membrane) and caudally to the primitive pit. At the point where the pit forms an indentation in the epiblast, the neurenteric canal temporarily connects the amniotic and yolk sac cavities.

The cloacal membrane is formed at the caudal end of the embryonic disc. This membrane, which is similar in structure to the oropharyngeal membrane, consists of tightly adherent ectoderm and endoderm cells with no intervening mesoderm. When the cloacal membrane appears, the posterior wall of the yolk sac forms a small diverticulum that extends into the connecting stalk. This diverticulum, the allantoenteric diverticulum, or allantois, appears around the 16th day of development. Although in some lower vertebrates the allantois serves as a reservoir for excretion products of the renal system, in humans, it remains rudimentary but may be involved in abnormalities of bladder development.

**FATE MAP ESTABLISHED DURING GASTRULATION**

Regions of the epiblast that migrate and ingress through the primitive streak have been mapped, and their ultimate fates have been determined . For example, cells that ingress through the cranial region of the node become prechordal plate and notochord; those migrating at the lateral edges of the node and from the cranial end of the streak become paraxial mesoderm; cells migrating through the midstreak region become intermediate mesoderm; those migrating through the more caudal part of the streak form lateral plate mesoderm; and cells migrating through the caudalmost part of the streak contribute to extraembryonic mesoderm (the other source of this tissue is the primitive yolk sac [hypoblast].

**GROWTH OF THE EMBRYONIC DISC**

The embryonic disc, initially flat and almost round, gradually becomes elongated, with a broad cephalic and a narrow caudal end. Expansion of the embryonic disc occurs mainly in the cephalic region; the region of the primitive streak remains more or less the same size. Growth and elongation of the cephalic part of the disc are caused by a continuous migration of cells from the primitive streak region in a cephalic direction. Invagination of surface cells in the primitive streak and their subsequent migration forward and laterally continues until the end of the fourth week. At that stage, the primitive streak shows regressive changes, rapidly shrinks, and soon disappears.

That the primitive streak at the caudal end of the disc continues to supply new cells until the end of the fourth week has an important bearing on development of the embryo. In the cephalic part, germ layers begin their specific differentiation by the middle of the third week, whereas in the caudal part, differentiation begins by the end of the fourth week. Thus gastrulation, or formation of the germ layers, continues in caudal segments while cranial structures are differentiating, causing the embryo to develop cephalocaudally.

**Clinical Correlates**

**Teratogenesis Associated with Gastrulation**

The beginning of the third week of development, when gastrulation is initiated, is a highly sensitive stage for teratogenic insult. At this time, fate maps can be made for various organ systems, such as the eyes and brain anlage, and these cell populations may be damaged by teratogens. For example, high doses of alcohol at this stage kill cells in the anterior midline of the germ disc, producing a deficiency of the midline in craniofacial structures and resulting in holoprosencephaly. In such a child, the forebrain is small, the two lateral ventricles often merge into a single ventricle, and the eyes are close together (hypotelorism). Because this stage is reached 2 weeks after fertilization, it is approximately 4 weeks from the last menses. Therefore, the woman may not recognize that she is pregnant, having assumed that menstruation is late and will begin shortly. Consequently, she may not take precautions she would normally consider if she knew she was pregnant.

Gastrulation itself may be disrupted by genetic abnormalities and toxic insults. In caudal dysgenesis (sirenomelia), insufficient mesoderm is formed in the caudalmost region of the embryo. Because this mesoderm contributes to formation of the lower limbs, urogenital system (intermediate mesoderm), and lumbosacral vertebrae, abnormalities in these structures ensue.

Affected individuals exhibit a variable range of defects, including hypoplasia and fusion of the lower limbs, vertebral abnormalities, renal agenesis, imperforate anus, and anomalies of the genital organs. In humans, the condition is associated with maternal diabetes and other causes.

Situs inversus is a condition in which transposition of the viscera in the thorax and abdomen occurs. Despite this organ reversal, other structural abnormalities occur only slightly more frequently in these individuals. Approximately 20% of patients with complete situs inversus also have bronchiectasis and chronic sinusitis.

**Tumors Associated With Gastrulation**

Sometimes, remnants of the primitive streak persist in the sacrococcygeal region. These clusters of pluripotent cells proliferate and form tumors, known as sacrococcygeal teratomas, that commonly contain tissues derived from all three germ layers (Fig. 5.9). This is the most common tumor in newborns, occurring with a frequency of one in 37,000. These tumors may also arise from primordial germ cells that fail to migrate to the gonadal ridge

**FURTHER DEVELOPMENT OF THE TROPHOBLAST**

By the beginning of the third week, the trophoblast is characterized by primary villi that consist of a cytotrophoblastic core covered by a syncytial layer. During further development, mesodermal cells penetrate the core of primary villi and grow toward the decidua. The newly formed structure is known as a secondary villus.

By the end of the third week, mesodermal cells in the core of the villus begin to differentiate into blood cells and small blood vessels, forming the villous capillary system. The villus is now known as a tertiary villus or definitive placental villus. Capillaries in tertiary villi make contact with capillaries developing in the mesoderm of the chorionic plate and in the connecting stalk. These vessels, in turn, establish contact with the intraembryonic circulatory system, connecting the placenta and the embryo. Hence, when the heart begins to beat in the fourth week of development, the villous system is ready to supply the embryo proper with essential nutrients and oxygen.

Meanwhile, cytotrophoblastic cells in the villi penetrate progressively into the overlying syncytium until they reach the maternal endometrium. Here they establish contact with similar extensions of neighboring villous stems, forming a thin outer cytotrophoblast shell . This shell gradually surrounds the trophoblast entirely and attaches the chorionic sac firmly to the maternal endometrial tissue. Villi that extend from the chorionic plate to the decidua basalis (decidual plate: the part of the endometrium where the placenta will form; are called stem or anchoring villi. Those that branch from the sides of stem villi are free (terminal) villi, through which exchange of nutrients and other factors will occur.

The chorionic cavity, meanwhile, becomes larger, and by the 19th or 20th day, the embryo is attached to its trophoblastic shell by a narrow connecting stalk .The connecting stalk later develops into the umbilical cord, which forms the connection between the placenta and embryo.